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Mesoporous polymer network produced from *N*-vinylpyrrolidone and triethylene glycol dimethacrylate as potential macromolecularly imprinted material and oligopeptide carrier

Natalia V. Fadeeva,* Evgenia I. Knerelman, Galina I. Davydova, Nina S. Emel'yanova and Svetlana V. Kurmaz

Institute of Problems of Chemical Physics, Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russian Federation. Fax: +7 496 522 3507; e-mail: natali-vi@inbox.ru

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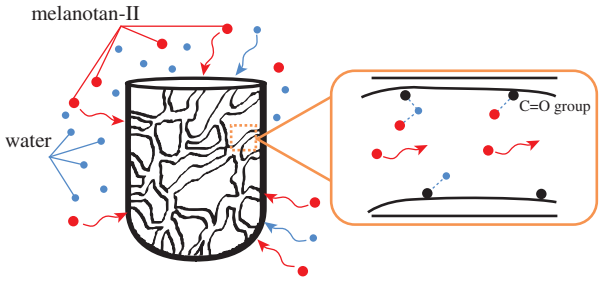
An original approach is proposed for creating the molecularly imprinted polymer and the carrier of protein molecules. Specifically, a thermally and chemically stable branched *N*-vinylpyrrolidone copolymer was used as the model of an imprint biomolecule and the porogen in the three-dimensional radical copolymerization of *N*-vinylpyrrolidone and triethylene glycol dimethacrylate. Removal of the branched copolymer left cavities available for binding biomolecules to the copolymer matrix due to its mesoporous structure.



Mesoporous polymer networks

Keywords: *N*-vinylpyrrolidone, molecularly imprinted polymers, carrier, oligopeptide, mesoporous polymer network, non-covalent biomolecular imprinting, melanotan II, adsorption.

One of the current trends in polymer chemistry is the creation of molecularly imprinted polymers (MIPs) capable of repeatedly recognizing imprint molecules or those related to them in structure, shape and size.[1] However, researchers are faced with the problems, such as preserving the native structure of imprint biomolecule, the formation of clear imprint and the availability of binding sites during the synthesis of polymer matrix by the method of non-covalent biomolecular imprinting. To overcome these difficulties, the model compounds, that are similar to the biopolymer in chemical nature, size, *etc.*, and stable during polymerization, are used as imprint macromolecules. The problem of access to the binding site is solved by the obtaining of porous matrices.[2] In addition to the complicated synthesis of MIPs for biomolecules, it is necessary to solve the problems associated with their application, namely delivery to the body, mitigation of adverse effects,[3–5] prolonged release, *etc.*[6–8] In this regard, the actual task is to develop a polymer matrix with adjustable porosity and functionality, which is capable of not only selectively isolating MIPs from solutions, but also delivering them.

The aim of this work was to study the prospects of the copolymer produced from *N*-vinylpyrrolidone (VP) and triethylene glycol dimethacrylate (TEGDM) as MIP and a carrier of protein molecules.

For this purpose, a synthetic analogue of the α-melanocyte-stimulating hormone, melanotan II (MTII), with the molecular weight of ~1 kDa, consisting of the amino acid sequence Ac–Nle–cyclo[Asp–His–D-Phe–Arg–Trp–Lys]–NH_2, was used. It is involved in the regulation of physiological processes along with others.[9–12] In particular, it affects skin pigmentation,[13–16] modulation of immune responses,[17,18] eating behavior,[19–21] energy homeostasis,[22] including blood glucose level,[20,23,24] *etc.*[25,26]

The optimized geometry of the MTII molecule (Figure 1) was obtained by quantum chemical calculations using the TPSSh/6-31G* method within the Gaussian 09 software package (see Online Supplementary Materials). It is seen that the intramolecular hydrogen bond exists between the oxygen atom of the C=O group and the hydrogen atom forming the N–H bond, and the molecule has cyclic structure. Moreover, the MTII structure contains many different functional groups, *e.g.*, C=O, N–H and NH_2, that can participate in non-covalent binding to the free and/or hydrated lactam C=O groups in the VP units forming a copolymer network.

Polymer matrix was prepared by the method of non-covalent macromolecular imprinting.[27] The three-dimensional radical bulk copolymerization of VP and TEGDM was carried out in the presence of the soluble thermodynamically compatible branched copolymer[28] which was removed thereafter using a 'good' solvent (Scheme S1, see Online Supplementary Materials). The branched copolymer was synthesized by the radical copolymerization of VP and TEGDM in toluene in the presence of 1-decanethiol as a

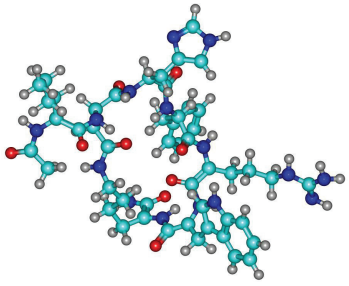


Figure 1 The optimized geometry of the MTII molecule.

