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Polymeric Sensory Systems Based on Molecular Imprinting for Identification and Separation of Molecules and Bigger Biological Objects

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Molecular imprinting of synthetic polymers based on noncovalent interactions play an important role in producing biomimetic receptors that challenge their natural model and also in producing modern drug assays. Formation of a prearranged complex in the presence of noncovalent interactions between the template and receptor is the main idea of processing. A resulting polymer binds the template with an appreciable selectivity over structurally related compounds. A potential use of the imprinted polymers is inconceivable. A molecularly imprinted polymer can be used as polymer particles and films; as a single recognition element and as multi-binding sites and assays. Examples of molecular imprinting in a thin polymeric film and on the polymer surface are presented.

Keywords: atomic force microscopy; fluorescence quenching; molecular imprinting of polymers; optical sensors; surface molecular imprinting

INTRODUCTION

Molecular imprinting is becoming a powerful technique for the preparation of synthetic polymers containing recognition sites for certain target molecules. It has become an effective method for encoding on

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a molecular scale the information on template molecule in bulk polymer and/or at the surface of the polymer matrix. The procedure of this technique involves incorporation of a template molecule to the polymerization mixture as it is schematically shown in Figure 1. The pre-assembly of the functional monomers around the template

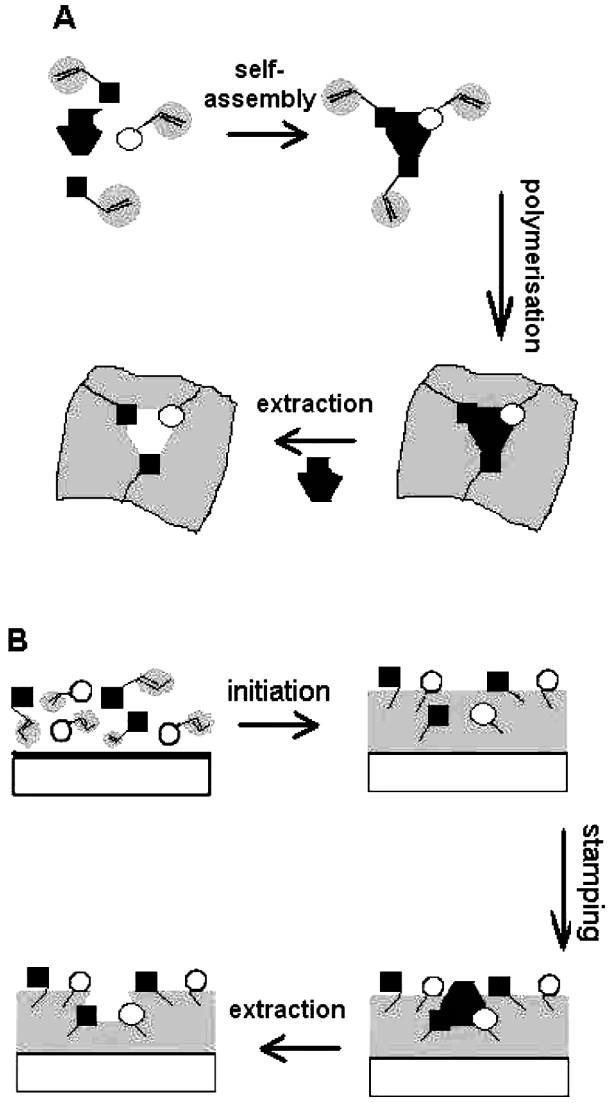


FIGURE 1 Schematic presentation of the molecular imprinting technology.

which is driven by their molecular interaction forces, leads to supramolecular structure formation which is then "freezing" by the co-polymerisation with the cross-linker. After the processing, the template molecules are removed from the rigid polymer. The copolymerization with template builds up a 3D functionalized molecular cavity in the polymeric network which is then able to recognize the template. The polymerization in bulk (see Figure 1A) produces monolithic membranes but also can be grounded to particles and can be used for filing of chromatographic columns [1–2]. The molecular imprinting on polymer surface as shown schematically in Figure 1B, may produce matrices with surface imprinted microorganisms for separation and identification in food and pharmaceutical industry [3].

