Polyelectrolyte capsules for controlled binding/release of fluorescent probe*

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Nanocapsules containing pyrene, a fluorescent probe, are formed on micelles of a cationic surfactant by layer-by-layer deposition of oppositely charged polyelectrolytes (polyacrylic acid/polyethyleneimine). Approaches, which allow to evaluate the release time of pyrene from the capsules are developed. The synthesized capsules make it possible to slow down the deactivation process of the excited pyrene molecule by almost an order of magnitude by adding a quenching compound to the bulk medium and through that, prolong its operation as fluorescent probe.

Key words: polyelectrolyte, encapsulation, pyrene, permeability, fluorescence quenching.

The development of polyelectrolyte micro- and nanocapsules for organic and biological substrates, which allow to regulate their reactivity and stability, making it possible to carry out biotransport and improve their operation properties is a promising direction in modern technology. Among the multitude of areas of practical application of polyelectrolyte capsules, their use as containers for the delivery of diagnostic and medicinal agents and gene material should be distinguished.[1—4] In this case the polyelectrolyte capsules act as an alternative to self-emulsifying delivery systems of medicinal agents, such as liposoms, micelles, emulsions, microemulsions.[5—7] The main advantages of nano- and microcapsules is their ability to be heavily loaded with the target compound, the availability and variety of materials for their formation, the controllability of their properties through the choice of components of the shell and their number.[1,8] An important parameter is the permeability of the shell, since variation of this characteristic makes it possible to regulate the release rate of the medicinal agents or the duration of spectral response of the diagnostic probes.

One of the most important approaches to capsule formation is based on the successive absorption of oppositely charged polyelectrolytes onto a substrate from a solution, the so-called layer-by-layer method.[9—11] This procedure is characterized by simplicity, high reproducibility, cost effectiveness, swiftness of the process, the ability to obtain homogenous roughly nanometer-sized particles. Apart from that, a wide range of natural (polysaccharides, polypeptides) and synthetic (polyacrylic acid, sodium polystyrenesulfonate, polyallylamine hydrochloride) polyelectrolytes are available for the development of these capsules.[12—16] All of this demonstrates the benefit of the layer-by-layer method in comparison with other used procedures (polymerization, coacervation, template procedure, *etc.*).[17—20] Undoubtedly, the main benefit of polyelectrolyte capsules is the ability to control their protective properties and substrate release processes through a directed choice of materials for the formation of the shell, as well as conditions for their formation and operation.

The encapsulation of charged organic compounds is not difficult. However the encapsulation of uncharged substrates with low molecular weight remains a problem which needs to be solved for each individual case separately. Works[21—23] demonstrated the preliminary solubilization of uncharged compounds with micelles of an ionic surfactant, after which layer-by-layer absorption of polyelectrolytes onto the formed charged surfactant—substrate complex was carried out.

The goal of the present work is the improvement and further development of the technology of polyelectrolyte encapsulation for the protection and prolongation of the release of uncharged low-molecular-weight compounds, as well as the development of new procedures of quantitative control of the substrate binding—release processes. In order to do this, based on polyacrylic acid (PAA) and polyethyleneimine (PEI) capsules are synthesized, containing pyrene, a fluorescent probe, which is used as a convenient model of hydrophobic spectral probes and poorly soluble therapeutic agents. The encapsulation procedure includes a preliminary step of solubilization of pyrene with micelles of cetyltrimethylammonium bromide (CTAB), which is a typical cationic surfactant, for which its aggregative properties, solubilization activity, and absorption on the interface are well studied. At this, a dispersed phase is formed, the particles of which (surfactant—pyrene) acquire a positive charge, that makes it easier

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to apply the first layer of the oppositely charged polyelectrolyte. The testing of the capsule properties included determining their size and charge, development and approbation of procedures for the evaluation of permeability of the walls, determination of the factors, which allowed to control the operational characteristics of the capsules.

