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Polyelectrolyte Capsules with Tunable Shell Behavior Fabricated by the Simple Layer-by-Layer Technique for the Control of the Release and Reactivity of Small Guests

Lucia Ya. Zakharova,*[,†] Alsu R. Ibragimova,[†] Elmira A. Vasilieva,[†,‡] Alla B. Mirgorodskaya,[†] Ekaterina I. Yackevich,[†] Irek R. Nizameev,[†] Marsil K. Kadirov,[†] Yuri F. Zuev,[§] and Alexander I. Konovalov[†]

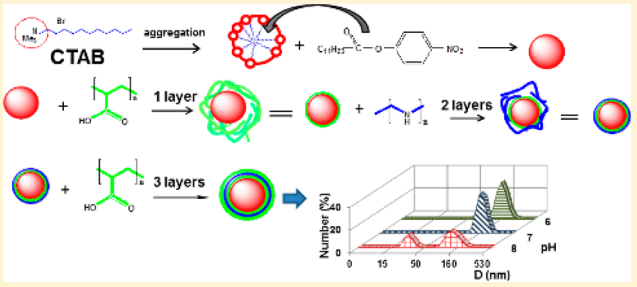
[†]State Budgetary-Funded Institution of Science, A. E. Arbuzov Institute of Organic and Physical Chemistry of Kazan Scientific Center of Russian Academy of Sciences, 8, ul. Arbuzov, 420088 Kazan, Russian Federation

[‡]Kazan National Research Technological University, 68, ul. K. Marx, Kazan, 420015, Russia

[§]State Budgetary-Funded Institution of Science Kazan Institute of Biochemistry and Biophysics of Kazan Scientific Center of the Russian Academy of Sciences, pob 30, Kazan, 420111 Russia

Supporting Information

ABSTRACT: A novel simple protocol for the layer-by-layer coating of uncharged organic substrates (hydrophobic carboxylic acid esters, CAEs) and control of their loading/release behavior has been developed. The approach involves the preliminary treatment of CAEs with the cationic surfactant cetyltrimethylammonium bromide followed by poly(acrylic acid)/polyethyleneimine alternate deposition. The basic hydrolysis of the substrates is used to spectrophotometrically control the loading/release behavior through monitoring the absorbency of the reaction product *p*-nitrophenolate ion. Unlike the reactivity of free CAEs, highly sensitive to the solution pH, and the presence of micellar catalysts, the reaction rate of the loaded substrates is unaffected by reaction conditions and can be administered by the capsule design (numbers of deposition cycles, adjusted pH, ultrasonication). The developed protocol makes it possible to omit the use of the sacrificial template and stages of its removal. Capsules corresponding to the biorelevant size criterion, with diameter of ≤200 nm, are obtained. They can be successfully applied for sustaining the dosage of different specimens with the desirable rate and for the control of the guest reactivity by tuning the shell permeability.



INTRODUCTION

Polyelectrolyte multilayer micro- and nanocapsules have attracted much attention in the field of nanomedicine, catalysis, food industry, cosmetics, magneto-optical applications, etc.[1−13] Encapsulation markedly improves technological and functional parameters of compounds, in particular decreases their toxicity and volatility, masks the color, taste, and odor, protects them from the outside, and prolongs the functional activity of the loads, thereby extending their applicability. In biomedical applications, special attention is paid to the targeted delivery of drugs with poor water solubility.[14−16] Therefore, in recent years, lipid based formulations including liposomes, micelles, emulsions, microemulsions, and self-emulsifying drug delivery systems have received considerable attention and practical use.[17−21] Their hydrophobic environment may be used as a cargo space for encapsulation of a variety of sparingly soluble therapeutic and diagnostic agents. Emulsification techniques allow fabrication of emulsion droplets within the nanoscale, which is relevant for their biomedical applications. Liposomes are regarded as ideal drug carriers, since their surface characteristics can be easily altered and their size is controllable. They can accommodate both hydrophilic and hydrophobic drugs within an interior aqueous phase and lipid phase in their shells. However, there exist a set of serious problems associated with these carriers which are exhaustively outlined in ref 16, such as impossibility to use the same protocol for making solubilized forms of different drugs; insufficient storage stability; difficulties with controlling the release rate of the drug; and problems with the scaling up of the technology. Although those problems have been largely overcome after much effort in this area, practical use of the liposome or block copolymer formulations is still limited. One of the most promising approaches to eliminate the drawbacks of the above formulations is the use of layer-by-layer (LbL) technology for carrier fabrication and surface modification. Recently,[14,22,23] a novel approach to the preparation of nanocapsules with the use of emulsions or liposomes as cargo followed by LbL deposition directly on the droplets of dispersed phase has been demonstrated.