In the copolymerisation involving a mono-functional monomer and/or multi-functional monomer, one of them being a receptor is strongly interacting with the template that, during the analytical process can transform the information on the event in the form of an electrical or optical signal. Due to an increasing number of samples that have to be measured immediately and automatically also in combination with screening and monitoring, there is an ever-increasing demand for high-performance of optical sensing systems. The use of optically active molecule as a functional monomer offers a possibility of noninvasive observations of the material response. We have developed the MIPs as an optical sensor of nucleotide-cAMP using as a functional monomer suitable derivative of styrylpiridine [4]. Although the experimental MIPs were very effective as a sensing system, the styrylpiridine derivative as a receptor and reporter group in one was not effective enough because of rather weak fluorescence emission. Obviously, looking for a new fluorescent receptor we are searching for a dye representing stable photophysical properties and strong fluorescence. The solution studies of fluorescence from the pyrazolequinoline derivative in the presence of nucleotides showed [5-6] that this dye, popular in nonlinear optics applications [7], could also be a useful dye for sensing. Our preliminary studies on the optical sensory system for nucleotides with the use of the pyrazolequinoline receptor: 1,3-diphenyl-6-vinyl-1H-pyranole-[3,4-b]-quinoline (PAQ) as a functional monomer incorporated into the polymerising mixture have proved its usefulness. The dye takes position inside the molecularly imprinted cavity to be important then in the analytical work. The dye-receptor interacting with the template, informs us on the event of adsorption by a change of fluorescence intensity. On the other hand, the presence of fluorosensor inside the imprinted cavity gives a possibility to follow not only the recognition event but also to interrogate the cavity internal structure.

The importance of cyclic nucleotides especially guanosine 3′,5′-cyclic monophosphate (cGMP) (the second messenger in a living cell) for human life, was the reason why we focused attention on polymeric sensors of the cyclic nucleotides. In this article a newly developed detection system for nucleotides [8] based on a linear polymer chain is discussed in comparison with the data obtained by the "classical" way of molecular imprinting during polymerization. The molecular imprinting on the polymer surface is also presented.

EXPERIMENTAL

Molecular Imprinting Based on a Commercially Available Linear Polymer

Among the variability of methodological solutions [9–12] we developed our own technique of molecular imprinting to obtain a fluorescent polymeric film as a sensing system for nucleotides [8]. Looking for an easy and inexpensive method for preparation of the MIPs we developed a methodology of molecular imprinting based on a linear polymer chain of polymethyl methacrylate (PMMA). The processing to obtain molecularly imprinted polymers (MIPs) adopted for these purposes is conceptually shown in Figure 2. This

