Application of nanostructural modification of polymer surface by molecular imprinting with proteins towards increased selective adsorption

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Introduction

Because globalization and growing needs to control impurities and toxicity, fast and effective measurements in pharmaceutical and food industry, great increase of new analytical methods are observed. The needs of continuous control and monitoring with high precision of many industrial processes stimulated of development of new technologies based on molecular imprinting of polymers (MIPs). The literature showing that during the last decade especially the technology of molecular imprinting of polymers in mass and in thin layer films has been developed [1÷3].

The sensing materials that were synthesized based on molecular imprinting up today are able to identify number of chemical compounds and drags. This type of sensing materials were synthesized by us during the last 15 years and also presented in this journal [3]. Development of new analytical methods for medical diagnosis caused interest in molecular imprinting with biological objects like proteins and microorganisms. Because of great potential applicability of the biosensing materials it is important for the specifically binding sites to be accessible for the biological objects. For these reason the surface molecular imprinting of polymers (SMIP's) appeared to be more useful. The sensing materials together with fluorescence microscopy technique give opportunity to see the molecules, to follow dynamics of the processes that the molecules access, to observe the distribution of the molecules and to calculate the concentration. The fluorescence microscopy showing the processes that the biological materials undergo noninvasively [4]. The studies with use of the fluorescence microscopy may be done with resolution of the microscopy.

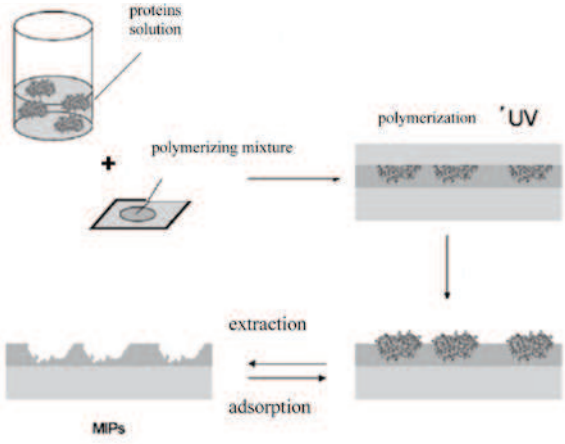


Fig. 1. Schematic of surface molecular imprinting of polymers

Technology of molecular imprinting at the polymeric surface is shown schematically in Figure 1. The technology SMIPs was presented in this journal previously [3]. The template could be the atom, ion, molecule, complex but also the macromolecule like peptide and microorganism. After removing of the template from the crosslinked polymer there is left empty site that is able to absorb the molecule like template. The template has the preference to be adsorbed in comparison to others present in the environment. The preferentiality is because of shape and size but mostly because of the functional groups distribution at the binding sites. Technology of molecular imprinting started by Wulff [5] appeared to be useful in pharmaceutical industry for purification and separation of compounds [1, 6÷7]. Especially when the pharmaceutical molecule has two enantiomeric forms it can be that one of the form positively affect the organism but the other not, as it was in case of talidomid. Actually the enantiomeric compounds can be separated with use of the MIPs [6]. The ability of MIPs for selective separation of molecules make them very attractive and the technology useful. MIPs can be useful in the sensors recognizing pathogenic microorganisms and toxins in living organisms and in environment. Molecularly imprinted polymers may increase the new medicines development, because they help in purification and separation of the new pharmaceutics being very effective as a solid phase in chromatography. The MIPs can work as solid phase particles with imprinted recognition sites of the medicines as well as the substrates or semi-products [1, 6÷8].

Together with increase of knowledge in biochemistry of living processes there is increase of new diagnostics tests based on MIPs to measure some particles and/or molecules concentration. Usually they are cheaper and much faster than the traditional methods of measurements. Molecular imprinting of bigger biological objects like peptides and microorganisms is from the practical point of view, due to molecular imprinting at the surface of polymers [9]. We have discussed this technology previously [3]. The nanostructural modification of polymer surface noticeably changes the properties of the polymer surface in comparison to unmodified surface. This modification leading to enhanced adhesion of the molecules and microorganisms that were template during the imprinting process [10]. The molecular interactions at the interface between the biological system and the polymer over the processing leading to increase of number of the specific binding sites. The molecular imprinting at the surface of polymers is due to use of stamping technique. That we were using previously for molecular imprinting of the microorganisms [3].