Experimental

The formation of polyelectrolyte capsules was carried out using polyacrylic acid (MM 1800) and branched polyethyleneimine (MM 25000) (Sigma-Aldrich). Commercial samples of pyrene were used as substrates (Fluka, ≥99%). In order to apply charge, a preliminary treatment of pyrene with a solution of CTAB was carried out. Cetylpyridinium bromide (CPB) was used as a fluorescent quencher. The registration of electronic spectra of pyrene was carried out in a solution of a non-ionic surfactant — Brij-35. All listed surfactants (Sigma-Aldrich) contained no less than 99% of the main compound and were used without additional purification. Water purified on a Direct-Q 5 UV (pH 6.8—7, χ = 2—3 μS cm^{-1}) was used for the preparation of solutions.

Synthesis of polyelectrolyte capsules. A solution of pyrene in dichloromethane (0.1 mL, 0.0192 mol L^{-1}) was added to an aqueous solution of CTAB (8 mL, 0.01 mol L^{-1}) with continuous stirring (magnrtic stirrer, 750 rpm). The mixture obtained was placed into a refrigerator for 30 min, then a precipitate formed was separated by centrifugation at the rate of 8000 rpm (centrifugation time 15 min). Then, the precipitate was resuspended into water (pH 6.0), followed by the addition of an aqueous solution of PAA (8 mL, 1 mg mL^{-1}, pH 6.0). After stirring the mixture for 3 min, the formation of loose flake-like particles was observed, which were separated after prolonged (45 min) centrifugation at the rate of 8000 rpm. For depositing the next layer, the precipitate obtained was diluted with an aqueous solution of PEI (8 mL, 1 mg mL^{-1}, pH 6.0), and followed the procedures described above.

Physicochemical studies of capsule properties. The sizes and the zeta-potential of the obtained polyelectrolyte capsules were determined on a Malvern ZetaSizer Nano photon correlation spectrometer of dynamic and electrophoretic light scattering (Malvern Instruments, Great Britain). The source of laser radiation was a gas He—Ne laser (power 10 mW, wavelength 633 nm, light scattering angle 173°, time of pulse accumulation 5—8 min).

Electronic spectra of pyrene were obtained on Specord 250 Plus spectrophotometer (Analytik Jena, Germany) in the 200—400 nm range at 25 °C. The solutions under study were placed into thermostatic quartz cells with an absorbing pathway of 0.5 or 1 cm.

Fluorescence spectra of pyrene were recorded on a Cary Eclipse scanning spectrofluorometer (Varian, Australia). The measurements were carried out in a quartz cell with an optical pathway of 1 cm at 25 °C (the wavelength of excitation of pyrene 335 nm, the parameters of the excitation and emission slit 5 nm). The measurements of luminescence were carried out in the absence of and in the presence of a quencher (CPB).

Results and discussion

It should be noted that the reported procedure of encapsulation of hydrophobic fluorescent probes using the technology which included the preliminary treatment of substrates with ionic surfactants has not been described in literature previously. However in work[24] the synthesis of multilayer polyelectrolyte film applied onto a solid substrate containing pyrene was carried out. The first step of this method included the simultaneous mixing of pyrene, PAA and CTAB in highly alkaline solutions. Then, a layer of polycation was applied onto the formed absorptive layer. An attempt to use this procedure for the preparation of a microcapsule was not successful, since already during the first stage finely dispersed precipitates were formed, which could not be separated even by prolonged centrifugation, which made it impossible to continue the process. For the synthesis of polyelectrolyte capsules based on PAA and PEI, containing pyrene, we used a method which was developed and successfully applied by us earlier to fatty acid esters. The details of the synthesis, which included the solubilization of the substrate with CTAB micelles in the first stage, are described in the Experimental section, and the scheme of the process is given on Fig. 1.

Capsules PAA—PEI obtained by applying charged polyelectrolytes onto the pyrene—CTAB complex were characterized using the standard methods right after the synthesis: by measuring the zeta-potential and particle size. It is shown that three-layer capsules obtained at pH 6.0 and dispersed in an aqueous solution are the particles with a zeta-potential of −32 mV and a hydrodynamic diameter of 120 nm (Fig. 2). Apart from that, the system contains particles with a size of ~530 nm, which can form as a result of adhesion of several small capsules. Dispersed capsules stored over a week have unchanged characteristics.

