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Molecularly Imprinted Thin Polymeric Film as a Fluorescent Sensor for Nucleotides

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The technology of molecular imprinting of polymers (MIPs) was used to obtain optical sensors for nucleotides. The methacrylates were used as functional monomers and 1,3-diphenyl-6-vinyl-1H-pyrazole-[3,4-b]-quinoline (PAQ) was used as a fluorescent receptor and the nucleotides: guanosine 3′,5′-cyclic monophosphate (cGMP) and adenosine 3′,5′-cyclic monophosphate (cAMP) were a template. The optical sensor obtained as a thin-layer polymeric films was investigated by steady-state fluorescence spectroscopy. The calculated association constant of the cGMP imprinted sensor is $K_a = 1.7 \times 10^4\, M^{-1}$ *and of cAMP imprinted sensor* $K_a = 9.9 \times 10^4\, M^{-1}$.

Keywords: fluorescence spectroscopy; MIPs; nucleotides; optical sensors; pyrazolequinolines

INTRODUCTION

At present, a wide range of optical sensors based on fluorescence measurements have found application in medicine and biology, mainly due to their non-invasive character of work [1]. Molecular imprinting (MIPs) is a technology, which creates template-shaped cavities in the polymer matrices with memory not only of the shape but also of the electronic structure of the template molecules. This technology is based on the recognition system used by enzymes for a substrate, which is called the "lock and key" model. In recent decades, the

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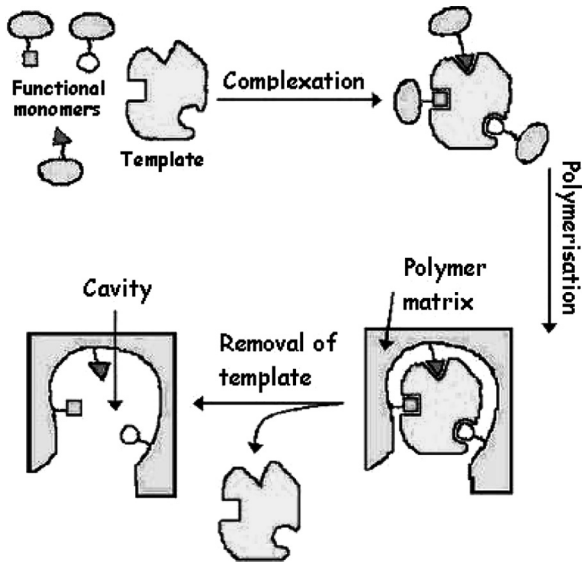
molecular imprinting technique has been developed for use in receptors, chromatographic separations [2,3], fine chemical sensing, etc. Taking advantage of the shape selectivity of the cavity [4], the use in catalysis for certain reactions has also been facilitated [5].

Molecularly imprinted materials are usually prepared using a template molecule together with functional monomers that assemble around the template molecule by interaction between functional groups of both the template and monomers. Then, the mixture is cross-linked during copolymerisation as shown in Scheme 1. The functional monomers are polymerized to form an imprinted matrix. After polymerisation the template molecule is removed from the matrix, leaving behind a cavity complementary in size and shape to the template. After extraction the obtained molecular cavity can work as a selective binding site for a specific template molecule [6]. In the optical sensor interactions of the template with the receptor group or molecule result in an analytical signal which gives information on molecular recognition. This effect depends mostly on the mechanisms of interactions between them.

In the case of a fluorescent receptor the fluorescence signal informs us about changes inside the cavities and molecular recognition. In the present study PAQ with the functional vinyl group was used as a receptor, while cyclic guanosine monophosphate (cGMP) and cyclic



SCHEME 1 Molecular imprinting formation.

adenosine monophosphate (cAMP) were the template. Our early investigations indicate quenching of fluorescence in the presence of nucleotides when studied in solution [7]. Pyrazolequinoline derivatives were investigated as active layers in electroluminescent devices [8]. Recently, we used PAQ as a fluorescent receptor in the polymeric sensory system [9]. This molecule has strong and stable fluorescence when it is incorporated into the polymer film. The molecularly imprinted polymeric sensors presented previously [9] were characterized by good selectivity and reproducibility. We report here steady-state fluorescence measurements of optical polymeric sensors with built-in pyrazolequinoline derivative as a fluorosensor.