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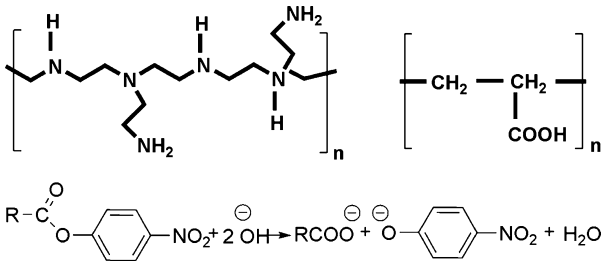
The LbL strategy assumes the encapsulation of an active component into the cavity formed by alternating deposition of polycations and polyanions.[24−28] Polyelectrolyte capsules engineered in such a way are highly competitive in the field of drug targeting.[4,7,8,16,29,30] The major advantages of these microcapsules are their loading capacity and the possibility to precisely tailor their properties by choosing the components of the capsules. Considerable amounts of macromolecular therapeutics can be encapsulated inside these capsules, which can be engineered as multicompartmentalized carriers.[5] Also, their mechanical strength can be tailored by varying the number of coating layers, by inclusion of nanoparticles, or by thermal treatment.[31] They can be easily administered by injection and by topical administration as well as by oral intake and can be internalized by many different cell types and release their payload in a sustained fashion after a specific physical, chemical, or biological stimulus.

Herein, we focus on the development of a novel simple protocol for the encapsulation of small water-insoluble guests and their sustained release. Although synthetic polyelectrolytes are used in the formulation, we consider that some findings may be helpful for the development of drug carriers in a follow-up study. Advantages of the formulation proposed and their possible potentiality in drug delivery are determined by the tasks of the study, which are as follows: (1) to simplify the procedure of encapsulation by excluding the use of the sacrificial template and stages of its removal; (2) to obtain capsules corresponding to the size criteria of biorelevant applications, i.e., ≤200 nm, because only nanometer-sized delivery vehicles can be used for long circulation;[14,29,30] (3) to design the capsules with the trigger-free release of small guests by tuning the parameters of the polyelectrolyte shell; (4) to demonstrate that, regardless of the nature of the loaded substrates and the reaction conditions, shell parameters are the only factor controlling the release and reactivity of cargos; (5) to show the applicability of the approach for encapsulation of water insoluble small guests. The latter problem remains poorly investigated, with only few LbL strategies focused on low-molecular-weight organic or bioorganic guests.[32−39] Meanwhile, a growing number of drugs are low-molecular-weight lipophilic substances.

These tasks determine the choice of building blocks for capsule formation. Electrically neutral hydrophobic carbonic acid esters (CAEs) (Scheme 1) were used as loaded substrates and templates at one time. Their reactivity in aqueous molecular and micellar solutions was investigated in our earlier works, including the pH, salt, and medium effects on the ester cleavage.[40−43] This makes it possible to use the esters as spectral probes and quantitatively monitor their concentration during the fabrication and functioning of capsules. The obtained kinetic data on the basic hydrolysis of substrates are used herein for the development of the spectrophotometry method for the express monitoring of the loading efficiency, reactivity, and/or stability and release of entrapped substrates. As polyelectrolytes, polyethyleneimine (PEI) and poly(acrylic acid) (PAA) were used. They bear functional groups capable of H-bonding, and therefore their charge character may be easily changed by the variation of solution pH, thereby providing the basis for stimulus responsive behavior. To impart a charge character to the substrates, they were solubilized in the aqueous micellar solutions of cationic surfactant cetyltrimethylammonium bromide (CTAB) yielding the CAE loaded CTAB particles hereinafter noted as CAE@CTAB.

Thus, we propose a novel simple LbL technique for embedding low-molecular-weight organic guests with poor water solubility, in which the substrate treated by a cationic surfactant plays simultaneously the role of the template as well as of the container load. Capsules fabricated in such a way are assumed to be of nanoscale dimension and to demonstrate tunable release behavior. In addition, the express method for monitoring the permeability of capsules will be proposed.

Scheme 1. Schematic Representation of Structural Formulas of Polyelectrolytes and the Hydrolysis of CAEs[a]



[a]R = n-C_9H_{19} (p-nitrophenyl caprinate, PNPC), n-$C_{11}H_{23}$ (p-nitrophenyl laurate, PNPL), n-$C_{13}H_{27}$ (p-nitrophenyl myristate, PNPM).

EXPERIMENTAL SECTION

Chemicals. Commercial CTAB (Sigma-Aldrich), p-nitrophenyl caprinate (PNPC), p-nitrophenyl laurate (PNPL), and p-nitrophenyl myristate (PNPM) (Fluka) of 99% purity were used as received. Cetyldimethylhydroxyethylammonium bromide (CHAB) was synthesized through the reaction of 2-dimethylaminoethanol with hexadecyl bromide according to refs 44 and 45. The structure of CHAB was proved by elemental analysis and IR and ^{1}H NMR spectroscopy. PEI and PAC (Aldrich) of 99% purity with molecular masses of 25000 and 1800 respectively were used. ^{1}H NMR spectra of polyelectrolytes are given in Figures S1 and S2 in the Supporting Information.

