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Molecularly Imprinted Hybrid Adsorbents for Adenine and Adenosine-5′-triphosphate

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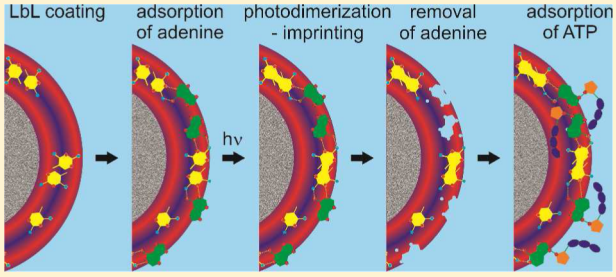
Supporting Information

ABSTRACT: Submicrometer-sized silica gel particles were coated with a polyanion and a polycation bearing thymine chromophores. The polymer-coated particles were found to selectively adsorb adenine and adenosine-5′-triphosphate (ATP), as compared to other nucleobases and nucleotides, respectively. The adsorption was enhanced by the irradiation of the particles in the presence of adenine which resulted in the molecular imprinting of adenine. ATP adsorption was strongly pH-dependent.



INTRODUCTION

Adenine is one of the five nucleobases (together with guanine, cytosine, thymine, and uracil) which are constituents of DNA and RNA, biopolymers encoding genetic information of the organisms.[1] Adenine is a part of many important biomolecules, such as adenosine, which is found in all cells, including glia and neurons. It plays important roles in the regulation of synaptic transmission and neuronal excitability in the central nervous system. Adenosine-5′-triphosphate (ATP) is mainly involved in the energy transport in the cell. Cell cycle events, e.g., proliferation, require the presence of a minimum ATP level in the cell. If the ATP level is reduced, but is above 15% of the normal level, the cell proliferation is arrested, while decreasing it further down to below 15% of the normal level results in the cell death.[2] That finding serves as the basis of the application of ATP-depleting agents in the therapy of cancer which may result in the tumor growth inhibition or regression, respectively.[3]

It was found that the ATP assay may be used to determine the metabolic rate before and after administration of the cytotoxic drugs to the breast tumor in vitro,[4] thereby enabling determination of the susceptibility of a patient to a chemotherapeutic drug. This allows reducing the number of unsuccessfully administered drugs which are devastating for the patient's health. Also, a positive correlation was observed between the ATP level in the tumor and the number of axillary lymph nodes involved[5] which is a parameter allowing the survival prognosis.

Modified nucleosides (including different adenosine derivatives) were identified in the urine more than a century ago. They are excreted in the urine of healthy subjects at very low concentrations, but in cancer patients their levels have been shown to be much higher and related to the type and progression of a cancer. Therefore, they are considered to be potential tumor markers.[6,7]

Moreover, it is known that ATP and other adenine-containing compounds play a significant role in the regulation of coronary blood flow,[8,9] protection of myocardium,[10] metabolism of heart,[11] and other functions which maintain the homeostasis of the cardiovascular system. ATP is considered as a sensitive biomarker of myocardial ischemia[12] and for monitoring therapeutic effects of anti-ischemia drugs.[13]

The methods typically used for the detection and quantification of adenine-based biomarkers include liquid chromatography,[14,15] electrophoresis,[16] and mass spectrometry,[17] the latter also coupled with other techniques.[18−20] However, these methods require complex and time-consuming sample preparation, including manual enrichment and cleanup, usually performed by solid-phase extraction. The advent of specific and sensitive immunoassay methods was a significant progress in this area and allowed direct analysis of the samples, simplified the quantification, and considerably shortened the analysis time.[21] However, the serious drawbacks of this method are cross-reactions, particularly in the case of urinary nucleosides, resulting in the loss of accuracy, and the results need a confirmation/verification with the use of an alternative method/test. In addition, immunoassay methods require the preparation of specific antibodies, which are unstable, costly, and require a time-consuming production procedure. Thus, further development in this field is obviously needed.

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Recently, the application of a molecularly imprinted polymer (MIP), imprinted with 1-methyladenosine (1-MA), a urinary cancer marker, was proposed.[22] The adsorbent was obtained by polymerization of methacrylic acid as a functional monomer, and a mixture of acetonitrile and water was applied as a porogen. The material was found to be highly selective when applied for direct extraction of 1-MA from spiked human urine and allowed to preconcentrate it, while interfering compounds were not adsorbed.

Even more recently, we have developed another, well-defined system based on the application of a hybrid adsorbent composed of silica gel particles coated with ultrathin polymeric layers.[23] The adsorbents were obtained from amphiphilic terpolymer containing thymine moieties and poly(allylamine hydrochloride) (PAH) and were fabricated by the layer-by-layer (LbL) deposition technique on the silica gel-based particles. The polymers bore thymine chromophores along the chains. The presence of these chromophores allowed selective adsorption of adenine-containing compounds since thymine is a base complementary to adenine with which it forms hydrogen bonds (Watson−Crick interaction) (Figure 1). Moreover, the

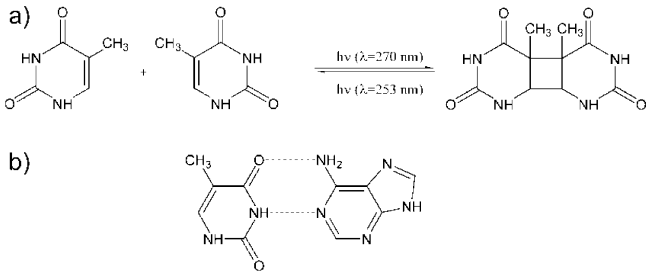

Figure 1. (a) Photodimerization of thymine. (b) Hydrogen bonds in the thymine−adenine pair.

thymine chromophores undergo photodimerization when irradiated with UV light. Photodimerization of the thymine chromophores attached to polymers coated on the silica gel particles, performed in the presence of adenine-like molecules, allowed their photochemical molecular imprinting. It should be noted that a photodimerization reaction does not involve the atoms in the thymine molecule which are involved in the formation of hydrogen bonds with adenine; therefore, photodimerization should not adversely affect formation of adenine−thymine pairs.

We have found that the polymer-coated particles efficiently adsorbed adenine derivatives. Their adsorptive capability increased even further upon molecular imprinting of adenine and was selective toward cytosine.