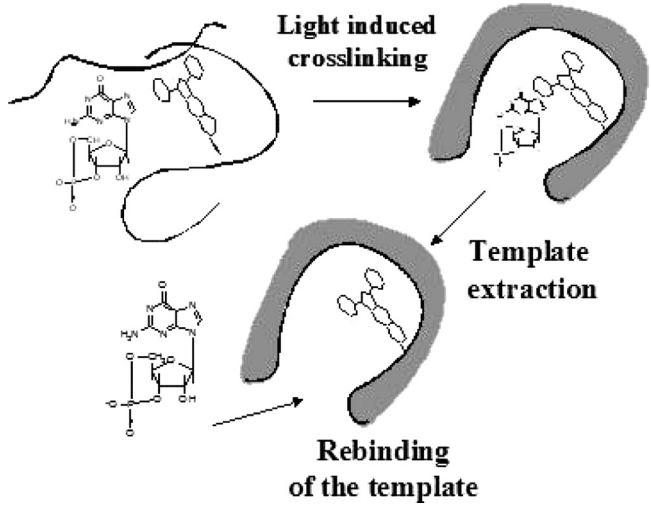


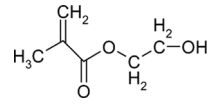
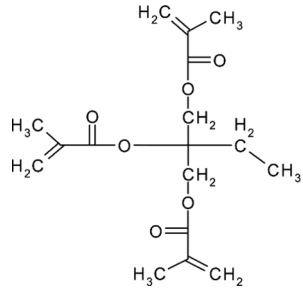
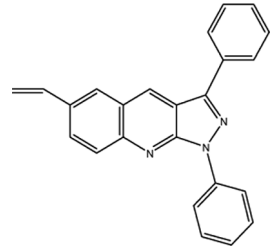
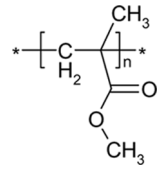
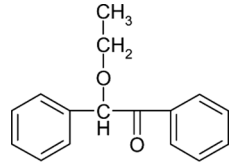
FIGURE 2 Schematic illustration of the MIPs preparation based on linear polymer chain (PMMA).

processing involves three steps: (1) complexation between a fluorescent functional monomer and a template, (2) light induced cross-linking of the polymer and the complex in presence of a photoinitiator, the complex being fixed by the 3D network and (3) extraction and readsorption and/or analytical work. The structures of compounds that were used in this approach are gathered in Table 1. Although, in general, the method of preparation was described in detail in a previous report [8] some details are needed to be depicted. A mixture of precursors, containing 10^{-4} M solution of PAQ in THF and 10^{-4} M solution of cGMP in DMSO 1:1 v/v was purged with argon for 0.5 h to eliminate oxygen. The mixture was stored in darkness overnight at room temperature to stabilize and to enable complexation. Then, the solution was mixed with 5% w/w PMMA dissolved in THF and all was left overnight to equilibrate, appropriate supramolecular structures to be formed in the mixture. Although PMMA dissolved slightly better in THF than in DMSO, we did not observe a noticeable inhomogeneity in the distribution of fluorescence from the film, that was shown previously [8]. The final mixture was cast on quartz plates using spin coating and/or drop casting methods. The polymer films were exposed to UV irradiation with a maximum at 350 nm. The films were subsequently stored in a vacuum oven for 24 hours at the pressure of 0.06 cm Hg at 60°C. The corresponding non-imprinted polymer (NIP) was prepared in the same way except that no template was added. After that the films were extracted in 10 cm^3 of deionized water until no change of fluorescence was observed, 24 hours of washing was enough. Subsequent incubation of the film in 10 cm^3 of aqueous solution of nucleotide for 24 hours provided re-adsorption of the template. The influence of the nucleotides adsorption on the MIPs fluorescence was studied by steady-state and time-correlated fluorescence microscopy. The same procedure was followed for the non-imprinted polymer.

Materials

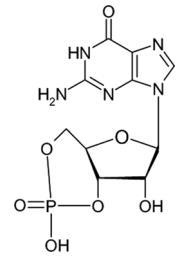
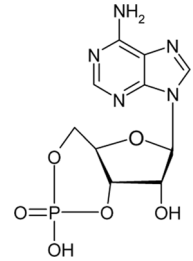
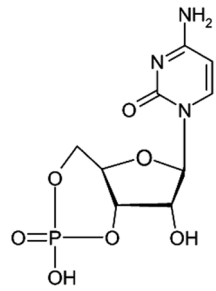
The polymer PMMA of 1×10^5 M_w was obtained from Solvent Dwory Sp z o.o. 1,3-diphenyl-6-vinyl-1H-pyranole-[3,4-b]-quinoline (PAQ) was prepared as described elsewhere [13]. The photoinitiator BEE and sodium salts of cyclic nucleotides: – adenosine 3′,5′-cyclic monophosphate (cAMP), guanosine 3′,5′-cyclic monophosphate (cGMP) and guanosine 5′-monophosphate disodium salt (GMP), cytidine 3′:5′ cyclic monophosphate (cCMP), guanine (2-amino-6-hydroxypurine). Tetrahydrofuran (THF) and dimethyl sulfoxide (DMSO) were purchased