The pH-metric measurements were carried out in a thermostatically controlled cell at (25 ± 0.1) °C by using an "I-130 Ionomer" pH-meter with an uncertainty less than 0.05 pH units.

Protocol of the Capsule Formation (Figure 1). The dispersion obtained by the dropwise addition of 0.78 mL of 0.01 M ethanol solution of CAE to 0.01 M CTAB micellar solution (for details, see the Supporting Information) was

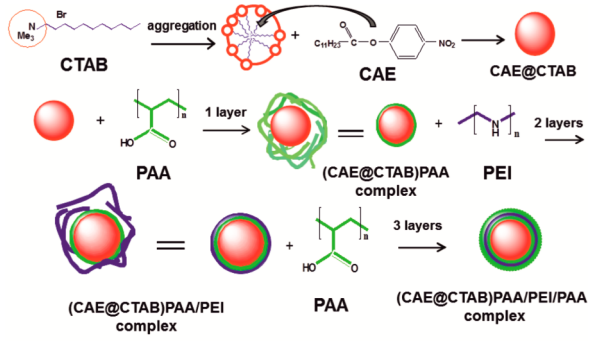


Figure 1. The schematic representation of the encapsulation process.

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centrifuged at 13000 rpm for 15 min using the MPW-35 apparatus and then decanted. In this way, CAE-loaded CTAB micelles (CAE@CTAB) were obtained. Then, 8 mL of 1 mg/mL PAA aqueous solution adjusted to pH 6 to 8 with 1 M NaOH was poured into the as-prepared CAE@CTAB sample with continuous stirring. After the first step of polyelectrolyte coating the sample was centrifuged to remove the excess of polyanion, and then resuspended in water. After that the above treatment sequence was repeated using PEI. After the deposition of the final polyelectrolyte layer the sample was washed with 4 mL of bidistilled water and stirred. In all protocols, the poly(acrylic acid) layer was terminal, so that the negatively charged particles are formed in all cases. The dispersion of capsules has pH of 6.6 to 7.0, which corresponds to physiological conditions. Specific conditions of treatment (pH value, the number of polyelectrolyte layer, etc.) were chosen depending on the task resolved. In special experiments, an additional 30−40 min ultrasonication (Elmasonic S 15 H; operating frequency 35 kHz) of the samples was carried out after each coating (for details, see the Supporting Information). The amount of the encapsulated ester was monitored by spectrophotometry through the difference between the initial quantity of the CAE and that left in the solution after the coating. To this end, after each coating procedure an aliquot was taken off from the supernatant solution decanted after centrifugation, 2-fold diluted with CTAB solution (from 0.005 to 0.01 M), and poured in strong alkali (0.1 M NaOH). In these conditions, there occurs a rapid basic hydrolysis of CAE, which may be monitored spectrophotometrically based on absorption of the product of the hydrolysis of CAE, *p*-nitrophenolate anion (PNP), at 400 nm. The concentration of PNP produced coinciding with the unloaded CAE was calculated as follows: $D_{400}/(\varepsilon \times l)$; here ε is the extinction equal to 18000 M^{-1} cm^{-1} and l is the pathway.

The capsules formed were stable for at least a month, which was supported by data on their size and permeability. To test the capsule integrity, an aliquot of dispersion of capsules with CAE loaded was taken, and their parameters, including the size, the zeta potential, and the substrate release profile, were monitored every five days. Hydrodynamic diameter changed by not more than 5%, while the release of CAEs differed by ca. 3−6%.

Kinetic Study. A Specord UV−vis spectrophotometer with temperature-controlled cell holders was employed. The rate of hydrolysis of substrates was measured spectrophotometrically by means of monitoring the absorbency of *p*-nitrophenolate ion at λ = 400 nm. In the case of encapsulated substrates, an aliquot of the aqueous dispersion of capsules was introduced into water or a micellar solution of adjusted pH value. Generally, a 1:1 dispersion/solution ratio was used. The rate of hydrolysis of substrates in terms of half-life values was measured spectrophotometrically by means of monitoring the absorbency at λ = 400 nm through a certain period, until the reaction was completed and PNP release stopped.

Dynamic Light Scattering Measurements. DLS measurements were performed by means of the PhotoCor Complex and Malvern Instrument Zetasizer Nano. The measured autocorrelation functions were analyzed by the Malvern DTS software, the DynaLS program, and the second-order cumulant expansion methods. The zeta potential Nano-ZS (MALVERN) using laser Doppler velocimetry and phase analysis light scattering was used for the zeta potential measurement. DLS and zeta potential titration involved no fewer than ten measurements in 5 to 10 runs, so that ≥50 scans of at least three independent experiments were obtained. Only multiply reproducible results were taking into account; thereby they differed by less than 4%. Additional details are given in ref 46 and the Supporting Information.