The present paper reports our studies on adsorption of nucleobases and nucleotides by the particles coated with the thymine-bearing anionic polymer used in the previous study[23] and a novel cationic thymine-bearing polymer. The influence of adenine molecular imprinting on the adsorption of different nucleobases and nucleotides by the polymer-coated particles was studied. Although the materials obtained are far from being practically applicable, the results indicate that the combination of the principles on which they are based (complementarity of nucleobases, molecular imprinting, and LbL method of fabrication) yielded interesting adsorptive properties and that these materials may be further improved.

RESULTS AND DISCUSSION

Polymers Used in the Studies. The aim of the research was to obtain materials which could efficiently adsorb adenine and adenine-containing compounds of biomedical interest so that they could be detected and/or assayed as biomarkers. For this purpose, polymers bearing thymine moieties were synthesized. Thymine is a nucleobase known to undergo photodimerization when irradiated with UV light (Figure 1a).[24] Thus, polymers bearing thymine chromophores may undergo photo-cross-linking, which, if performed in the presence of a low molecular weight compound (a template), may enable fabrication of MIPs that can selectively adsorb the template molecules.[23] Moreover, thymine attached to the polymer forms a pyrimidine−purine complementary base pair with adenine stabilized by two hydrogen bonds (Figure 1b). The nucleobase pair formation is expected to enhance the imprinting effect and add to the selectivity of the adsorption of adenine-containing compounds. Indeed, molecularly imprinted surfaces covered with a thymine-bearing polymer selectively adsorb adenine, as found in our previous studies.[23] The presence of the thymine chromophores in the polymers is evidenced by the presence of the 270 nm absorption band in the UV−vis spectra of their solutions (see Supporting Information). For the current studies, we have chosen two types of thymine-bearing polymers, i.e., a polycation and a polyanion. They could be used in the fabrication of ultrathin polymeric multilayers using a LbL method. The thymine-bearing polycation (AM) is a copolymer of thymylethyl acrylate (TEA) and methacryloyl aminopropyltrimethylammonium chloride (MAPTAC), while the thymine-bearing polyanion (ADT) is a terpolymer of sodium 2-acrylamido-2-methyl-1-propanesulfonate (AMPS), *N*-dodecylmethacrylamide (DodMAm), and thymylethyl methacrylate (TEMA) (see Supporting Information). Multilayers were formed using one or two thymine-bearing polymers. The polycations and polyanions used were assigned A*x*M and ADT*x* acronyms, respectively, where *x* is the molar content of a thymine-bearing monomer.

Preparation of Polymeric Multilayers via the LbL Method. To obtain polymeric multilayer films on the quartz plates, AM−ADT and PAH−ADT polymer pairs were used. As the quartz substrate and surface of Stöber particles (see below) are negatively charged, they were treated with cationic PAH or AM. The adsorbed PAH or AM formed the first monolayer and reversed the substrate surface charge to the positive value. Then, the negatively charged ADT was adsorbed to form the first bilayer. That process was repeated several times. The formation of subsequent bilayers on the quartz plates was confirmed by the measurement of UV−vis absorption spectra which revealed a linear increase of the absorbance at the maximum of the absorption band of the thymine chromophore, λ_{max} = 270 nm (see Supporting Information). As expected, the PAH−ADT22 films, i.e., those obtained with only one layer formed by a thymine-bearing polymer, contained less thymine chromophores per unit area than the AM−ADT22 films, in which both polymers in a bilayer bear thymine moieties. On the other hand, although A17M contains less thymine-bearing monomer than A25M, the films of A17M−ADT22 contain more thymine groups per unit area than A25M−ADT22 films as evidenced from their stronger absorption at 270 nm (see Supporting Information). This results from the fact that A17M is a polycation with a greater charge density, and the surface coated with this polymer is expected to be more charged than

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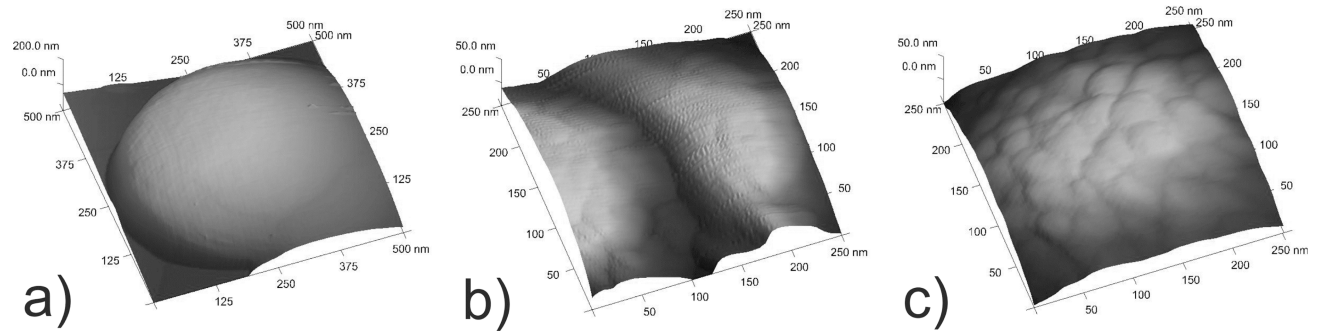

Figure 2. AFM images of uncoated particles (a) and particles coated with nonirradiated A25M (b) and coated with A25M and photoimprinted with adenine (c).

that coated with A25M. In fact, the zeta potential value of the surface of Stöber particles coated with this polymer is +35.6 mV, while that of A25M-coated particles is +26.1 mV. Thus, it may be expected that A17M interacts stronger with a negatively charged support or an ADT layer, and its layer is thicker than that of A25M. Since some studies indicate that it may be possible to obtain a multilayer composed of many layers of a single polyelectrolyte,[25] the deposition of multilayers composed of A17M or A25M was also attempted (see Supporting Information); however, almost constant absorption of the plates indicates that the deposition of only one layer of these cationic polymers is possible without a polyanionic interlayer.

Photodimerization of Polymeric Thymine Chromophores in Solution and in the LbL Films. Photodimerization of the thymine chromophores attached to the polymer chain was studied, both in the solution and after deposition in the form of the LbL films. Irradiation of the aqueous polymer solutions resulted in a decrease of the intensity of the absorption band with a maximum at λ_{max} = 270 nm in the UV−vis spectrum, indicating photodimerization of the thymine moieties. After 5 h of irradiation, the fraction of photodimerized thymine chromophores ranged from 66 to 74% (see Supporting Information).