TABLE 1 Components of Polymerizing Mixtures Involved in these Approaches

Monomers
2-Hydroxyethyl methacrylate (HEMA) 
Trimethylolpropane trimethacrylate (TRIM) 
1,3-diphenylo-6-vinyl-1H-pyrazole-[3,4-b]-quinoline (PAQ) 
polymethyl methacrylate (PMMA) 
Initiators
benzoin ethyl ether (BEE) 

(*Continued*)

TABLE 1 Continued

Template	
Guanosine 3′,5′-cyclic monophosphate (cGMP)	
Adenosine 3′,5′-cyclic monophosphate (cAMP)	
Cytidine 3′,5′-cyclic monophosphate (cCMP)	

from Sigma-Aldrich Co. and used as received. Aqueous solutions of nucleotides were maintained using double-distilled, deionized water.

Methods

The fluorescence signal from cGMP imprinted and non-imprinted polymer films was collected using the MicroTime 200 confocal lifetime microscope, from Picoquant GmbH (Berlin, Germany). The setup includes an inverted optical microscope (IX-71; Olympus, Japan). The sample holder was positioned on a XY piezo-scanning stage with a

scanning range of $80 \times 80\,\mu m$ and resolution of 1 nm. The images were obtained by scanning the sample over a 100X (Olympus) objective. The emitted photons were collected in epi-fluorescence mode, after passing appropriate filters, by a single photon avalanche diode (SPAD).

The sample was excited by a diode laser, 5 mW power, with an average emission in range 370–380 nm, maximum intensity at 375 nm, purchased from PicoQuant GmbH. The Microtime 200 software was used for system control, data acquisition and processing.

RESULTS AND DISCUSSION

The molecularly imprinted polymers were obtained with the procedure presented in Figure 2 as thin-layer films on quartz glass plates and then studied as it was. The films were transparent for visible light. In agreement with Figure 2, we considered the following steps in the analytical work: 1) the polymerization, 2) extraction, and 3) re-adsorption of the template. The fluorescence images were done at three steps. For comparison we show also the data of nonimprinted (blank) polymeric films subjected to the same procedure.

Figure 3 shows the integrated fluorescence emission from both cGMP imprinted polymer and from nonimprinted polymer after the three steps of processing of the MIPs: i) polymerization, ii) extraction, and iii) re-adsorption. We repeated the extraction and re-adsorption twice for the same film. Although there are some fluctuations of the fluorescence intensity between the first and second re-adsorption, we expect that the material is stable enough to be used repeatedly for longer. Figure 3 shows us also the intensity of fluorescence when the MIPs is incubated in the presence of other molecule than the template, nucleotides of different electronic structure, adenosine 3′,5′-cyclic monophosphate (cAMP) and cytidine 3′:5′ cyclic monophosphate (cCMP), the structures of these nucleotides are shown in Table 1. The selectivity factor of adsorption of cGMP imprinted polymer against the other nucleotides can be calculated from the data of fluorescence measurements. The selectivity factor was formulated as follow:

$$a_{Template/Quencher} = \frac{I_0 - I_{Template}}{I_0 - I_{Quencher}} \quad (1)$$

where I_0 and $I_{Template}$ and I_Q are respectively the fluorescence intensity after washing of the polymer and after incubation in presence of the template-cGMP and after incubation in presence of the other nucleotide or molecule which are quenching of the polymer