AFM Study. An atomic force microscope (MultiMode V, USA) was used to investigate the morphology of the particles, in analogy with ref 46. 250−350 kHz cantilevers (Veeco, USA) with silicon tips were used in all measurements. The AFM imaging was performed after water evaporation, on a mica surface.

RESULTS AND DISCUSSION

Capsule Formation. *Optimization of pH Conditions for Capsule Fabrication.* The effectiveness of polyelectrolyte interactions in the process of the shell formation mainly depends on their electrostatic attraction and increases with the charge density of polycation and polyanion. Both polyelectrolytes under study demonstrate a pH-dependent behavior, and therefore the solution pH may control their charge character.[47−49] Although the PEI/PAA pair may be used for the fabrication of capsules based on hydrogen bonding,[50] this study is focused on LbL assemblies based on electrostatic force. To choose the optimal pH values providing the highest fractions of protonated amino groups of PEI and dissociated carboxy groups of PAA, potentiometric titration of the polyelectrolyte solutions has been carried out. As can be seen from the pH dependence in Figure S3 in the Supporting Information, the pH range from 6 to 8 corresponds to the maximum contributions of the anionic form of PAA (50−90%) and the cationic form of PEI (40−80%). To accomplish this, the polyelectrolyte deposition is carried out within the above pH range.

It should be mentioned that CTAB exerts practically no effects on the acid−base properties of PEI (Table S1 in the Supporting Information), while PAA at pH >6 dissociates to an anionic form and may produce an insoluble polyelectrolyte complex with cationic surfactants. Therefore, a careful removal of precipitates by centrifugation occurred at each coating stage.

Characterization of the Capsules Obtained. The process of the substrate encapsulation is shown in Figure 1. The amount of the encapsulated ester was controlled by spectrophotometry based on the difference between the initial quantity of the CAE taken and that left in the solution after each coating procedure. As can be seen from Table S2 in the Supporting Information, ca. 19% of nonsolubilized substrate remains in solution after the completion of the first stage (the CAE@CTAB preparation), while ca. 1 to 2% of the substrates is lost under the polyelectrolyte deposition. Totally, ca. 23% of the unloaded substrates remains in the collected supernatant fractions after a 3-cycle deposition, which yields a 0.225 mg/mL loading efficiency.

The two main properties of capsules that should be monitored are their zeta potential and size. Since CAE@CTAB particles bear a positive charge, their coating by means of the alternate deposition of polyelectrolytes began with the oppositely charged PAA. The zeta potential measurements were taken in order to confirm whether the LbL self-assembly was successful. Figure 2 exemplifies the zeta potential data for the PNPL encapsulation as a function of the number of layers. The recharging of capsules occurs depending on the outermost layer, which strongly supports the fact that the coating occurs through the polyanion/polycation alternation.

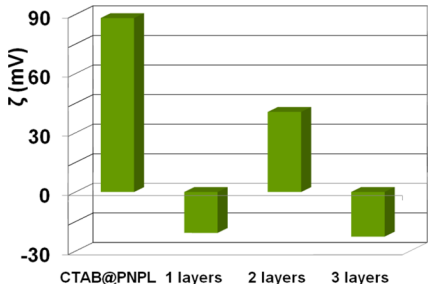

Figure 2. The values of zeta potential for the PNPL-loaded capsules fabricated at pH 7.0 as a function of the number of layers starting with the CTAB@PNPL sample; 25 °C.

Figure 3, Figures S4–S6 in the Supporting Information, and Table 1 show DLS data exemplified by the PNPL-loaded

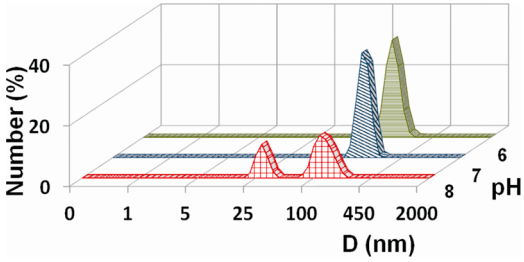

Figure 3. Size distribution analysis as a function of pH adjusted at the LbL coating exemplified by three-layered PNPL-loaded capsules, 25 °C.

capsules formed when several factors vary. For the sake of comparison, data for PNPM- and PNPC-loaded capsules are given in Figure S7 in the Supporting Information. As can be seen, the mean sizes of the particles are within the range of 50–200 nm, which correspond enough to biorelated criteria. As mentioned above, the optimal pH predicted from potentiometric titration data lies in the range of 6 to 8. As can be seen from Figure 3 and Table 1, the most suitable size parameters are obtained at pH 6.0. Under these conditions, three-layered capsules with the hydrodynamic diameter (D_H) of ca. 175 nm and low polydispersity are obtained. An increase in pH to 7.0 results in the formation of larger particles with D_H = 256 nm. Although the monomodal size distribution for the particles remains, their polydispersity increases. Further increase in the basicity is followed by the appearance of several populations, including the low particles of ca. 30 nm and the larger ones of D_H >100 nm (Figure 3). This is probably due to the fact that at pH 8.0 PAA becomes completely dissociated, while only 20% of the PEI amino groups are protonated. As a result, particles of two types are formed, i.e., the smaller ones contributed by strong electrostatic interactions, and the larger ones obtained through the assembly of the strong and weak polyelectrolytes.