The stamping technique was used here for molecular imprinting of peptides. In this study we have combined the method with a micro-contact imprinting approach reported for slightly bigger pentameric protein [11]. Micro-contact approach to protein imprinting uses as a monolayer only a minimal mass of protein. Micro-contact imprinting has advantages in that little or no template remains 'trapped' under the polymer film's surface by completion of polymerization. We report here our approach to obtain of molecular imprinting at polymer surface of proteins like albumin group: α-lactalbumin and ovalbumin.

The albumins are rather low molecular mass peptides, well soluble in water and salt solutions in range of pH from 4 to 8.5. The albumins group includes α-lactalbumin, ovalbumin and albumins of plants. The albumins are main component (60%) of plasma proteins, α-lactalbumin is a component of mammals milk, molecular mass is 14 178 Da, ovalbumin molecular mass is 44 000 Da. The noticeable difference of molecular mass and as a consequence the difference in size of α - laktalbumin and owoalbumin, and similarities of the structure prompted us to choose the proteins as a biological material in the studies on molecular imprinting of proteins at polymeric surface and selectivity of adsorption of the imprinted sites.

Adsorption of proteins at polymeric surface, modified and unmodified, was studied using fluorescence microscopy of Nikon Eclipsse 2000U with CCD camera and computer system with related program for image processing.

Staining of the proteins by fluorophore and then incubation of the MIPs in presence of the proteins gave possibility for observation of the polymer surface. The proteins have been stained by styrylpyridine (dimethylaminstyrylpyridine) fluorophore, suggested for proteins by the „Molecular Probes" [4]. Additionally we were studied the fluorophore photo physical properties and interactions with the proteins extensively [12].

The proteins molecules are adsorbed at the MIPs surface by interaction with functionalized binding sites that were imprinted there. The strong interactions and enhanced adhesion of the protein which was a template during processing leading to selective adsorption at the modified polymeric surface.

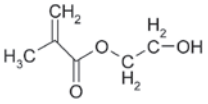
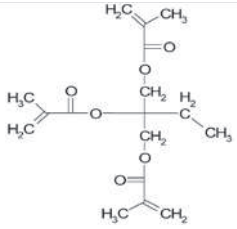
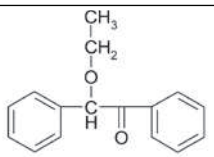
The system of surface modified polymer after incubation with the protein, which was stained by the fluorophore, excited by light of wavelength characteristic for the marker of the protein is fluorescent. The intensity of the fluorescence depends on concentration of the proteins adsorbed at the modified polymeric surface, the MIPs. The fluorescence is measured with the CCD camera and analytical program. We will present here the studies of adsorption of the two proteins: α-lactalbumin and ovalbumin at polymeric surface modified by molecular imprinting. The selectivity of proteins adsorption at the surface of the sensing material will be discussed, also specific interactions of protein with polymeric surface. The specific binding sites formed during of imprinting processing are effective in interaction with the template molecule and not with the others.

Preparation of the polymerizing mixture and polymeric films

Polymeric matrices used for analytical studies of proteins adsorption were prepared using methacrylate monomers TRIM and HEMA, tetrahydrofuran as a solvent and bensoin ethyl ether as initiator of polymerization were used. The chemical structure of the substrates is shown in Table 1. To the film preparation, about 60 μl of the polymerizing mixture was poured onto microscopy glass plate. The plate was then irradiated using UV lamp emitting of broad spectrum with maximum wavelength at 350 nm. The copolymerization was in atmosphere of inert gas (argon) during 20 sec.

Table 1

Precursors of polymerization used to MIPs preparation

monomers	
2-hydroxyethyl methacrylate (**HEMA**)	
trimethylolpropane trimethacrylate (**TRIM**)	
photoinitiator	
benzoin ethyl ether (**BEE**)	
template&analyt	
α - laktoalbumina	14 176 Da
owalbumina	44 000 Da

Preparation of protein stamp (template)

Matrices were imprinted with two proteins: ovalbumin and α-lactalbumin. The aqueous protein solutions of concentrations of 0,03% by weight were prepared. Such low concentration of proteins was chosen in order to obtain homogeneous and single-layer surface. The stamp/template was made by pouring of the solutions, in amount 200 μl onto microscopy cover glass and left for 24 hours at room temperature to dry.

Preparation of surface molecularly imprinted polymers

After irradiation of the film during 20 sec, the film was covered by the stamp and left for 24 hours in the inert gas atmosphere at room temperature. Then the template protein was extracted by washing in water. Polymer control sample, non-imprinted, was prepared with the same procedure except none template was used during of the processing.