The most important characteristic of nanocapsules is their permeability. In each individual case it is necessary to choose the methods of controlling the rate of substrate release from the capsules and the means of regulating it. Taking into account the fact that pyrene has a characteristic absorption spectrum in the visible and UV regions, spectrophotometry is a convenient method for analyzing the properties of the formed polyelectrolyte capsules. In order to increase the solubility of pyrene, a solution of non-ionic surfactant Brij-35 was used, which is capable of efficient solubilization of low-polar organic compounds.[25,26] Several bands with absorption maxima at 232, 241, 262, 274, 321, and 337 nm can identified in the spectrum of pyrene in a solution of this surfactant. The last band is located in the region with the least influence of interfering factors, therefore it is chosen as the main signal. In the first stage the extinction coefficient of this compound (ε_{337} = 37500 L mol^{-1} cm^{-1}) was determined from the spectra recorded at various pyrene concentrations using the Beer–Lambert–Bouguer law ($D = \varepsilon lC$).

However, in the course of this work it turned out that the absorption spectra of encapsulated and free pyrene are little different, and optical spectroscopy is not suitable for monitoring the release process of this substrate from the

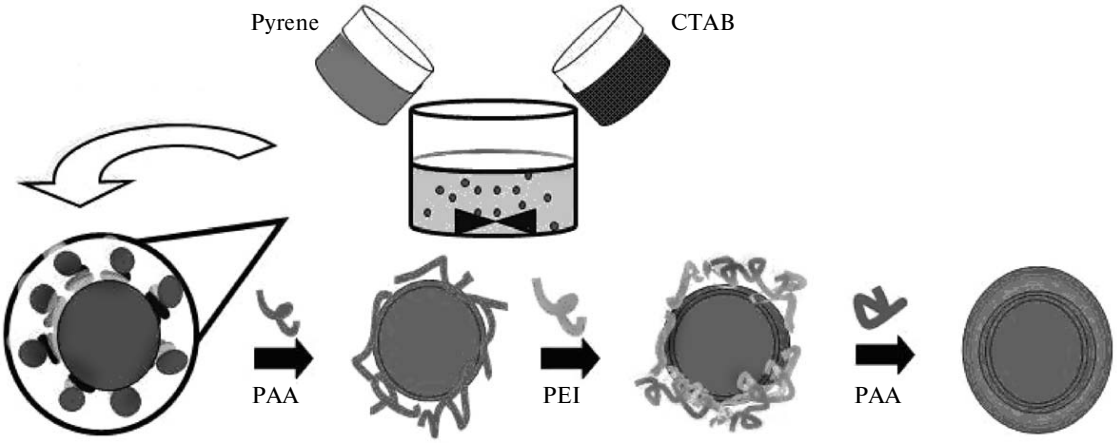

Fig. 1. A scheme of the synthesis of polyelectrolyte capsules containing pyrene.

capsules, but can still be useful for determining its loses during synthesis. This way, the analysis of pyrene content in washings, obtained at different stages of capsule formation made it possible to determine that its net loses during encapsulation are no greater than 30% from the initial amount.

The ability of pyrene to act as a fluorescent probe can be used to determine its release rate from the capsules into the bulk medium. In order to accomplish this, we registered the emission spectra of this compound confined in the three-layer PAA—PEI capsules over set periods of time. A typical example spectrum of encapsulated pyrene is given in Fig. 3, *b*. It is known that the position of the peaks in the fluorescence spectrum of pyrene depends little on the solvent, whereas the ratio of their intensities is sensitive to the change in medium properties and, first of all, to the microenvironment polarity. The parameter, which allows to evaluate the influence of the medium, is the ratio of intensities of the first peak at 373 nm (I_1) and the third peak at 384 nm (I_3).[27,28] The ratio I_1/I_3 is sensitive to micropolarity in the localization zone of the fluorescent probe and, for pyrene dissolved in water, has the highest value (~1.8), whereas in hydrocarbon solvents it is less than 0.6 (see Ref. 28). Based on the results of the change of the ratio I_1/I_3 in time (see Fig. 3), the release rate of