Then, the PAH−ADT and AM−ADT multilayer films (seven bilayers) formed on a quartz support have been irradiated under the same experimental conditions (see Supporting Information). Also in this case photodimerization of the thymine groups was observed, which was completed within 10 h of irradiation, and the fraction of photodimerized thymine groups after that time ranged from 61% for A25M to 87% for PAH−ADT22. This fraction is unexpectedly high compared to that for the polymers in solution taking into account the limited mobility of the thymine chromophores attached to the polymeric chains forming the films. It may be explained by the fact that thymine moieties have a tendency to stack on each other giving rise to a significant hypochromic effect, as often observed for solution systems.[26] Therefore, some portion of thymine moieties may exist as stacked in the multilayer, which may be formed when the film is prepared by the LbL technique. Such a pairwise interaction of thymine may be favorable for its photodimerization.

Stöber Silica Gel Particles Coated with Thymine-Containing Films. Silica gel particles were obtained from tetraethyl orthosilicate (TEOS) using the well-known Stöber method, as described in the Experimental section. The size of the particles synthesized was about 700 nm, as found from SEM images (see Supporting Information). The photographs revealed that almost perfectly spherical silica gel particles were formed with a very smooth surface. As expected, these particles do not adsorb any of the nucleobases or ATP.

The particles were coated with thymine-containing polymers. Since the uncoated particles are negatively charged (their zeta potential was found to be −80 mV), the particles could be coated with cationic AM films and with a polymeric bilayer composed of PAH and ADT, as well as with an AM−ADT bilayer. The topology of the particle surface was further monitored using AFM microscopy (Figure 2). The images for uncoated particles revealed a smooth surface in agreement with SEM microphotographs. The surface of the particles coated with the polymers shows large structures. After irradiation of the polymer-coated particles, both in the presence and in the absence of adenine, their roughness increased significantly due to the formation of much smaller hemispherical structures (see Supporting Information).

Adsorption of ATP on Polymer-Coated Silica Gel Particles. We have further studied the possibility to use the particles coated with a thymine-containing polymer as the outermost layer to adsorb adenine-containing compounds. ATP was chosen due to its importance in the physiological processes in living organisms. ATP is a strongly negatively charged molecule, whose charge ranges from −4 at pH ≥ 7 to −2 at pH ≤ 3.[27] Thus, it may be expected that electrostatic attraction or repulsion strongly influences its adsorption process by the particles coated with the polycationic or polyanionic outermost layer, respectively. Also, these interactions should strongly depend on pH.

As expected, it was found that both PAH−ADT- and AM-coated particles adsorb ATP (Figure 3). We have observed that 30 min is sufficient for the adsorption process to reach

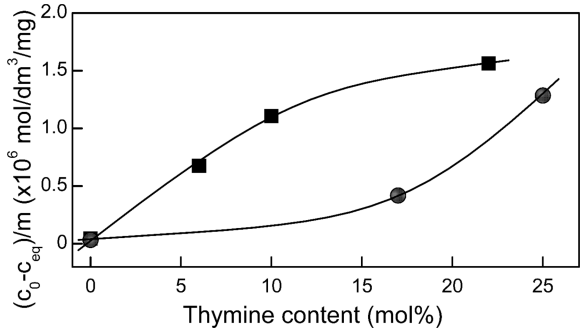

Figure 3. Dependence of the amount of ATP adsorbed on the thymine content in the ADT used in PAH−ADT films (■) and in AM (●) polymer coating the particles at pH = 3 for a unit mass of the particles (c_0 = 7.6 × 10^{-5} mol/dm^3, mass of particles was ∼10 mg).

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equilibrium and that uncoated particles practically do not adsorb molecules containing adenine moieties.

As we have shown in Figure 3, the amount of ATP adsorbed by the silica gel particles coated with these polymers as the outermost layer grows with increasing content of thymine chromophores in both ADT and AM polymers. This is an indication of the formation of adenine−thymine base pairs and the confirmation of the role of the thymine chromophores in the process of ATP adsorption. Quite unexpectedly, the particles coated with anionic ADT adsorb ATP stronger than those coated with cationic AM. This suggests that electrostatic interactions are not the only factor controlling the adsorption process and that the pyrimidine−purine bidentate hydrogen bonding is so stable that it may not be dissociated by an electrostatic repulsion between the polyanion and ATP once the complementary hydrogen bond is formed. A much greater amount of ATP adsorbed by A25M, i.e., a copolymer with a higher thymine-bearing monomer content and lower content of MAPTAC (a cationic monomer), than by A17M indicates that the adsorption ability of these polymers toward ATP results from the balance between the electrostatic and specific, chemical interactions. Indeed, the specific interactions between ATP and thymine moieties serve as a driving force for ATP adsorption from its solution.

With a view of the potential applications of the particles, e.g., as adsorbents in the chromatographic columns which could be used for the solid-phase extraction and preconcentration of ATP, by analogy to other polymeric systems,[14,16,22] the effect of the mass of the adsorbent (the PAH−ADT22 adsorbent was selected) on the amount of ATP adsorbed was tested (Figure 4).

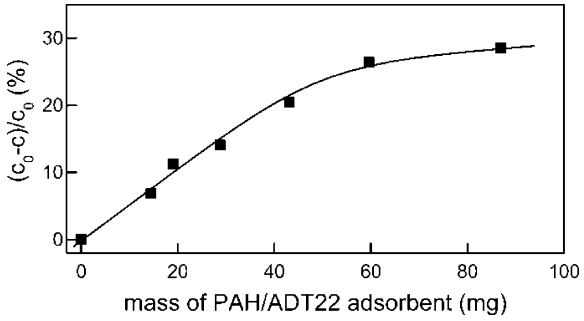

Figure 4. Dependence of the amount of ATP adsorbed on the mass of the silica gel particles coated with PAH−ADT22 (V = 3.5 mL, c_0 = 7.5 × 10^{-5} mol/dm^3, pH = 3).

As we have shown, 60 mg of the adsorbent decreased the ATP concentration by about 25%. For a greater mass of the adsorbent used, the decrease of ATP concentration was lower.

Dependence of ATP Adsorption on pH. We have measured the adsorption of ATP by a PAH−ADT22 coated adsorbent. At pH = 7, ATP molecules bear a strong negative charge, i.e., −4, and this is probably why they are only weakly adsorbed by the silica gel particles coated with the PAH−ADT22 bilayer whose zeta potential is also strongly negative, i.e., −70 mV (Figure 5).