Ultrasonication is often used for the treatment of condensed systems to obtain homogeneous vehicles, monodisperse samples, and uniform nanoparticles.[51,52] To test this factor, a 30–40 min auxiliary ultrasonication of layer deposition was performed, with the assumption of a decrease in the capsule size when treated. However, only a slight effect on the particle size occurs upon ultrasonication (Table 1, Figure S6 in the Supporting Information). These data are in agreement with ref 39, which reports that a decrease in the particle size involves a longer ultrasonication (for >60 min), while a shorter treatment may even be followed by an increase in the size of particles. The authors treated these data from the viewpoint of the initial bridging of larger particles with a polyelectrolyte, while a prolonged ultrasonication leads to the uniform distribution of polyelectrolyte over particles and their separation from each other.

Generally, the introduction of a polyelectrolyte layer is followed by ca. 3 nm increment in the capsule size.[24] Meanwhile, the LbL assemblies with no sacrificial substrate probably demonstrate a different size behavior compared to the other modifications of the method. Supportive of the above small size changes upon ultrasonication, an increase in the number of layers from three to five exerts almost no effect on the size of the particle or even results in a slight decrease of

Table 1. Characterization of the Capsule Size Parameters under the Variation of Conditions of the Capsule Fabrication (Adjustable pH, Ultrasonication, Number of Layers) and Capsule Testing (Aging, Bulk Solution pH)

no. of a sample	adjustable pH	number of layers	time of ultrasonication, min	mean diam,[c] nm	polydispersity index
		Effect of Adjustable pH			
1	6.0	3	0	175 (65)	0.115
2	7.0	3	0	256 (68)	0.245
4	8.0	3	0	120; 28	0.590
		Effect of the Ultrasonication and Number of Layers			
6	6.0	3	30	183 (70)	0.240
7	6.0	3	60	171	0.263
8	6.0	5	0	50; 189	0.299
9	6.0	5	30	105	0.41
		Effect of the Bulk Solution pH and the Aging			
10	6.0 (6.4[a])	3	0	168	0.145
11	6.0 (11.7[a])	3	0	287	0.768
12	6.0 (6.4[a])[b]	3	0	164	0.126
13	6.0 (11.7[a])[b]	3	0	217	0.468
14	6.0 (6.4[a])	3	30	154	0.434
15	6.0 (11.0[a])	3	30	214	0.680
16	6.0 (6.4[a])[b]	3	30	168	0.251
17	6.0 (11.0[a])[b]	3	30	160	0.397

[a]Value of bulk solution pH. [b]24 h aging. [c]Averaged over ten measurements. The AFM data are given in the brackets.

their diameter (Table 1, Figure S6 in the Supporting Information). This is probably due to the more compact layer packing occurring with an increase in the number of layers. Such behavior is assumed to occur at the outset of coating (for the first 5−7 layers), which is generally characterized by an inconsiderable increase in the shell thickness.[32,53]

AFM Data. AFM data are rather supportive of the DLS results and demonstrate a nanometer dimension of the particle size. While due to differences in their physical background these two methods may give mismatch results, mostly they are in rather good agreement. Somewhat lower sizes obtained by the AFM as compared to the DLS method probably originated from the fact that (1) the latter yields hydrodynamic diameters, which involve solvate shells of particles; and (2) shrinkage of the capsules may occur upon the solvent release in the case of the AFM method. Figure 4 and Figures S8 and S9 in the Supporting Information show the AFM images of the samples obtained under different conditions. The PNPL@CTAB sample shows no distinct nanosized particles (Figure S8 in the Supporting Information), while the coating of the PNPL without preliminary CTAB treatment results in the clustering of the particles and their ordering, which is especially evident in Figure S8c in the Supporting Information for the five-layered capsules. The most uniform spherical capsules of ca. 65 nm in diameter are fabricated at pH 6.0, which is exemplified by the AFM image for the three-layered PNPL-loaded capsules in Figure 4. As can be seen in Figure 4b, ultrasonication of the sample results in some increase in the polydispersity, while the mean size little changes. In the case of the LbL deposition, occurring under pH 7.0 rather uniform roughly spherical particles are formed with the diameter of ca. 68 nm, although a clustering can be observed on the scanning of some section of the three-layered sample (Figure S9 in the Supporting Information). On the transition from three- to five-layered samples they become more ordered and uniform with a mean size of 72 nm (Figure S9 in the Supporting Information).