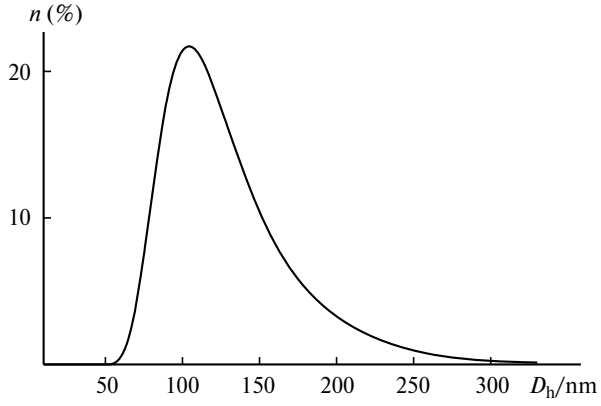

Fig. 2. A size distribution of three-layer PAA—PEI capsules containing pyrene formed at a pH 6.

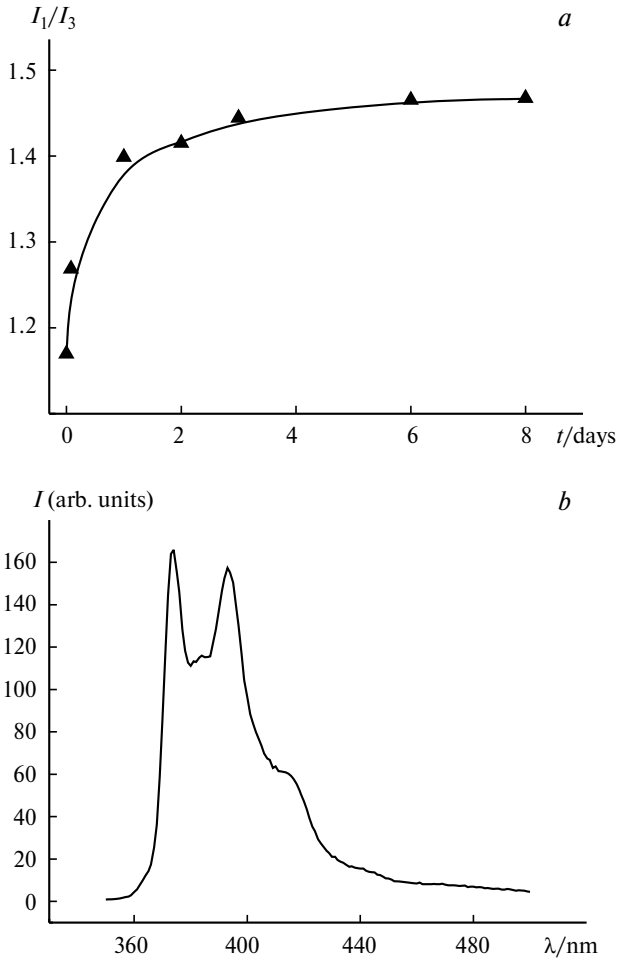

Fig. 3. (*a*) A time dependence of the change in the ratio of the intensities of the first and the third peaks (I_1/I_3) in the fluorescence spectra of encapsulated pyrene (25 °C, pH 7.0). (*b*) Fluorescence spectrum of encapsulated pyrene.

pyrene from capsules into the bulk medium can be determined. Using this method it was found that at pH 7.0 pyrene is completely liberated from freshly formed capsules over three days, however, the greatest changes occur in the system during the first day.