EXPERIMENTAL

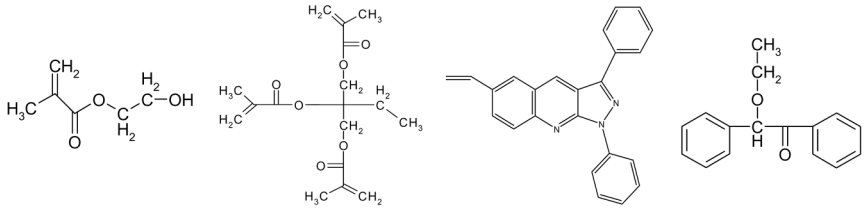
Materials

2-hydroxyethyl methacrylate (HEMA) as a functional monomer and trimethylolpropane trimethacrylate (TRIM) as a crosslinking agent were obtained from Sigma-Aldrich Co. 1,3-diphenyl-6-vinyl-1H-pyrazole-[3,4-b]-quinoline (PAQ) was prepared as described elsewhere [10]. A photoinitiator – benzoin ethyl ether (BEE), adenosine 3′,5′-cyclic monophosphate (cAMP), guanosine 3′,5′-cyclic monophosphate (cGMP) used as a template and cytidine 3′,5′-cyclic monophosphate (cCMP), guanine (2-Amino-6-hydroxypurine), adenine (6-aminopurine) – compounds used to selectivity investigation, were purchased from Sigma-Aldrich Co. Structures of these compounds are specified in Figure 1. Tetrahydrofuran (THF) and methanol were obtained from Sigma-Aldrich Co. The solvents were of spectroscopic grade and checked for impurities by absorption and fluorescence measurements.

Preparation of Molecularly Imprinted Polymeric Films

Preparation of the polymeric sensor involves three steps: (1) formation of a fluorosensor-template complex, (2) light-induced polymerization with addition of the photoinitiator and (3) extraction and re-adsorption of the template. The complexation took place in a mixture of 10^{-5} M PAQ in THF and 10^{-5} M cGMP or cAMP in methanol in a 1:1 v/v ratio. (The solvents had similar boiling point in the range from 65 to 67°C). Then, the solution was mixed with HEMA, TRIM and benzoin ethyl ether dissolved in methanol. The final mixture was cast on quartz plates using the drop-casting method in the presence of argon, and was exposed to UV irradiation (1 minute) with a maximum at 350 nm. The films were subsequently stored in a vacuum oven for 24 hours

Functional monomers



Templates

Compounds used to selectivity investigation

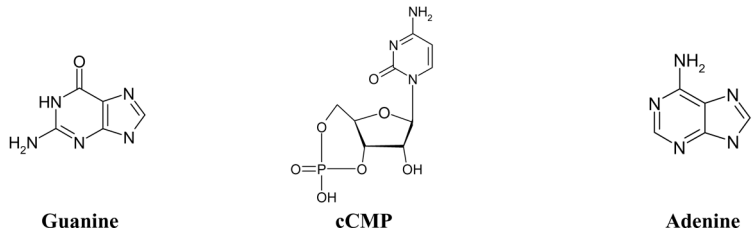


FIGURE 1 Structures of component used to MIPs preparation: *functional monomers* – **HEMA** – 2-hydroxyethyl methacrylate, **TRIM** – trimethylolpropane trimethacrylate, **PAQ** – 1,3-diphenyl-6-vinyl-1H-pyrazole-[3,4-b]-quinoline, photoiniciator – **BEE** – benzoin ethyl ether; *templates* – **cGMP** – guanosine 3′,5′-cyclic monophosphate, **cAMP** – adenosine 3′,5′-cyclic monophosphate; *compounds used to selectivity investigation* – **Guanine**, **cCMP** – cytidine 3′,5′ cyclic monophosphate, **Adenine**.

at the pressure of 0.06 cm Hg at 65°C. The corresponding non-imprinted polymer (NIP) was prepared in the same way, but methanol was added instead of the template. The films were extracted in distilled water for 24 hours. An aqueous solution of nucleotide of appropriate concentration was used for incubation of the film. 24 hours were sufficient for re-adsorption of the template. To determine association constants, a series of concentrations of nucleotides

(10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} M) in water was prepared for the analytical process. The non-imprinted polymer was treated using the same procedure.