At pH = 3, the charge of ATP is less negative by 2 units, so it is significantly adsorbed by the adsorbent particles (Figure 5) due to the formation of thymine−adenine hydrogen bonds and possibly also interactions with hydrophobic domains formed by dodecyl groups.[23]

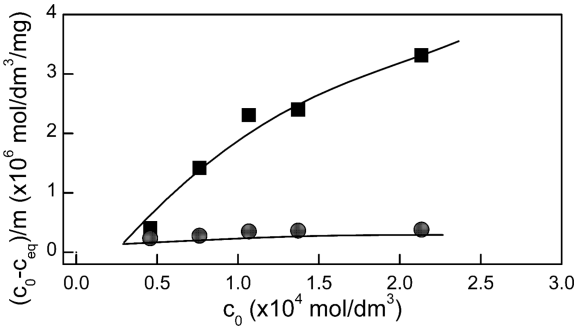

Figure 5. Plot of the dependence of the concentration decrease of ATP on its initial concentration per PAH−ADT22 adsorbent unit mass at pH = 3 (■) and pH = 7 (●).

Adsorption of ATP by the silica gel particles coated with a polycation, i.e., A25M, was also studied (Figure 6). The zeta

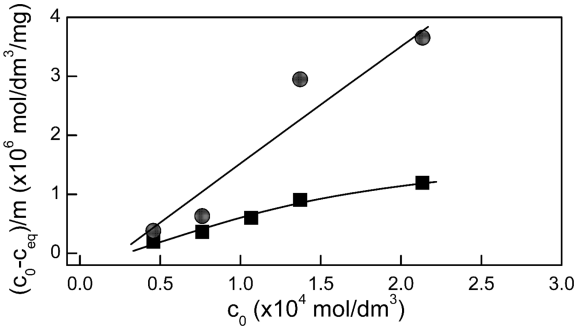

Figure 6. Plot of the dependence of the concentration decrease of ATP on its initial concentration per A25M adsorbent unit mass at pH = 3 (■) and pH = 7 (●).

potential of their surface was found to be +24 mV, thus there should be an attractive interaction between the particles and the molecules of the nucleotide. Therefore, an opposite effect of pH on adsorption was found, as expected. At both pH = 3 and pH = 7 ATP was adsorbed; however, at pH = 7 adsorption was much stronger due to a stronger attractive electrostatic interaction.

Adsorption of ATP by the Adenine-Imprinted Particles. The adsorption of ATP by the silica gel particles imprinted with adenine was studied using particles coated with PAH−ADT22 or coated with A25M. For the particles coated with PAH−ADT22, a significant increase in the amount of ATP adsorbed was found for imprinted particles, compared to both uncoated and coated nonirradiated particles (Figure 7a). The imprinted particles adsorb about twice as much ATP as the coated nonirradiated ones. This finding confirms the expectation that photodimerization of a polymeric thymine chromophores does not affect their ability to form hydrogen bonds with the adenine moiety. For A25M-coated particles, a similar imprinting effect was found (Figure 7b); however, in this case, the imprinted particles adsorb much less ATP than the imprinted ones coated with PAH−ADT22.

Studies on the Selectivity of Adenine Adsorption vs Other Nucleobases. The adsorption of adenine in comparison with cytosine, guanine, and thymine by the polymer-coated microspheres was studied using the mixture of all four nucleobases. The microspheres covered with A25M−ADT10 and A25M−ADT10−A25M were applied as adsorbents; thus, both the polyanion and the polycation used to coat the

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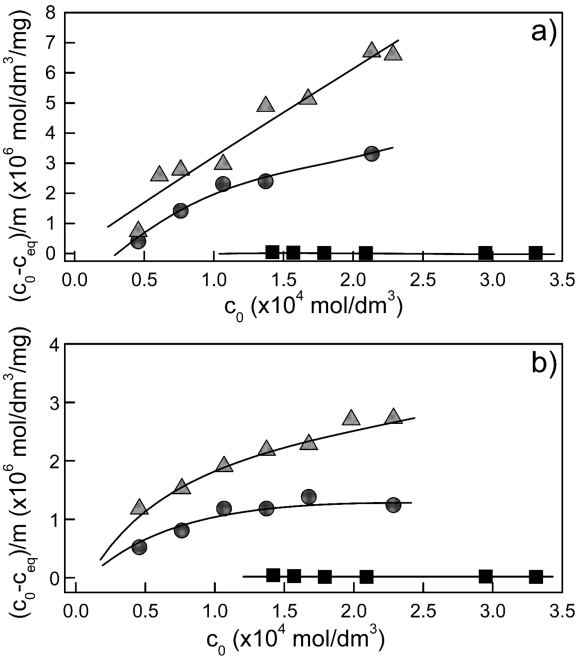

Figure 7. Adsorption of ATP at pH = 3 by uncoated particles (■) and particles coated with PAH−ADT22 (a) and A25 M (b) nonirradiated (●) and imprinted with adenine (▲).

microspheres contained thymine moieties. Both types of adsorbents showed the strongest absorption capacity for adenine. For microspheres coated with either A25M−ADT10 or A25M−ADT10−A25M bilayers, adsorption of adenine was the highest compared to that of other nucleobases, both for nonimprinted and adenine-imprinted materials. Nonimprinted A25M−ADT10 coated microspheres (Figure 8a) adsorbed about a half of the mass of adenine per unit mass of the

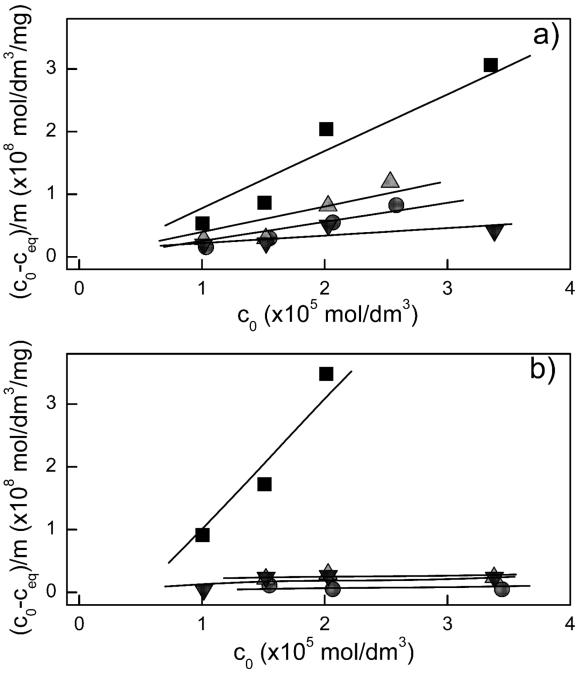

Figure 8. Adsorption of adenine (■), cytosine (●), guanine (▲), and thymine (▼) by microspheres coated with A25M−ADT10 (a) nonimprinted and (b) imprinted with adenine.