An alternative procedure that we used for studying the permeability of the capsule walls is the fluorescence quenching technique. It involves measuring the intensity of fluorescence of encapsulated pyrene when a quenching compound capable of deactivating the excited molecule was added to the bulk medium. Cetylpyridinium bromide, frequently used for this purpose, served as a quencher. Fluorescence quenching of free (unencapsulated) pyrene in the presence of CPB occurs over time. However, the rate of this process decreases considerably if the pyrene is enclosed into a polyelectrolyte capsule and the quencher is added to an aqueous medium. The quenching rate in this case will be determined by the diffusion of pyrene through the capsule shell and be dependent on its properties. The fluorescence spectra of free and encapsulated pyrene in the presence of the quencher are given in Fig. 4. A comparison of the change of the fluorescence intensity of pyrene in the presence of CPB over time (Fig. 5) shows that at pH 7.0 a three-layer polyelectrolyte capsule PAA—PEI slows down its reaction with the quencher by an order of magnitude. Such a protective role of the capsule can provide a prolonged action of pyrene, which broadens the range of its use as a diagnostic probe.

An important problem for the encapsulation of organic compounds is the necessity of controlling the release rate of the substrate. The variation of pH turned out to be one of the possible ways of solving this problem. This is explained by the fact that the pH value determines the polyelectrolyte charge and, therefore, the strength of electrostatic interactions between the capsule layers. For example, for the PAA—PEI pair an effective contact of the polyelectrolyte layers is provided at pH 6—7 both for capsule formation and during operation. However, if it is necessary to speed up the release of the substrate from the capsules, the pH of the medium must be changed, weakening the interaction of the shell layers. Fluorescence spectra at pH 2.3 were measured for pyrene that is immobilized in

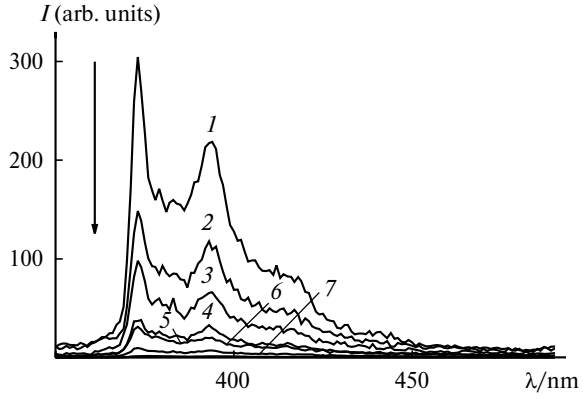

Fig. 4. Fluorescence spectra of encapsulated pyrene without a quencher (*1*), right after the addition of CPB (*2*), after 1 (*3*), 24 (*4*), 48 (*5*), 72 (*6*), 120 h (*7*) (C_{CPB} = 0.053 mmol L^{-1}, 25 °C).

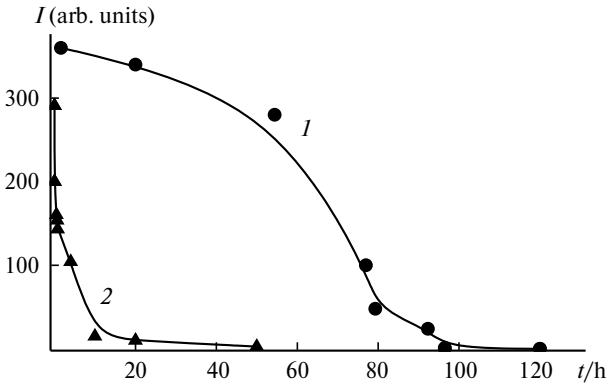

Fig. 5. A time dependence of the fluorescence intensities of encapsulated (*1*) and free (*2*) pyrene at λ = 373 nm in the presence of CPB (0.053 mmol L^{-1}, 25 °C).