RESULTS AND DISCUSSION

The electronic absorption and steady-state fluorescence emission spectra of the PAQ [11] and fluorescence quenching of this molecule in the presence of nucleotides: guanosine 3′,5′-cyclic monophosphate (cGMP) and adenosine 3′,5′-cyclic monophosphate (cAMP) in solution were previously discussed [7,12]. The absorption spectrum of the PAQ comprises a high-energy strong absorption centred at 290 nm and broad low-energy band at around 390 nm. This second band is due to a charge transfer and it is sensitive to the environment of interaction. The emission spectra were collected at the excitation wavelength of 390 nm.

The quenching of fluorescence from the film after incubation with nucleotides was observed. The dependence on the concentration of nucleotide has a linear character in the solvent and allowed us to calculate Stern-Volmer constants. Figure 2 shows integrated fluorescence from the thin-layer cGMP imprinted polymeric films during the step-by-step processing also when the film was incubated in the

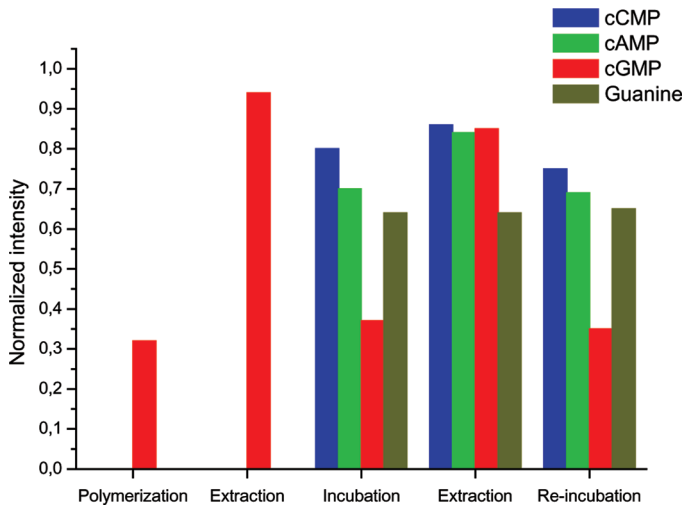


FIGURE 2 Integrated intensity of fluorescence of cGMP imprinted polymer in step by step processing, after incubation in presence of 10^{-4} M nucleotides in water.

presence of different nucleotides. Intensity is normalized in respect to fluorescence intensity of non-imprinted film (NIP). Molecules, which were chosen for this experiment, have a similar chemical structure to the template (structures in Fig. 1). We did not observe a significant change occur in the shape of the fluorescence spectra during the whole experimental cycle. We observed only changes of the fluorescence intensity depending on the molecule which the film was incubated with. The electronic structure and the distribution of the functional groups were fixed by the polymerization that the obtained cavities acted selectively. Cavities present in the polymeric film absorb other nucleotides, which is connected with the change of fluorescence.

Similar investigations were performed for cAMP-imprinted films. Figure 3 shows the integrated fluorescence of step-by-step processing. Molecularly imprinted polymeric sensors are characterized by good reproducibility. The washing and re-adsorption cycle was repeated twice and we expect that the material would work longer.

These measurements allow us to calculate selectivity factors from the following equation:

$$a_{\text{Template/Quencher}} = \frac{I_0 - I_{\text{Template}}}{I_0 - I_{\text{Quencher}}} \tag{1}$$

where:

I_0 – intensity of MIPs fluorescence after extraction;

I_{Template} – intensity of MIPs fluorescence in the presence of molecules used as a template;

I_{Quencher} – intensity of MIPs fluorescence in the presence of other nucleotides – quenchers.

Values of calculated selectivity factors for cGMP-imprinted polymeric films and cAMP-imprinted films are shown in Tables 1 and 2, respectively. Factor a obtained for cAMP-imprinted film incubated with cGMP is lower than one. This suggests that in spite of cavities produced by cAMP, cGMP better interacts with the pyrazolequinoline receptor. As we know from our previous studies, in solution cGMP is a stronger quencher than cAMP. This is proved by Stern-Volmer constants determined from the linear dependences of relative intensity change with the concentration of these nucleotides. The Stern-Volmer constants are $K_{SV(cGMP)} = 3.1 \times 10^3\,M^{-1}$, $K_{SV(cAMP)} = 1.2 \times 10^3\,M^{-1}$. This is compatible with the result obtained for the polymeric sensory system. Significant quenching was observed for cGMP-imprinted film in contrast with cAMP-imprinted film.