adsorbent compared to those coated with A25M−ADT10−A25M microspheres (Figure 9a). On the other hand, the

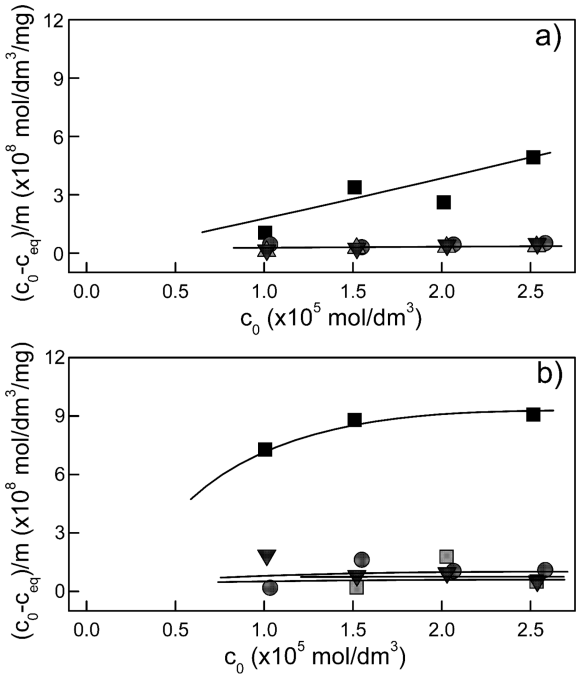

Figure 9. Adsorption of adenine (■), cytosine (●), guanine (▲), and thymine (▼) by microspheres coated with A25M−ADT10−A25M (a) nonimprinted and (b) imprinted with adenine.

A25M−ADT10−A25M microspheres adsorbed only adenine, while their absorption of the other three nucleobases was negligible (almost zero); therefore, they showed much higher selectivity than A25M−ADT10 coated microspheres (Table 1), which displayed measurable adsorption of cytosine, guanine, and thymine, although much lower than that of adenine. Then it was investigated whether the adenine adsorption capacity and selectivity may be enhanced by molecular imprinting of the above microspheres using adenine as a template. For both types of microspheres, a significant increase of adsorption capacity with spectacular improvement of selectivity was observed (Figure 8b and Figure 9b, Table 1) with molecularly imprinted A25M−ADT10−A25M coated microspheres showing greater adenine adsorption capacity than the A25M−ADT10 coated microspheres.

Studies on the Selectivity of ATP Adsorption vs Other Nucleotides. For the studies on the selectivity of ATP adsorption, the A25M− or PAH−ADT10-coated microspheres were used. Nonimprinted A25M-coated microspheres adsorbed a similar amount of ATP and guanosine 5′-triphosphate (GTP) and twice as much as that of thymidine 5′-triphosphate (TTP), while cytidine 5′-triphosphate (CTP) was not adsorbed at all (Figure 10a). Imprinting with adenine improved the ATP adsorption capacity. However, since the adsorption capacity for CTP and TTP increased also and that for GTP did not change (Figure 10b), the selectivity of ATP adsorption increased compared to GTP and TTP and decreased compared to CTP (Table 2)

On the other hand, nonimprinted PAH−ADT10-coated microspheres adsorbed ATP more strongly than nonimprinted A25M-coated microspheres and selectively compared to all three other nucleotides (Figure 11a, Table 2). The stronger

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Table 1. Selectivity of Adsorption of the Nucleobases vs Adenine by A25M−ADT10 and A25M−ADT10−A25M Adsorbents, Both Nonimprinted and Imprinted[a]

	A25M−ADT10			A25M−ADT10−A25M		
	n_{MIM}/n_{NIM}	$(n_{Ad}/n_{Nu})^{NIM}$	$(n_{Ad}/n_{Nu})^{MIM}$	n_{MIM}/n_{NIM}	$(n_{Ad}/n_{Nu})^{NIM}$	$(n_{Ad}/n_{Nu})^{MIM}$
adenine	1.71	1.00	1.00	2.84	1.00	1.00
cytosine	0.09	3.71	67.70	2.39	5.96	7.09
guanine	0.36	2.50	13.15	2.39	8.54	7.78
thymine	0.52	4.03	11.86	5.80	6.55	4.18

[a] n_{MIM} and n_{NIM}: number of moles of the nucleobase adsorbed at $c_0 = 2 \times 10^{-5}$ mol/dm^3 by 1 mg of the microspheres molecularly imprinted and nonimprinted with adenine, respectively. For unavailable c_0 values, interpolated values were used. $(n_{Ad}/n_{Nu})^{NIM}$ and $(n_{Ad}/n_{Nu})^{MIM}$: ratios of the number of moles of adenine and a nucleobase adsorbed at $c_0 = 2 \times 10^{-5}$ mol/dm^3 by 1 mg of the microspheres molecularly imprinted and nonimprinted with adenine, respectively.

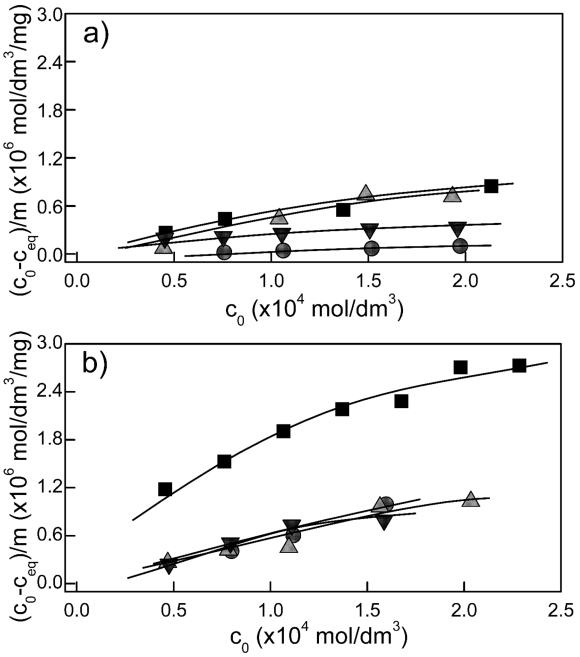

Figure 10. Adsorption of ATP (■), CTP (●), GTP (▲), and TTP (▼) by microspheres coated with A25M (a) nonimprinted and (b) imprinted with adenine.