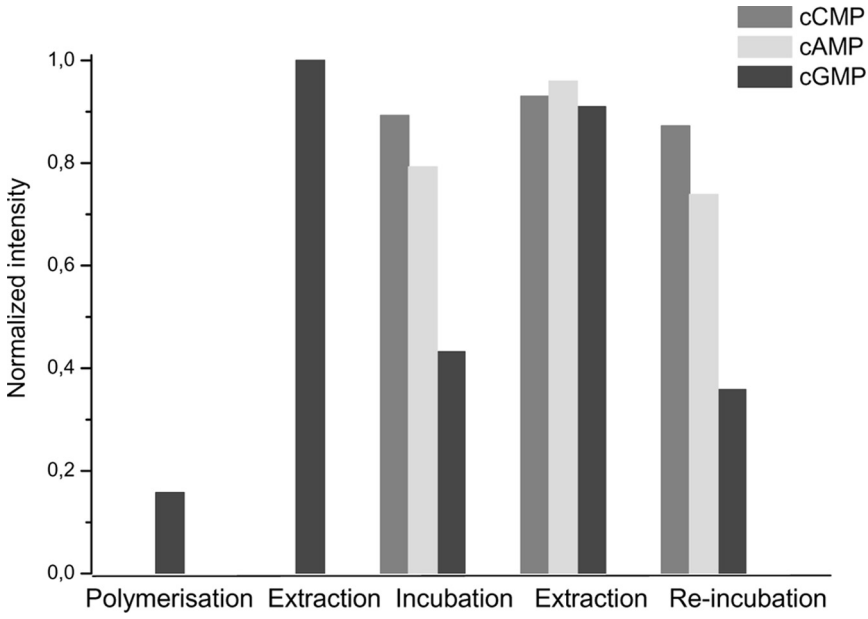


FIGURE 3 Histogram of fluorescence intensity of cGMP imprinted MIPs, obtained with use PMMA for three steps: polymerization, extraction and readsorption.

fluorescence. The selectivity factors are $a_{T/Q} = 2.5$ for adsorption of cAMP and $a_{T/Q} = 5.1$ for cCMP. We can compare the histograms of fluorescence intensities, shown in Figure 3 with the data in Figure 4, which are obtained for MIPs copolymerized classically. The classical MIPs were polymerized from the mixture of functional monomers: HEMA, TRIM, benzoinethyl ether-initiator and PAQ – fluorescent receptor and the template-nucleotide cGMP, 1.5, 15, 7.5, 38, 38 of volume fraction respectively, structures of the components are gathered in Table 1.

At first sight the histogram in Figure 4 matches the fluorescence intensities of MIPs prepared with linear polymer PMMA. The obtained selectivity factor for "classically" polymerized MIPs and then incubated with cAMP $a_{T/Q} = 2.4$ and similarly for the "classical" MIPs incubated with cCMP $a_{T/Q} = 4.1$. These parameters are slightly lower than for the MIPs obtained from PMMA. Although the reasons of the differences are unknown, one can note that the "classical" procedure introduces better fitting of the functional groups of the monomers with template. The high molecular weight of the linear polymer leads to a decrease of mobility of the functional