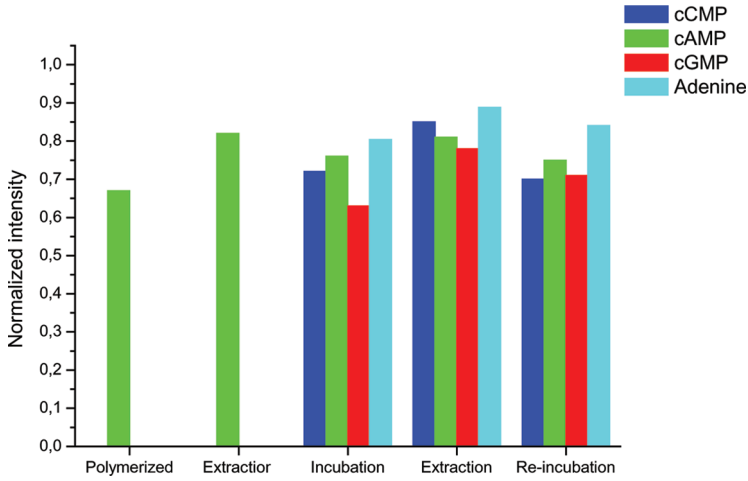


FIGURE 3 Integrated intensity of fluorescence of cAMP imprinted polymer in step by step processing, after incubation in presence of 10^{-4} M nucleotides in water.

Dependence of I_0/I versus concentrations of the used nucleotide is shown in Figure 4. One can see that the quenching process has non-linear character. The graph in Figure 4 may be described by the modified Stern-Volmer equation [13].

$$\frac{I_0}{I} = \frac{1 + K_a[Q]}{1 + \frac{k_{FQ}}{k_F} K_a[Q]} \tag{2}$$

where:

I_0 – fluorescence intensity without the quencher (nucleotides);
I – fluorescence intensity in the presence of nucleotides;
K_a – association constants;
k_{FQ} – process rates of fluorescence from F-Q complex;
k_F – process rates of fluorescence from the fluorophore.

TABLE 1 Selectivity Factor for cGMP-Imprinted Film in Presence of Different Nucleotides

cGMP/Nucleotide	a
cGMP/cAMP	2.38
cGMP/cCMP	4.07
cGMP/Guanine	1.90

TABLE 2 Selectivity Factor for cAMP-Imprinted Film in Presence of Different Nucleotides

cAMP/Nucleotide	a
cAMP/cGMP	0.53
cAMP/cCMP	5.00
cAMP/Adenine	1.25

As we can see in Figure 4, incubation of polymeric film in series of solutions caused a decrease of fluorescence intensity in almost every case. Exception was incubation in 10^{-7} M solution. In this case the concentration is probably too small and the molecules of nucleotides are not visible to the sensory system. This solution acts as a solvent which washed the rest of adsorbed nucleotides.

Equation (2) was used to fit the plot and to calculate the association constants. Calculated K_a-values are given in Table 3. A reference sample was a non-imprinted polymeric film (NIP) treated in the same way as the imprinted film. Significant differences of K_a for cGMP and cAMP imprinted films may be cause by different mobility of the compounds during of the processing. The driving forces are certainly responsible for enhanced K_a for cAMP, but the reason is not known yet.

CONCLUSIONS

Molecularly imprinted polymeric sensors are characterized by good reproducibility and this material may be used repeatedly. The