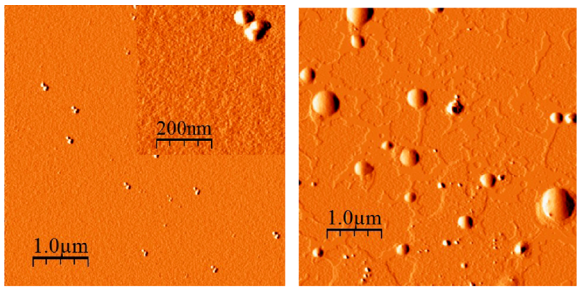

Figure 4. AFM images of the PNPL@CTAB loaded three-layered capsules fabricated under pH 6.0 without (a) (inset presents the image of higher magnification degree) and with (b) ultrasonication.

The Reactivity of Encapsulated CAEs (ECAEs). The permeability of capsules is the key factor responsible for their functional properties and technological applicability, including the protection of guests from unfavorable environmental effects, sustained dosing, prolongation of the drug activity, control of the reactivity, etc. As reviewed in ref 24, the permeability of capsules toward small guests depends on several factors, among which the number of layers plays a paramount role. In the present work, the reactivity of CAEs is explored for the elucidation of the role of permeability as a crucial factor determining the reaction rate of entrapped substrates. The use of CAEs as probes provides possibilities for the development of an easy and simple protocol for the spectrophotometry control of their loading and release on each stage of the LbL deposition. To test permeability, the basic hydrolysis of CAEs (Scheme 1) released was explored by monitoring the absorbency at λ = 400 nm corresponding to one of the products of the hydrolysis, namely, the *p*-nitrophenolate ion (see the Supporting Information for details).

It should be noted that partial disintegration of polyelectrolyte capsules under basic conditions in the presence of cationic surfactants may be expected (see for example, data in Table 1 indicating that swelling the capsules may occur at pH 11.0). It is known, that a set of physical and chemical stimuli such as temperature,[53,54] pH,[25,31,53,55] ionic force,[25,31,55] the presence of cationic surfactants,[56] and the treatment by ultrasound of special output power[31] may be involved to develop the responsive LbL materials. Therefore we are aware that capsules can be modified under the conditions of their testing. Within the framework of the present study, we tried to minimize the effect as follows: low values of pH (although in basic range of pH) and surfactant concentrations were used; all the experiments on the release of substrates were carried out under similar conditions, so that the contribution of these factors to the release profiles can be ignored upon discussing the other factors varied. Importantly, special conditions are required to switch on and off the release of substrates.[30,55] It is noteworthy that the top layer may demonstrate a specific response to the stimuli as compared to the deeper part of the shell.[9,31]

According to refs 40 and 41, the rate of hydrolysis of hydrophobic CAEs in water at neutral pH is low. However, it can be increased by the alkalization of solution or by the addition of aggregated surfactants, with the reaction rate being strongly dependent on the structure and concentration of surfactants. Cationic surfactants are well-known micellar catalysts for nucleophilic substitution due to the electrostatic attraction of nucleophilic anionic reagents to the positively charged micellar surface.[40] High catalytic effects toward ester bond cleavage are documented for cationic surfactants with hydroxyalkylated head groups.[57,58] The 275-fold and ca. 500-fold acceleration of hydrolysis of PNPL occurs in the 0.005 M CTAB and CHAB solution respectively as compared to basic hydrolysis in the surfactant free solution.[58] The rate enhancement may occur in nonionic micelles as well, which is documented in ref 59. Therein a 50-fold acceleration of the PNPL hydrolysis in the Triton-X-100 micelles has been described. The micellar catalysis of CAEs in cationic micelles under alkali conditions is shown in Figures S10−S12 in the Supporting Information. As can be seen from Figures S10 and S11 in the Supporting Information, the increase in the observed rate constant (k_{obs}) occurs in the series myristate $\leq$ laurate < caprinate for both the CTAB and CHAB micelles. For all esters the rate of basic hydrolysis is higher in CHAB as compared to CTAB micelles. In the case of PNPL, k_{obs} increases by a factor of 2 to 7 depending on pH on transiting from CTAB to CHAB. The solution pH strongly affects the reaction rate, resulting in a 3- and 7-fold increase in k_{obs} for CHAB and CTAB micelles respectively with an increase in pH from 9.2 to 10. Thus, two points should be emphasized based on the data characterizing the chemical reactivity of non-encapsulated (hereinafter the term "free substrate" is used) CAEs. First, the micelle-catalyzed basic hydrolysis of CAEs may

provide a basis for the express analysis of their loading and release behavior. Second, the rate of ester cleavage markedly differs depending on the chemical structure of CAEs, the solution pH, and the nature and concentration of surfactants.