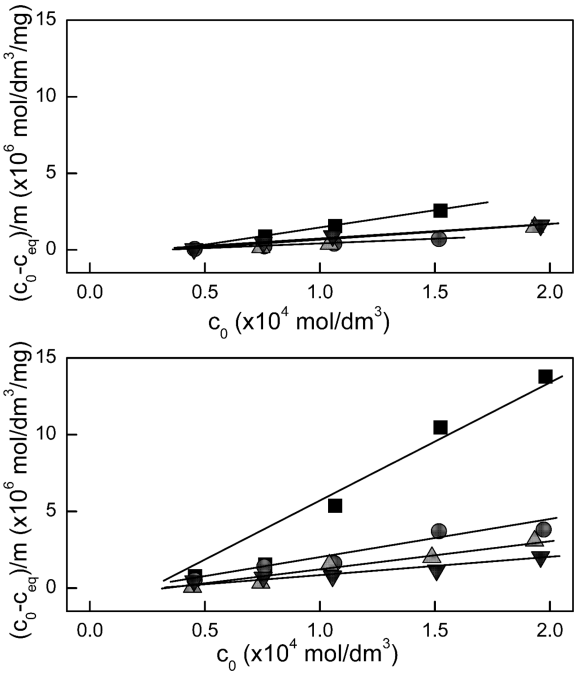

Figure 11. Adsorption of ATP (■), CTP (●), GTP (▲), and TTP (▼) by microspheres coated with PAH−ADT10 (a) nonimprinted and (b) imprinted with adenine.

adsorption of negatively charged ATP by PAH−ADT10-covered microspheres which have a negatively charged surface than by positively charged A25M is somewhat surprising and suggests that the adsorption capacity for ATP may be enhanced by hydrophobic interactions between ATP and dodecyl groups of ADT10. Imprinting adenine on PAH−ADT10-coated microspheres increased adsorption of all nucleotides but most significantly for ATP, thereby increasing the selectivity (Figure 11b, Table 2). Thus, one may conclude that molecular imprinting of adenine on the microspheres coated with thymine-containing polymers, both a polyanion and a polycation, allowed preparing of adsorbents selectively binding ATP vs other nucleotides.

CONCLUSIONS

Silica gel particles were coated with a polyanion (ADT) and/or a polycation (AM) containing thymine moieties. The coated

Table 2. Selectivity of Adsorption of the Nucleotides vs ATP by Two Adsorbents, Both Nonimprinted and Imprinted[a]

	A25M			PAH−ADT10		
	n_{MIM}/n_{NIM}	$(n_{ATP}/n_{NuTP})^{NIM}$	$(n_{ATP}/n_{NuTP})^{MIM}$	n_{MIM}/n_{NIM}	$(n_{ATP}/n_{NuTP})^{NIM}$	$(n_{ATP}/n_{NuTP})^{MIM}$
ATP	3.44	1.00	1.00	2,82	1.00	1.00
CTP	13.46	9.11	2.10	2,53	2,83	3,15
GTP	2.76	1.06	2.33	1,47	2,29	4,40
TTP	1.33	2.12	2.62	1,12	2,79	7,00

[a] n_{MIM} and n_{NIM}: number of moles of a nucleotide adsorbed at $c_0 = 1.5 \times 10^{-5}$ mol/dm^3 by 1 mg of the microspheres molecularly imprinted and nonimprinted with adenine, respectively. For unavailable c_0 values, interpolated values were used. $(n_{ATP}/n_{NuTP})^{NIM}$ and $(n_{ATP}/n_{NuTP})^{MIM}$: ratios of the number of moles of ATP and a nucleotide adsorbed at $c_0 = 1.5 \times 10^{-5}$ mol/dm^3 by 1 mg of the microspheres molecularly imprinted and nonimprinted with adenine, respectively.

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particles were found to adsorb adenine and ATP in contrast to noncoated ones. ATP adsorption was stronger at pH = 3 than at pH = 7 for PAH−ADT22-coated particles, while the opposite was observed for A25M-coated microspheres. A significant increase in the adsorption of adenine and ATP was found for the polymer-coated particles photoimprinted with adenine by irradiation of the particles with UV light (causing thymine photodimerization) in the solution of adenine. The adenine-imprinted particles selectively adsorb adenine vs other nucleobases and ATP vs other nucleotides.

EXPERIMENTAL SECTION

Materials. Tetraethyl orthosilicate (TEOS, 98%, Fluka), adenosine 5′-triphosphate disodium salt hydrate (ATP, ≥99%, Sigma-Aldrich), cytidine 5′-triphosphate disodium salt (CTP, ≥95%, Sigma), guanosine 5′-triphosphate sodium salt hydrate (GTP, ≥95%, Sigma), thymidine 5′-triphosphate sodium salt (TTP, ≥96%, Sigma), adenine (≥99%, Sigma-Aldrich), cytosine (≥99%, Sigma-Aldrich), guanine (≥98%, Sigma-Aldrich), thymine (≥98%, Sigma-Aldrich), sodium chloride (analytical grade, POCh), sodium citrate dihydrate (≥99%, Sigma-Aldrich), citric acid (≥99%, Sigma-Aldrich), sodium phosphate monobasic monohydrate (≥99%, Sigma-Aldrich), sodium phosphate dibasic heptahydrate (≥99.99%, Sigma-Aldrich), methanol (Chromasolv, ≥99.99%, Sigma-Aldrich), glacial acetic acid (Sigma-Aldrich), ammonium acetate (Sigma-Aldrich), ethyl alcohol (analytical grade, POCH), ammonium hydroxide (25% G.R., Lach-Ner), 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS, 99%, Aldrich), 2,2′-azobis(isobutyronitrile) (AIBN, >98%, Aldrich), poly(allylamine hydrochloride) (PAH, Aldrich, average M_w = 15 000 g/mol), 3-acrylamidopropyl trimethylammonium chloride solution 75 wt % in H_2O (Sigma Aldrich), 2-bromoethyl acrylate (94%, Alfa Aesar), 2,4-di-*tert*-butyl-*p*-cresol (>98%, Kanto Chemical), and *O*,*O*′-bis(trimethylsilyl)thymine (97%, Aldrich) were used as received. Water was distilled twice and deionized using the Simplicity Millipore Water Purification System. All solvents used were of HPLC grade.