Adsorption of proteins at the modified polymer surface

We examined adsorption of template or competitor protein to test the molecular imprinting of protein at the polymeric surface and how this modification of the surface affect properties like adsorption and extraction of the protein. Two proteins from the albumin group were applied, both are similar structure and soluble in water, differing in molecular mass and size; α-lactalbumin of molecular mass 14 178 Da and ovalbumin of molecular mass 44 000 Da. To investigate the binding of proteins to an imprinted film and to a control polymer made without a protein template we incubated the films in solution as follow:

- water solution of the protein of concentration 0,03% by weight
- aqueous solution of the fluorophore-marker of the proteins of 10^{-3}M concentration has been prepared. For doing the incubation we mixed the solutions in amount 1:1. The films were incubated with mild agitation, after which they were washed to remove unbound proteins.

The glass cover slip together with the film was placed at the microscopy table and the fluorescence intensity was measured. The microscopy worked under the same parameters during the all experiments. The intensity was measured from surface of the film 30×30 μm². Analyzing of adsorption and extraction kinetic the procedure of measurement of fluorescence intensity was repeated every two minutes. Figure 2 showing dependence of fluorescence intensity during adsorption of α - lactalbumin after incubation and measured from the polymer film that surface was modified with α-lactalbumin.

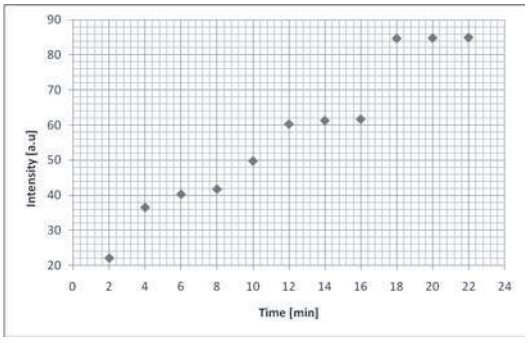


Fig. 2. Readsorption of α- laktoalbumin at the polimer surface modified with α- laktalbumin

Similarly Figure 3 showing dependence on time of intensity during adsorption of ovalbumin and measured from the polymer surface imprinted with ovalbumin.

The results of these rebinding experiments shown that adsorption of α-lactalbumin from the environment was completed after 15 min but of ovalbumin was after 20 min. After the rebinding the film was washed with distilled water and the measurements were done also every two minutes. Figures 4 and 5 are showing the dependences of extraction on

time. The results of these rebinding and extraction experiments shown that both processes are affected by the molecular mass of protein but there is additionally dependence on interactions between the biological material and the polymer.

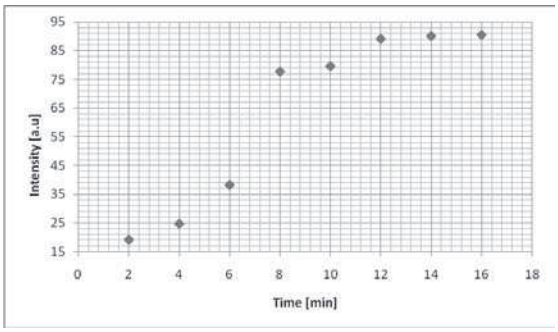


Fig. 3. Readsorption of ovalbumin at the polymer surface modified with ovalbumin

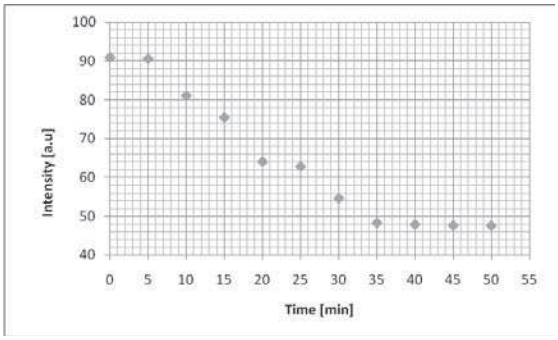


Fig. 4. Extraction of the polymer film modified with α- lactalbumin

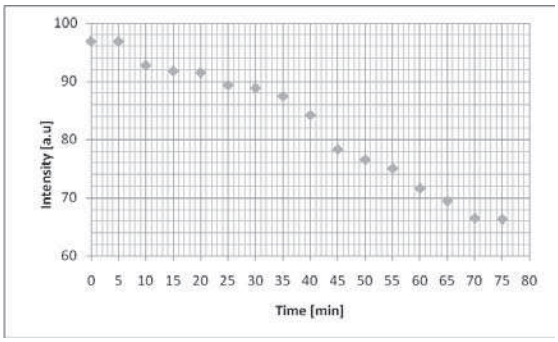


Fig. 5. Extraction of polymer which was modified with ovalbumin

Selectivity of protein adsorption and specific binding sites

To show that the rebinding of the imprinted polymer was a result of selectivity and specificity which coming out from molecular imprinting method, we decided to apply the imprinting of two proteins from group of albumin differing with molecular mass: α-lactalbumin and ovalbumin. The time of polymerization during the imprinting with template was established to be 30 min. for the both protein.