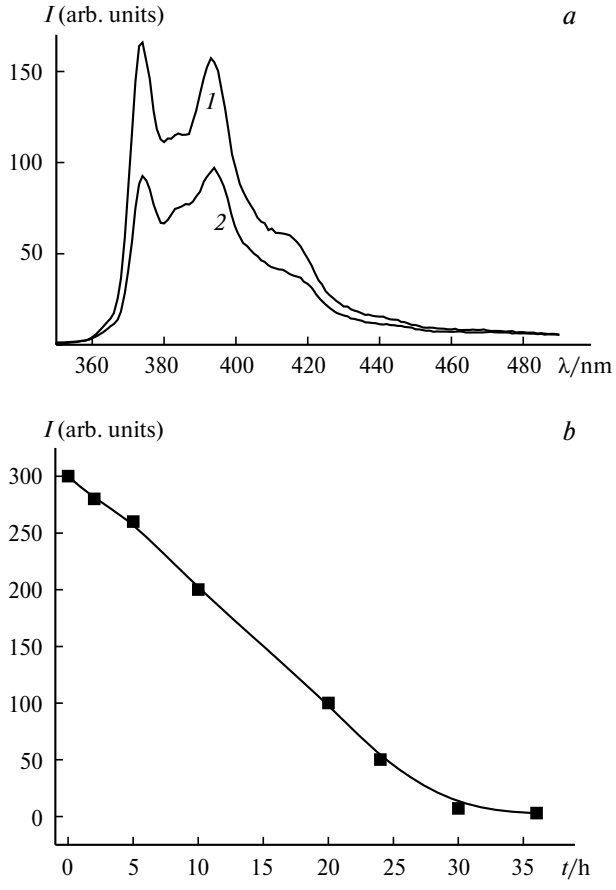

Fig. 6. (*a*) The change of the fluorescence intensity of encapsulated pyrene as a dependence of the medium pH: 2.3 (*1*), 7.0 (*2*). (*b*) A time dependence of the fluorescence intensities of encapsulated pyrene in the presence of CPB (0.053 mmol L^{-1}) in acid medium (pH 2.3, 25 °C, λ = 373 nm).

PAA—PEI capsules. The fluorescence intensity of encapsulated pyrene increases sharply on going from a neutral to the acid medium (Fig. 6), which indirectly indicates a loosening of the capsule wall. In the case of unencapsulated pyrene no influence of pH on the fluorescence spectrum was noted.

Apart from that, a faster release of pyrene from the capsule in an acid medium is indicated by a dependence, which reflects the change of micropolarity over time, as well as a comparison of the rate of fluorescence quenching in the presence of CPB: at pH 2.3 the release of pyrene from the capsules happens 3 times faster than in a neutral medium.

In conclusion, in the present work using the layer-by-layer method of applying polyelectrolytes the immobilization of pyrene in nanosized PAA—PEI capsules was carried out. Procedures for the control of pyrene release from the capsule are suggested. It was shown that the change of pH of an external medium can be the factor that influences the rate of substrate release from the capsule. It was found that the encapsulation of pyrene makes it possible to slow down the process of its deactivation by almost an order of magnitude with an addition of a quenching compound into the bulk medium. The obtained polyelectrolyte capsules satisfy one of the most important criteria of biocompatibility: their hydrodynamics diameter is less than 200 nm. This opens up prospects for their use for providing a prolonged use of pyrene or other diagnostic fluorescent probes.