Apparatus. UV measurements were carried out on a Hewlett-Packard HP 8452A diode array spectrophotometer in 1 cm optical path quartz cuvettes in the spectral range from 190 to 820 nm. HPLC chromatograms were obtained using a Waters HPLC system equipped with a Waters 2996 photodiode array detector and a BioBasic-8 5 μm, 4.0 × 250 mm column. Irradiation of the samples was carried out using a Rayonet photoreactor equipped with up to sixteen 21 W lamps with the emission intensity maximum at 300 nm. The zeta potential of the Stöber particles coated with PAH−ADT and AM−ADT films was evaluated using Zetasizer Nano ZS, Malvern Instruments Ltd. SEM images were obtained using a Hitachi S-4700 scanning electron microscope (Hitachi Company, Tokyo, Japan) with a Noran Vantage microanalytical system. Atomic Force Microscope (AFM) images were obtained with a NanoScope IV Multimode atomic force microscope (Veeco, Santa Barbara, CA) working in the tapping mode with standard Veeco silicon cantilevers for measurements in the air of normal spring constant of 40 N/m. AFM samples were prepared using the spin coating method on silicon wafers, which previously were purified in "piranha" solution (a mixture of H_2SO_4 and H_2O_2 in a 1:3 ratio) (*CAUTION! "Piranha" solution should be handled with extreme care!*) and rinsed with water.

Synthesis of the Terpolymer of Sodium 2-Acrylamido-2-methyl-1-propanesulfonate (AMPS), *N*-Dodecylmethacrylamide (DodMAm), and Thymylethyl Methacrylate (TEMA) (ADT). The terpolymer of sodium 2-acrylamido-2-methyl-1-propanesulfonate (AMPS), *N*-dodecylmethacrylamide (DodMAm), and thymylethyl methacrylate (TEMA) (ADT) was synthesized as described elsewhere.[23] In brief, a typical synthesis procedure was as follows: 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) (1.99 g, 9.60 mmol) was neutralized by an equimolar amount of Na_2CO_3 in DMF (30 mL). Then, DodMAm (0.709 g, 2.80 mmol), TEMA (0.191 g, 0.80 mmol) prepared according to the literature,[28,29] and AIBN (5 mg, 0.03 mmol) were added to this solution and transferred into a glass ampule. The solution was degassed on a high vacuum line equipped with a diffusion pump by six freeze−pump−thaw cycles, and then the ampule was sealed. Polymerization was carried out at 60 °C for 30 h. The polymer obtained was purified by 3-fold precipitation from methanol into a large excess of diethyl ether and subsequent dialysis against pure water for 1 week. The polymer was recovered by freeze-drying. Yield: 2.49 g (85.8 g). A series of terpolymers were obtained. In further studies, two polymers with the different monomer content were used, i.e., 50 mol % of AMPS, 40 mol % of DodMAm, and 10 mol % of TEMA (ADT10); and 67 mol % of AMPS, 11 mol % of DodMAm, and 22 mol % of TEMA (ADT22).

Synthesis of Thymylethyl Acrylate (TEA). 2-Bromoethyl acrylate (5.00 g, 27.9 mmol) and 2,4-di-*tert*-butyl-*p*-cresol (50 mg, 0.227 mmol) were added to *O*,*O*′-bis(trimethylsilyl)thymine (3.78 g, 14.0 mmol). The reaction was carried out at 60 °C for 10 days. Then, methanol (100 mL) was added to the reaction mixture to deprotect thymine oxygen atoms. The precipitate was filtered off, and the solvent was evaporated under vacuum. White solid was obtained, which was recrystallized from methanol. An amount of 1.58 g of thymylethyl acrylate (TEA) was obtained (yield: 50.6%).

Synthesis of the Copolymer of 3-(Methacryloyl)-aminopropyltrimethylammonium Chloride (MAPTAC) and *N*-(Acryloyloxyethyl)thymine (AM). A typical procedure of the polymer synthesis was as follows: 2,2′-azobis(isobutyronitrile) (AIBN, 2.74 mg, 0.0167 mmol), 3-(*N*-methacryloyl)-aminopropyltrimethylammonium chloride (MAPTAC, 1.224 g, 5.545 mmol), and thymylethyl acrylate (TEA, 0.496 g, 2.358 mmol) were dissolved in a mixed solvent of DMSO (6.3 mL) and water (1.6 mL). Polymerization was carried out at 60 °C for 20 h under argon. After polymerization, the solution was cooled to room temperature. The polymer was purified by dialysis against pure water for 1 day and freeze-dried. An amount of 1.091 g of the polymer (AM) was obtained. Two copolymers containing 17 and 25 mol % of TEA, respectively, were obtained (referred to hereafter as AM17 and AM25) as found from ^{1}H NMR measurements.

Preparation of Polyelectrolyte Multilayer Films. Polyelectrolyte (multilayer) films were assembled according to the well-known LbL method using either PAH or AM as a polycation and ADT as a polyanion. PAH−-ADT films were prepared using a 0.1 g/L solution of PAH in 1 M NaCl and 1.0 g/L solution of ADT in 6.25% NH_4OH. AM−ADT films were prepared using a 0.5 g/L AM solution in 0.1 M NaCl and 1.0 g/L ADT solution in 6.25% NH_4OH. Quartz substrates were cleaned prior to the polymer adsorption by immersion in freshly prepared "piranha" solution (i.e., a mixture of 30% H_2O_2 and concentrated H_2SO_4 in 1:3 ratio). (*CAUTION! Piranha solution should be handled with extreme care!*). Such prepared plates were stored under water before use and dried under argon before deposition of the first polymer layer. In the first step the negatively charged quartz plate was immersed in a polycation solution (PAH or AM polymers) under sonication for 5 min, then rinsed with distilled water, and dried in a stream of argon. Subsequently, a quartz plate covered with a PAH or AM layer was dipped into polyanion solution (ADT) under sonication for 5 min, rinsed with distilled water, and dried in a stream of argon to complete a first fabrication cycle. This preparation process was repeated until the desired number of bilayers was achieved. In each cycle, one bilayer was deposited on both surfaces of the substrate. The progress in the film deposition was followed by the measurement of the UV−vis absorption spectra.