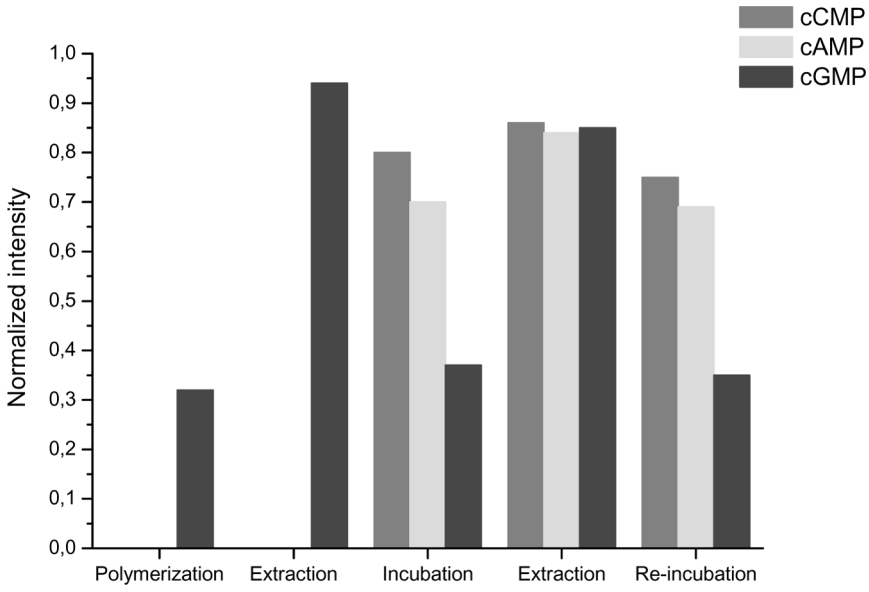


FIGURE 4 Histogram of fluorescence intensity of cGMP imprinted MIPs obtained by "classical" copolymerisation of following mixture of functional monomers:

groups immobilized at the polymer chain and also decreases the quality of the fit.

Molecular Imprinting on the Polymer Surface

For recognition and separation of bigger biological objects surface molecular imprinting of polymers (SMIPs) can be used. Surface molecular imprinting of polymers (SMIPs) is shown conceptually in Figure 1B. Additionally, the stamp technique was used to get the effect of molecular imprinting on the surfaces [14]. The idea of stamping technique on the polymer surface is shown in Figure 5. The polymerizing mixture was composed of TRIM, HEMA, initiator-benzoin ethyl ether and solvent THF, 10, 2, 1, 10 of volume fraction respectively. The thin-layer of liquid pre-polymerizing mixture was obtained by pouring of the mixture on microscopy glass using rotating base. The polymerization was initiated by a xenon lamp ($\lambda = 350\,\text{nm}$); 10 sec of irradiation in the inert gas atmosphere (argon) is sufficient to obtain an accurate pre-polymerized thin film. The thin-layer film was illuminated from the bottom and the glass was transparent for the light.

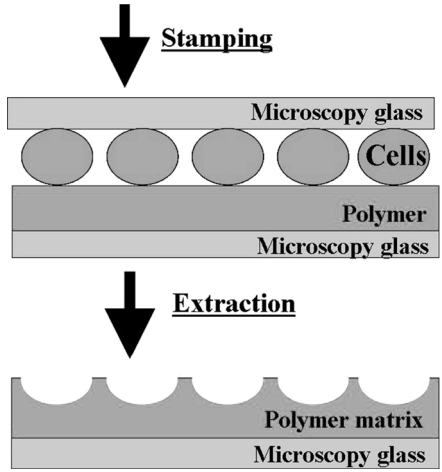


FIGURE 5 Stamping technique.

Then the stamp of biological material was used. An example of the stamp from yeast cells is shown in Figure 6. Then, the stamp and the film together are left at room temperature for 24 hrs. After the polymerization, the film was washed with water to remove the micro-organisms-template. The polymer surface studies were performed using two techniques: optical microscopy – Nicon and atomic force microscopy (AFM) with Solver Pro, NT-MDT in resonant mode. Figure 7 shows the image of polymer surface after imprinting with yeast cells of Madeira species. For comparison, Figure 8 shows an

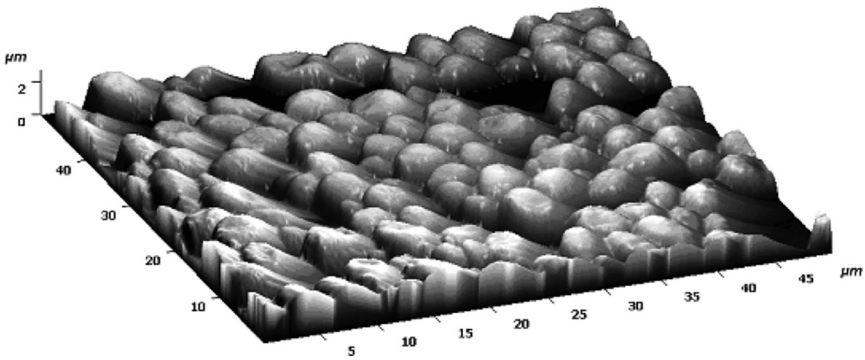


FIGURE 6 3D AFM image of stamp with yeast cells of Madeira species.