Another reactivity behavior may be observed for the ECAEs. In this case, apart from the above two main factors, i.e., the chemical reactivity of the substrates and bulk conditions, the reaction rate is assumed to be strongly controlled by the CAE diffusion through the capsule shells to the bulk solution, in which the hydrolysis occurs. Therefore the reactivity of loaded compounds may be administered by varying the permeability of capsules. The mechanism of permeability is discussed in ref 14, in which two different modes are distinguished, i.e., permeability through pores and that involving the so-called bulk diffusion through the polyelectrolyte multilayer shell. Figure 5 and Table 2 show the reactivity of ECAEs exemplified

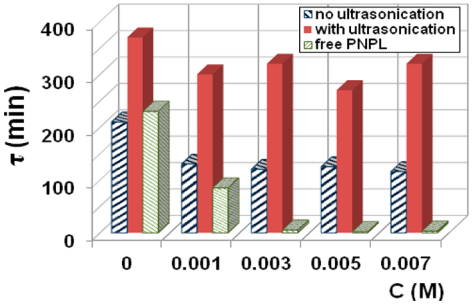

Figure 5. Half-life values for hydrolysis of PNPL in free and encapsulated (three-layered capsules fabricated under pH 7) states as function of CHAB concentration; pH 9.2; 25 °C.

Table 2. Half-Life Values for Hydrolysis of Free PNPL and PNPL Loaded into the Three-Layered Capsules Fabricated under pH 7.0 without Ultrasonication as Function of Bulk Conditions

	$\tau_{1/2}$, min				
	encapsulated PNPL			free PNPL	
	pH 9.2	pH 10	pH 11	pH 9.2	pH 10
surfactant-free alkali	200	180	150	290	230
CHAB 0.003 M	120	120	120	1.9	0.8
	100[a]				
	145[b]				
CTAB 0.003 M	120			16	1.5
Triton-X-100 0.003 M	135				30

[a]For PNPC. [b]For PNPM.

by PNPL under the variation of a number of factors. The other CAEs studied demonstrate a very similar behavior, which is evident from the data in Table 2. The half-life parameter is used as a quantitative characteristic of the hydrolysis rate. As bulk media, 0.0015−0.003 M CHAB solution at pH 9.2 was generally used, since this surfactant demonstrated a high catalytic activity (Figure S10 in the Supporting Information, Table 2). Surprisingly, unlike free CAEs, no obvious effect of the bulk solution on the reactivity of ECAEs has been revealed. As can be seen from Table 2, the half-life values of hydrolyses of the ECAEs undergo little changes ranging from 120 to 150 min regardless of the solution pH, the presence of surfactants, and their nature. Meanwhile under similar pH variation, the half-life of hydrolysis of free substrates may decrease by a factor of 7 in the presence of 0.003 M Triton-X-100 and by 2 orders of magnitudes in the 0.003 M CHAB solution. As follows from the data in Figure 5 exemplified by PNPL, the variation of CHAB concentration does not influence the reactivity of substrates. Thus, the only factor determining the reactivity of ECAEs is the permeability of capsules, which may be administered by their design as shown in Figure 5 and Figures S13 and S14 in the Supporting Information. The data given therein reveal that the adjusted pH, ultrasonication, and amount of polyelectrolyte layers strongly influence the reaction rate of the entrapped substrates.

The solution pH is of importance from the viewpoint of the capsule fabrication (Figure S1 in the Supporting Information), since it is the very factor that determines the charge density of macromolecules and hence the effectiveness of electrostatic interactions between adjacent layers. Therefore this parameter is responsible for the close packing upon coating the substrate, which is supported by the data in Figure S13 in the Supporting Information. The optimization of the process by the adjustment of solution pH within the range from 6 to 8 results in the maximum protection effect, when the reaction rate is only controlled by the substrate release. Indeed, the half-life value of basic hydrolysis of PNPL increases from 22 min (free PNPL) to 50 min (pH 6.0) and 120 min (pH 7.0). In the case of preliminary ultrasonication, half-life values of 180 min (pH 6.0) and 300 min (pH 7.0) are observed. With no pH adjustment a more loose packing is probably observed, which is reflected in the higher rate of hydrolysis, in particular, lower half-life values of 30 min (no treatment) and 150 min (30 min ultrasonication) occur (Figure S13 in the Supporting Information).

Another essential parameter is the amount of polyelectrolyte layers. As can be seen from Figure S14 in the Supporting Information, half-life value increases as follows: 50, 190, 340 min (with no treatment) and 180, 240, 400 min (30 min ultrasonication) with an increase of deposited layers in the series 3, 5, 7. Thus, more marked effects are observed upon the transition from three- to five-layered capsules (a 3.5-fold retardation of the hydrolysis of PNPL), while a further increase in the number of polyelectrolyte layers exerts a smaller effect.