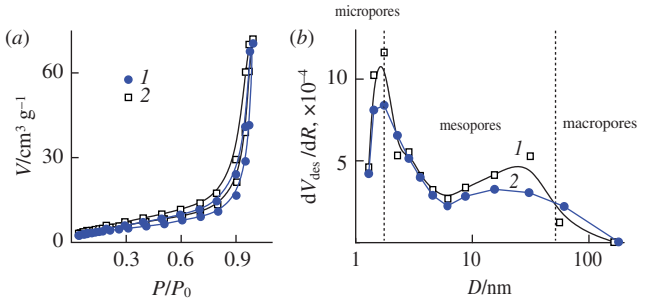


Figure 2 (*a*) Adsorption–desorption isotherms and (*b*) pore size distribution curves for polymer matrix I after (*1*) extraction of the branched copolymer with isopropyl alcohol and (*2*) water treatment.

chain transfer agent (Scheme S2) to avoid the formation of macrogel. The physico-chemical characteristics of the branched VP–TEGDM copolymer were determined (Table S1, see Online Supplementary Materials). Individual macromolecules, having a spherical particle shape and a diameter of about 4–10 nm according to TEM analysis (Figure S1, see Online Supplementary Materials) and DLS measurements (Figure S2), can be potentially used to produce traces of imprint macromolecules, whereas their nanoscale aggregates can be considered as the blanks of nanopores. Moreover, according to IR spectroscopy, the polar VP monomer molecules bind to the hydrated copolymer[29] to form the H-complex, that is the necessary condition for three-dimensional non-covalent macromolecular imprinting, *viz.* the formation of prepolymerization complex occurs.[2] After extraction of the branched copolymer with isopropyl alcohol, mesopores (2–50 nm) and imprint traces with the size close to the diameter of the single macromolecule remained in the monolithic (block) polymer matrix. Thus, the protocol for preparation of the polymer matrix formally met the criteria for creation of MIPs. In addition, we previously established their capability of sorbing PVP selectively, which was considered not only as a model of imprint macromolecules due to their close structure, chemical nature and size,[30,31] but also as a model of natural protein.[32]

To study the sorption of MTII, polymer matrix I was used that contained open mesopores and was characterized by the specific surface area $S_{sp} = 20$ m^2 g^{-1} and the pore volume $V_p = 0.11$ cm^3 g^{-1} (Figure 2, curve *1*). Polymer matrix II, prepared in the absence of the branched copolymer, was employed for comparison. Both polymer matrices were kept in water at room temperature for 10 days and dried in air to a constant weight. After the water treatment, the values of S_{sp} and V_p for polymer matrix I were 17 m^2 g^{-1} and 0.11 cm^3 g^{-1}, respectively. The S_{sp} value for matrix II was less than 1 m^2 g^{-1} and pores were practically absent.

The electron absorption spectroscopy was used to study the sorption of MTII by copolymer matrices I and II. The absorption spectrum of MTII in aqueous solution has an absorption band at the wavelength of ~280 nm (Figure S3), which is the superposition of individual absorption bands for functional groups in various amino acid residues. During the experiment, the shape and structure of this band did not change for 24 days, which indicated the oligopeptide stability in aqueous solution. The volume and diameter of an MTII molecule, calculated from the Chemcraft data, are ~4.5 nm^3 and ~2 nm, respectively. Therefore, the MTII molecules together with those of a solvent easily diffuse into the pores of polymer matrix I.

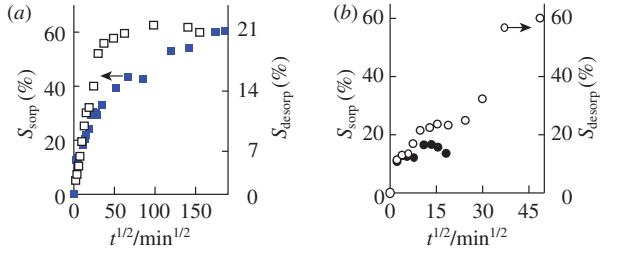


Figure 3 Time dependence of MTII sorption–desorption by (*a*) polymer matrix I and (*b*) polymer matrix II at 25 °C.

The dependence of MTII absorption by polymer matrices I and II on time is displayed in Figure 3 using the Fick equation coordinates. It was found that matrix I absorbs significantly more MTII (S_{max} = 60%) than matrix II, for which this value is only ~15%. Obviously, the reason is its porous structure, which makes the sorption centers, *viz.* the hydrated or free lactam C=O groups in the VP units, accessible for MTII binding. The sorption is stepwise in both the cases. This is due to the slow swelling of the copolymer matrices and the increase in diffusion of the MTII aqueous solution. Thus, copolymer matrix I binds the oligopeptide molecules, which indicates its potential as a MIP and a carrier of protein molecules.[2]

The MTII desorption [see Figure 3(*b*)] from polymer matrix II proceeded quickly and was stepwise. At the initial stage, about 22% of MTII was washed out, then the desorption rate increased again, and the desorption value reached ~57%. Meanwhile, the MTII desorption from polymer matrix I proceeded more slowly and the value of limiting desorption was only ~20%, despite the presence of pores in it, *i.e.*, there was MTII prolonged release from MIP. This is obviously due to the binding of MTII molecules to functional groups of the polymer matrix and the formation of H-complexes of different energy and strength.

Taking into account the new experimental data on the capability of copolymer matrix I to absorb the low molecular weight peptide from the aqueous solution and to release it slowly, we can conclude that nanoporous VP–TEGDM copolymer is promising as a MIP and a carrier of biomolecules, for example, globular peptides less than 10 nm in size.

The originality of the proposed approach is based on the fact that the template forming the imprint in the polymer matrix is not the biomacromolecule itself, but its model, which is a thermally and chemically stable branched copolymer. Removal of the branched copolymer forms cavities accessible for binding to the copolymer matrix due to its nanoporous structure. This approach can be universal for creating MIPs and carriers of protein molecules of various sizes, when choosing an appropriate model of the imprint macromolecule.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2020.11.016.

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