Synthesis of the Silica Gel Particles. Silica gel particles were obtained using a Stöber method.[30] Briefly, 3 mL of 16.7% ammonium hydroxide was added rapidly to a solution of 0.65 mL of TEOS in 11.35 mL of ethanol. The mixture was stirred vigorously with a magnetic stirrer for 24 h, and a suspension of silica gel particles was obtained. Ethanol and ammonium hydroxide were removed under vacuum. The silica particles were stored in the aqueous suspension.

Preparation of Hybrid Adsorbents Containing Thymine Moieties via LbL Polymer Deposition onto Silica Gel Particles. An amount of 5 mL of the suspension of silica gel particles in water prepared as described above was centrifuged at 10 000 rpm (7900*g*) for 3 min to separate the particles. Water was removed by decantation, and the particles were placed in 3 mL of polycation solution (0.1 g/L

of PAH or 0.5 g/L of AM). The sample was then sonicated for 10 min at 40 °C followed by centrifugation and decantation of the supernatant solution. The polycation-coated silica gel particles were washed with water by filling the centrifuge tube containing the coated particles with 5 mL of water, sonication for 2 min, centrifugation at 10 000 rpm for 3 min, and the decantation of water. The procedure described above was repeated 5 times. To obtain AM−ADT-coated particles, 3 mL of a 1 g/L ADT solution was then added to the AM-coated particles, and the sample was sonicated for 10 min at 40 °C. After that, the particle suspension was washed with water 5 times using the procedure described above. To obtain AM−ADT−AM-coated particles, the AM layer was supported on AM−ADT-coated particles in the same way as the first AM layer.

Adsorption of ATP by PAH−ADT-Coated Stöber Particles with Different Thymine Content in ADT. About 10 mg of Stöber particles coated with PAH−ADT prepared using ADT with different content of TEMA was added to 3.5 mL of 3×10^{-4} M adenosine 5′-triphosphate disodium salt (ATP) to adsorb an adenine-containing nucleotide. The adsorption was continued for 30 min under mixing. Bare silica gel particles (not coated with polymeric bilayers) were used as a reference material. The particles were separated by centrifugation, and the concentration of the adsorbed compound in the supernatant was determined using HPLC.

Influence of Mass of the Adsorbent on the Amount of ATP Adsorbed. The effect of amount of the adsorbent obtained by supporting ADT22 onto silica gel particles coated with PAH on the amount of ATP adsorbed was tested. Different amounts (10−90 mg) of the adsorbents were added to 3.5 mL of about 7×10^{-5} M ATP solution, and the adsorption was carried out for 30 min. Adsorbent was separated by centrifugation, and the concentration of ATP in the supernatant was determined using HPLC.

Preparation of Adsorbents Molecularly Imprinted with Adenine. Molecularly imprinted materials were prepared as follows: about 10 mg of silica gel particles coated with polymer(s), prepared as described above, were immersed in 9 mL of 0.022 mg/mL adenine solution in pH 7.0 phosphate buffer and equilibrated for 1.5 h. The equilibrated samples were then irradiated for 4 h in a Rayonet photoreactor equipped with four lamps with emission intensity maximum at 300 nm and the spectral range of 270−320 nm. Then the template compound was removed by washing out with water, as described above (at that step, however, the sample was not sonicated but stirred with a magnetic stirring bar). The washing procedure was repeated 9 times. The progress of adenine removal was followed by the measurement of UV absorption spectra of water used for washing. The washing process was completed when adenine was no longer detected in water used for washing. The reference (nonimprinted) particles were prepared by irradiating the same adsorbent in phosphate buffer (pH = 7) in the absence of adenine in the same conditions.

Studies on the Influence of pH on the Adsorption of ATP by PAH−ADT22-Coated and A25M-Coated Silica Adsorbents. Adsorption of adenosine 5′-triphosphate disodium salt (ATP) by both nonimprinted and adenine-imprinted particles coated with a PAH−ADT22 bilayer and A25M was studied. For that purpose, a defined amount of the adsorbent (about 10 mg) was added to 3.5 mL of 5×10^{-5}−5×10^{-4} M solution of ATP in the pH = 7 or pH = 3 buffer. The suspension was stirred using a magnetic stirrer at room temperature for 30 min. The amount of ATP adsorbed by the particles was determined using an HPLC chromatograph equipped with a UV−vis detector by the determination of its concentration difference in the solution before and after adsorption.

Selectivity of Adsorption of Nucleobases by A25M−ADT10-Coated and A25M−ADT10−A25M-Coated Silica Adsorbents. A defined amount of nonimprinted and imprinted A25M−ADT10 and A25M−ADT10−A25M-coated adsorbents (about 10 mg) was added to 3.5 mL of 1×10^{-5}−4×10^{-5} M solution containing all four nucleobases (adenine, cytosine, guanine, and thymine) in the pH 7 buffer. The suspension was stirred using a magnetic stirrer at room temperature for 30 min. The amount of the compound adsorbed by the particles was determined using an HPLC chromatograph equipped with a UV−vis detector as a difference of its initial and equilibrium concentration.

Selectivity of Adsorption of Nucleotides by A25M and PAH−ADT10-Coated Silica Adsorbents. The selectivity of adsorption of ATP, CTP, GTP, and TTP nucleotides was studied in a way similar to that of nucleobases; however, the adsorption from the solution of each nucleotide separately in the pH 3 buffer was studied rather than the adsorption from the nucleotide mixture. The initial concentration of the nucleotides ranged from 5×10^{-5} to 30×10^{-5} M.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

UV−vis spectra of the thymine-containing polymers, chemical structures of the compounds used, dependence of the absorbance of the polymeric multilayer film as a function of the number of bilayers, degree of photodimerization vs irradiation time, and SEM images of the particles. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

MIP, molecularly imprinted polymer; 1-MA, 1-methyladenosine; PAH, poly(allylamine hydrochloride); LbL, layer-by-layer; TEA, thymylethyl acrylate; MAPTAC, methacryloyl aminopropyltrimethylammonium chloride; AMPS, sodium 2-acrylamido-2-methyl-1-propanesulfonate; DodMAm, *N*-dodecylmethacrylamide; TEMA, thymylethyl methacrylate; TEOS, tetraethyl orthosilicate; GTP, guanosine 5′-triphosphate; TTP, thymidine 5′-triphosphate; CTP, cytidine 5′-triphosphate

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