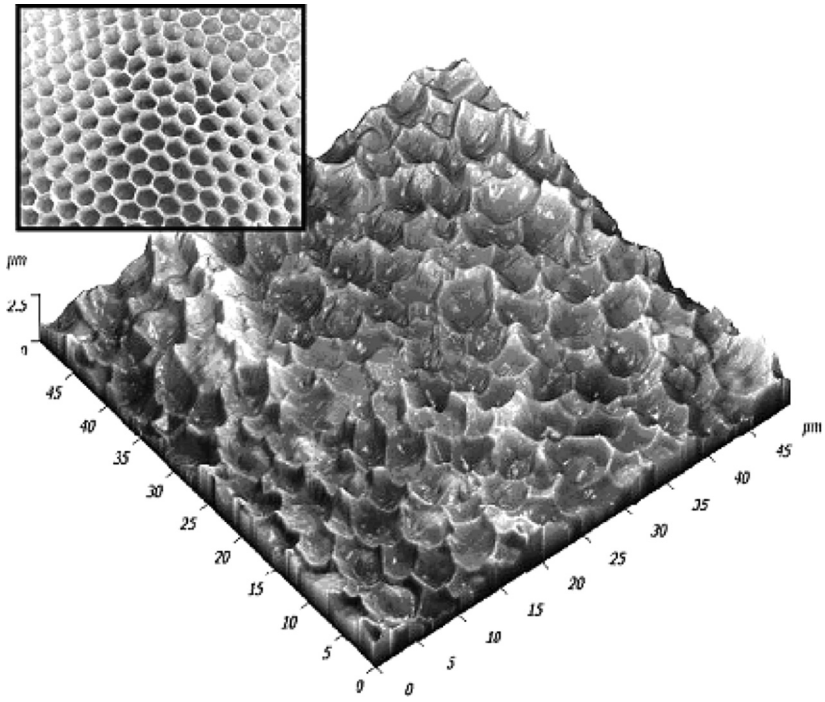


FIGURE 7 3D AFM image of polymer surface imprinted with yeasts Saccharomyces cerevisiae Madeira species. The insert is honeycomb.

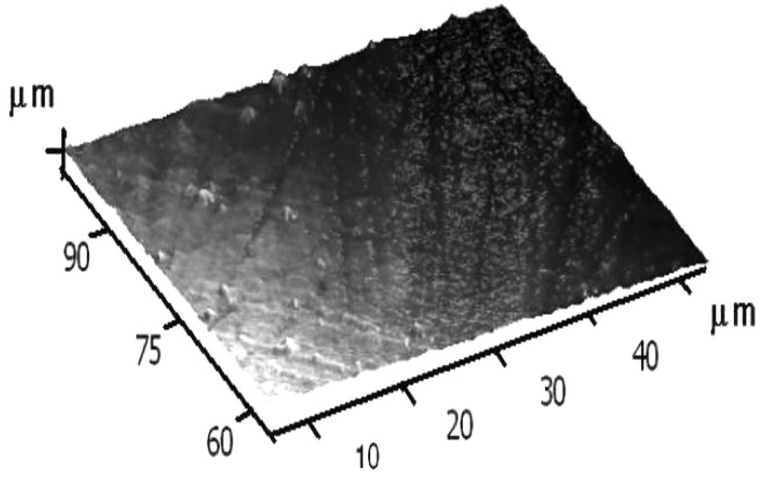


FIGURE 8 3D AFM image of non-imprinted polymer surface.