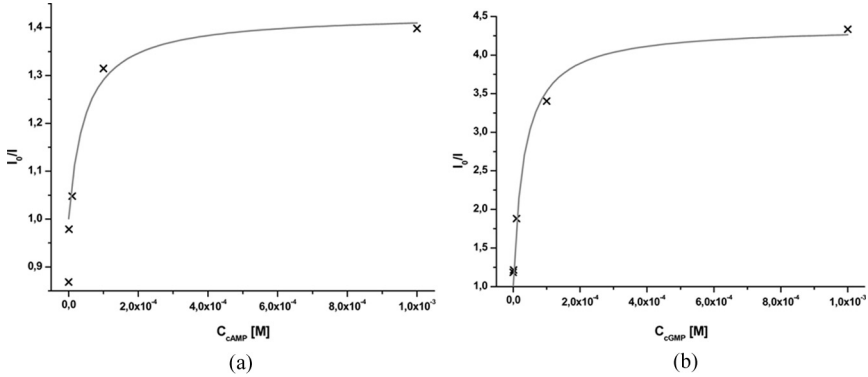


FIGURE 4 Dependence I_0/I versus concentration of nucleotides, adsorption for (a) cGMP-imprinted films incubated with cAMP; (b) cAMP-imprinted polymer incubated with cGMP. Plot was fitted by modified Stern-Volmer equation.

TABLE 3 Calculated Values of Association Constant for Two Imprinted Polymeric Films and for Reference Sample used for Comparison. (*Means that calculated error was higher than obtained value)

	$K_a \times 10^4$ [M^{-1}]	
	cGMP	cAMP
cGMP-imprinted	1.7 ± 1.3	3.0 ± 2.8
cAMP-imprinted	13.2 ± 2.8	9.9*
Reference	10.6 ± 5.8	3.8 ± 1.8

imprinted films are able to recognize the molecule, the structure of which was imprinted before, so that they selectively adsorb this molecule. The steady-state fluorescence measurements show quenching of fluorescence when the nucleotide/template was adsorbed into the imprinted cavity. Although the selectivity of adsorption of the template (cGMP or cAMP) when measured against some other nucleotides is satisfactory, the fluorescence quenching in the presence of another nucleotide is still noticeable. Calculated selectivity factors correspond to the factors obtained for the pyrazolequinoline molecule put into poly(methyl methacrylate) matrix. The pyrazolequinoline receptor incorporated in the polymer makes a possibly non-invasive measurement of the film. Calculated value of association constants for pyrazolequinoline receptor are comparable to the value obtained for other similar systems (e.g., vinylpyridine receptor [14]).

REFERENCES

[1] Ligler, F. S. & Rowe Taitt, C. A. (2002). *Optical Biosensors: Present and Future*, Elsevier: Washington, DC, USA.
[2] Kriz, D., Kriz, Ch. B., Andersson, L. I., & Mosbach, K. (1994). *Anal. Chem.*, *66*, 2636.
[3] Tamayo, F. G. & Martin-Esteban, A. (2005). *Journal of Chromathography A*, *1098*, 116.
[4] Simon, R., Collins, M. E., & Spivak, D. A. (2007). *Analytica Chimica Acta*, *591*, 7.
[5] Robinson, D. K. & Mosbach, K. (1989). *Journal of the Chemical Society, Chemical Communications*, *14*, 969.
[6] Yoshida, M., Hatate, Y., Uezu, K., Goto, M., & Furusaki, S. (2000). *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *169*, 259.
[7] Cywiński, P., Danel, A., & Wandelt, B. (2003). *Annals of Polish Chemical Society*, *2*, 975.
[8] Tao, Y. T., Balasumbramaniam, E., Danel, A., Jarosz, B., Tomasik, P., & Rasala, D. (2001). *Chem. Mater.*, *13*, 1207.
[9] Cywiński, P., Sadowska, M., Danel, A., Buma, W. J., Brower, A. M., & Wandelt, B. (2007). *Journal of Applied Polymer Science*, *105*, 229.

[10] Danel, A., Chaczatrian, K., & Tomasik, P. (2000). *ARKIVOC*, *1*, 51.
[11] Cywinski, P., Wandelt, B., Buma, W. J., & Danel, A. (2005). *Annals of the Polish Chemical Society*, *2*, 331.
[12] Cywiński, P., Sadowska, M., & Wandelt, B. (2005), *Zeszyty Naukowe – Fizyka*, *6/7*, 365.
[13] Connors, K. A. (1987). *Binding Constants: The Measurement of Molecular Complex Stability*, John Wiley & Sons Inc.: New York.
[14] Turkewitsch, P., Wandelt, B., Darling, G. D., & Powell, W. S. (1998). *Anal. Chem.*, *70*, 2025.