In all the cases, ultrasonication highly retards the release of substrates regardless of the other treatment conditions. Importantly, the contribution of this parameter is mostly appreciable if the other parameters are not optimized, i.e., if an unbuffered solution is used (a 7.5-fold retardation of the PNPL hydrolysis) (Figure S13 in the Supporting Information) and smaller layer numbers are adsorbed (a 3.5-fold retardation) (Figure S14 in the Supporting Information). On the contrary, the permeability of capsules undergoes little change upon ultrasonication if the other parameters provide an effective protection of the entrapped guests. Thus, only an inconsiderable increase in the half-life values of the hydrolysis of PNPL occurs upon ultrasonication in the case of 5- and 7-layered capsules (Figure S14 in the Supporting Information).

It is noteworthy that in the surfactant-free conditions the rate of hydrolysis of PNPL is lower as compared to the encapsulated PNPL under the same solution pH. This may be due to the fact that the presence of the hydrophobic lauryl group promotes the association of PNPL under the kinetic study, in which its concentration of 2×10^{-5} to 8×10^{-5} M is maintained. According to ref 60 in these conditions a marked retardation of the basic hydrolysis of PNPL occurs due to the aggregation and isolation of reaction centers from the nucleophiles located in the bulk phase. In the case of encapsulated PNPL its concentration in bulk solution would be controlled by the

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permeability of capsule shells, thereby providing a sustained release of PNPL and preventing it from the aggregation. In the presence of the surfactant, mixed PNPL-surfactant micelles are formed, which accelerate the hydrolysis, regardless of the substrate concentration.

Figure 6 summarizes the results of the influence of one of the key factors, i.e., the numbers of deposited layers in terms of the

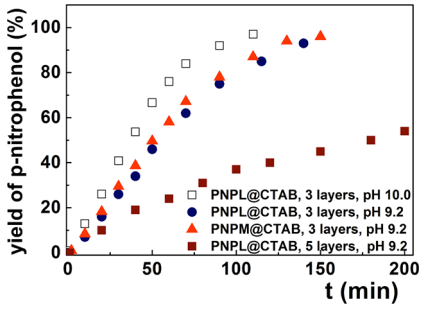

Figure 6. Release profile for ECAEs in terms of yielding the reaction product of released substrates as a function of the solution pH and numbers of polyelectrolyte layers; capsules were fabricated under pH 6.0 without ultrasonication; bulk conditions: 0.003 M CHAB; 25 °C.

release profile. It is quite evident that while the reaction conditions (the variation of solution pH and substrate structure) exert only little effect on the substrate reactivity, the shell behavior as such totally controls the release of substrates and hence the rate of their cleavage. This factor in combination with the above parameters of the adjusted pH and ultrasonication (Figures S13 and S14 in the Supporting Information) makes it possible to vary the continuation of the guest release from some minutes to many hours.

CONCLUSIONS

Thus, a novel simple protocol for the LbL coating of small uncharged guests and control of their loading/release behavior has been developed. The approach involves the step-by-step deposition of alternating poly(acrylic acid)/polyethyleneimine layers upon the organic uncharged substrates, namely, hydrophobic carboxylic acid esters previously treated with the cationic surfactant CTAB. The DLS and AFM studies revealed that the capsule size lies in the nanometer range of 100−200 nm, which is suitable for bioapplications. Carboxylic acid esters are also of biological importance, since they may be used as spectral probes for testing the ester cleavage process playing a significant role in the metabolism. The hydrolysis of substrates was used for monitoring the loading/release behavior through the absorbency of the product of the hydrolysis at λ = 400 nm corresponding to *p*-nitrophenolate anion. It appeared that the reactivity of the loaded substrates does not depend on the reaction conditions (solution pH, catalytic additives) and can be administered by the capsule design (numbers of deposition cycles, adjusted pH, ultrasonication). The developed trigger-free protocol can be successfully applied (1) to sustain the dosage of different specimens at a desirable rate, which can be attained by tuning the shell permeability; (2) to control the reactivity of organic compounds; and (3) to protect the entrapped guests from the environmental conditions.

ASSOCIATED CONTENT

Supporting Information

NMR spectra of polyelectrolytes; pH dependence of the fraction of their charged groups; AFM images of the particles; size distribution analysis for CAE-loaded capsules; kinetic data describing the reactivity of free CAEs, as well as some experimental details of the protocol used. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*A. E. Arbuzov Institute of Organic & Physical Chemistry, 8, ul. Arbuzov, 420088, Kazan, Russia. E-mail: lucia@iopc.ru. Fax: +7-843-2732253.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

LbL, layer-by-layer; CAEs, carboxylic acid esters; ECAEs, encapsulated carboxylic acid esters; CTAB, cetyltrimethylammonium bromide; CHAB, cetyldimethylhydroxyethylammonium bromide; PEI, polyethyleneimine; PAA, poly(acrylic acid); DLS, dynamic light scattering; AFM, atomic force microscopy; PNPC, *p*-nitrophenyl caprinate; PNPL, *p*-nitrophenyl laurate; PNPM, *p*-nitrophenyl myristate

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