Figure 6 shows histogram of intensity of fluorescence emission from polymer surface imprinted during copolymerization in presence of a template/ovalbumin and incubated in presence of A) ovalbumin and B) α-lactalbumin. Intensity of fluorescence which is measuring the step of adsorption of protein suggest lower adsorption of α-lactalbumin. The adsorption of ovalbumin is stronger by about 35%. These experiments demonstrate the selectivity in rebinding of ovalbumin to polymer imprinted with the same protein. The experiment of rebinding of the polymer film was done three times with the same protocol. The protein was extracted and were then again rebound with almost the same result.

That indicate that the imprinting method employed here is able to generate high stability materials exhibiting properties of recognition and selectivity in adsorption.

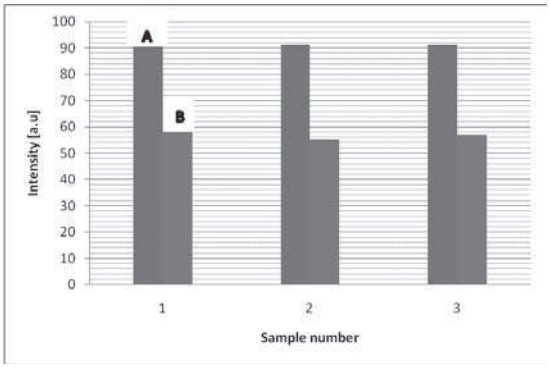


Fig. 6. Histogram of fluorescence intensity from the polymeric surface modified with ovalbumin after incubation in presence of: A) ovalbumin, B) α-lactalbumin

Conclusions

Surface molecularly imprinted polymers (SMIPs) are characterized by specificity of interactions with protein/template. This material selectively adsorb the protein that was used as a template during imprinting. The material of the polymeric sensing system is stable. The experiment of rebinding was repeated three times and the results shown in Figure 6 suggest good reproducibility. To be useful, for example as a sensor, the selective recognition shown by the imprinted polymer when challenged with single proteins must be maintained in the presence of a competitor protein. As approach to this situation we used the same procedure as was used for template ovalbumin rebinding to test competitor protein α-lactalbumin. The results of this study showed a significant difference in binding for the template and the competitor (Fig.6) even that the last was much lower in size.

Films with unmodified surface showing very low adsorption of any protein after incubation.

Literature

1. Allender C.J., Richardson C., Woodhouse B., Heard C.M., Brain K.R.: International Journal of Pharmaceutics 2000, **195**, 39-43.
2. Nicholls I.A., Rosengren J.P.: Bioseparation 2002, **10**, 301-305.
3. A) Wandelt B., Cywiński P., Sadowska M., Hachulka K.: Chemik 2007, **2,** 115-119, B) Wandelt B., Sadowska M., Hachulka K.: Chemik 2008, **2,** 65-69.
4. Haugland R.P.: *Handbook of Fluorescent Probes and Research Products, Molecular Probes* 2002.
5. Wulff G., Knorr K.: Bioseparation 2002, **10**, 257 – 276.
6. Glad M., Reinholdsson P., Mosbach K.: Reactive polymers 1995, **25**, 47-54.
7. Sellergren B., Allender Ch,J.: Advanced Drug Delivery Reviews 2005, **57**, 1733-1741.
8. Ye L., Haupt K., *Anal. Bioanal. Chem.* 2004, **378,** 1887.
9. Dickert F.L., Hayden O,. Lieberzeit P.: *Synthetic Metals* 2003, **138**, 65.
10. Hachulka K., Lekka M., Okrajni J., Ambroziak W., Wandelt B.: *Biosensors and Bioelectronics* 2010, **26**, 50–54.
11. Chou,P-C J. Rick, T-C: Chou *Analytica Chimica Acta* 2005, **542**, 20–25.
12. Wandelt B., Cywiński P., Darling G.D., Stranix B.R.: Biosensors & Bioelectronics 2005,**20**, 1728-1736.

English translation by the Authors

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