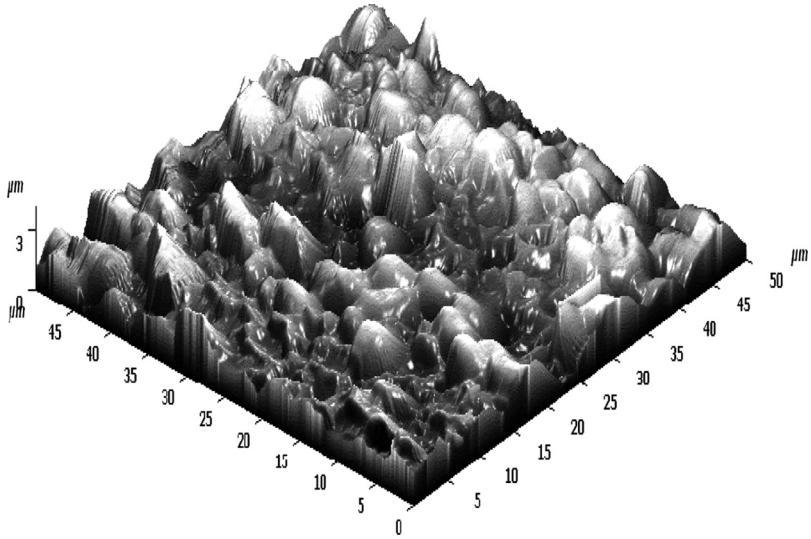


FIGURE 9 3D AFM image of the imprinted surface with yeast cells of Madeira species after re-adsorption.

image of the non-imprinted surface of polymer in the same scale, obtained with the same procedure except of the template.

The effectiveness and selectivity of re-adsorption of the cells at the surface of the film was studied after incubation of the film in aqueous suspension of particular cells for 30 min. In Figure 9 we

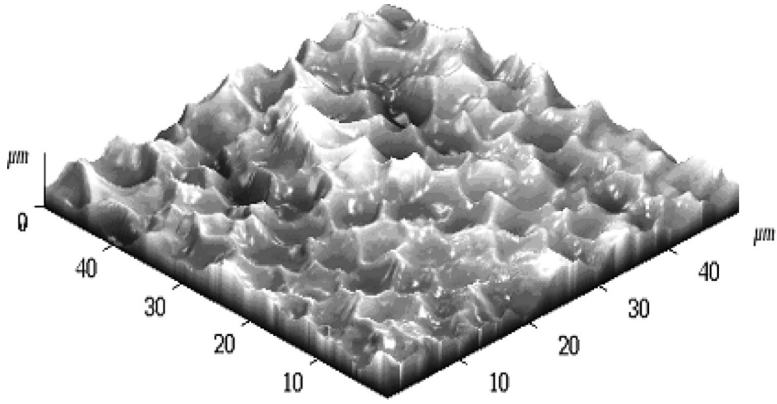


FIGURE 10 3D AFM image of polymer matrix imprinted with cells of Madeira species after incubation with cells of Syrena species.

can see the polymer surface imprinted with yeast cells of Madeira species after re-adsorption of the cells of Madeira species. For comparison, we can see the same imprinted polymer surface after incubation in the aqueous suspension of yeast cells of Syrena species in Figure 10.

CONCLUSIONS

The molecular imprinting based on linear polymers is a very convenient method and this type of sensor can be easily prepared in any laboratory. Low cost of the materials for preparation increases availability of this technology for small laboratories in food industry.

The molecularly imprinted polymer surface has the ability of selective adsorption of the same biological objects as template. These properties of selective adsorption of the SMIPs can be applied for identification of microorganisms. Bigger molecules like peptides and biological objects like microorganisms can be easily identified with the use of optical microscopy and/or AFM technique.

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