References

1. B. G. De Geest, S. De Koker, G. B. Sukhorukov, O. Kreft, W. J. Parak, A. G. Skirtach, J. Demeester, S. C. De Smedt, W. E. Hennink, *Soft Matter*, 2009, **5**, 282.
2. I. P. Kaur, H. Singh, *J. Controlled Release*, 2014, **184**, 36.
3. R. M. Hernández, G. Orive, A. Murua, J. L. Pedraz, *Adv. Drug Delivery Rev.*, 2010, **62**, 711.
4. A. S. Sergeeva, D. A. Gorin, D. V. Volodkin, *BioNanoScience*, 2013, **4**, 1.
5. S. S. Rane, B. D. Anderson, *Adv. Drug Delivery Rev.*, 2008, **60**, 638.
6. A. Sprunk, Cl. J. Strachan, A. Graf, *Eur. J. Pharm. Sci.*, 2012, **46**, 508.
7. V. P. Torchilin, *Adv. Drug Delivery Rev.*, 2006, **58**, 1532.
8. S. De Koker, R. Hoogenboom, B. G. De Geest, *Chem. Soc. Rev.*, 2012, **41**, 2867.
9. G. B. Sukhorukov, A. L. Rogach, M. Garstka, S. Springer, W. J. Parak, A. Munoz-Javier, O. Kreft, A. G. Skirtach, A. S. Susha, Y. Ramaye, R. Palankar, M. Winterhalter, *Small*, 2007, **3**, 944.
10. S. De Koker, L. J. De Cock, P. Rivera-Gil, W. J. Parak, R. A. Velty, Ch. Vervaet, J. P. Remon, J. Grooten, B. G. De Geest, *Adv. Drug Delivery Rev.*, 2011, **63**, 748.
11. M. M. de Villiers, D. P. Otto, S. J. Strydom, Y. M. Lvov, *Adv. Drug Delivery Rev.*, 2011, **63**, 701.
12. S. F. M. van Dongen, H.-P. M. de Hoog, R. J. R. W. Peters, M. Nallani, R. J. M. van Hest, *Chem. Rev.*, 2009, **109**, 6212.
13. G. Berth, A. Voigt, H. Dautzenberg, E. Donath, H. Mohwald, *Biomacromolecules*, 2002, **3**, 579.
14. C. E. Mora-Huertas, H. Fessi, A. Elaissari, *Int. J. Pharmaceutics,* 2010, **385**, 113.
15. S. Anandhakumar, V. Nagaraja, A. M. Raichur, *Colloids Surf. B*, 2010, **78**, 266.
16. R. Luo, S. S. Venkatraman, *Biomacromolecules*, 2013, **14**, 2262.
17. S. Aziz, J. Gill, P. Dutilleul, R. Neufield, S. Kermasha, *J. Microencapsul.*, 2014, **31**, 774.
18. J. Cui, M. P. van Koeverden, M. Müllner, K. Kempe, F. Caruso, *Adv. Colloid Interface Sci.*, 2014, **207**, 14.
19. M. Kukut, O. Karal-Yilmaz, Yu. Yagci, *J. Microencapsul.*, 2014, **31**, 254.
20. O. Shimoni, Ya. Yan, Ya.Wang, F. Caruso, *ACS Nano*, 2013, **7**, 522.
21. E. A. Vasilieva, A. R. Ibragimova, A. B. Mirgorodskaya, E. I. Yackevich, A. B. Dobrynin, I. R. Nizameev, M. K. Kadirov, L. Ya. Zakharova, Yu. F. Zuev, A. I. Konovalov, *Russ. Chem. Bull.* (*Int. Ed.*), 2014, **63**, 232 [*Izv. Akad. Nauk, Ser. Khim.*, 2014, 232].
22. G. Verma, P. A. Hassan, *Phys. Chem. Chem. Phys.*, 2013, **15**, 17016.
23. L. Ya. Zakharova, A. R. Ibragimova, E. A. Vasilieva, A. B. Mirgorodskaya, E. I. Yackevich, I. R. Nizameev, M. K. Kadirov, Yu. F. Zuev, A. I. Konovalov, *J. Phys. Chem. C*, 2012, **116**, 18865.
24. X. Liu, L. Zhou, W. Geng, J. Sun, *Langmuir*, 2008, **24**, 12986.
25. C. O. Rangel-Yagui, A. Pessoa Junior, L. C. Tavares, *J. Pharm. Pharmaceut Sci.*, 2005, **8**, 147.
26. K. Y. Cheng, J. W. Wong, *Environ Technol.*, 2006, **27**, 835.
27. K. Kalyanasundaram, J. K. Thomas, *J. Am. Chem. Soc.*, 1977, **99**, 2039.
28. E. D. Goddard, N. J. Turro, P. L. Kuo, K. P. Ananthapadmanabhan, *Langmuir*, 1985, **1**, 352.

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