

# Molecularly Imprinted Polymer for Serotonin Recognition

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## Abstract

Developing of new techniques for detecting important biologic molecules are one of the aims of molecular imprinting. Serotonin (5-hydroxytryptamine) is a biogenic amine that acts as a neurotransmitter in the central and peripheral nervous systems. It is present in a variety of organisms, ranging from humans to species such as worms that have primitive nervous systems, and mediates a variety of physiological responses in distinct cell types. Serotonin is one of the indolic compound which has a clinical importance. Serotonin imprinted polymers were synthesized by using different functional monomers. Best results were taken with 2-(trifluoromethyl) acrylic acid and methacrylamide. The binding capacities in different solvents were evaluated. The specificity of polymer was tested by real blood sample.

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## INTRODUCTION

Serotonin (5-hydroxytryptamine) is a biogenic amine that acts as a neurotransmitter in the central and peripheral nervous systems [1]. It is present in a variety of organisms, ranging from humans to species such as worms that have primitive nervous systems [2], and mediates a variety of physiological responses in distinct cell types. It is believed to play a role in the regulation of various cognitive and behavioral functions, including sleep, mood, pain, depression, anxiety, aggression, and learning [3-5].

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Disruptions in serotonergic systems are implicated as critical factors in mental disorders such as schizophrenia, depression, infantile autism, and obsessive compulsive disorder [6-7]. In clinical chemistry of serotonin, focus is also on the diagnosis and follow-up of carcinoid tumors. Platelet serotonin may be the most sensitive indole marker for the detection of carcinoid tumors that secrete only small amounts of serotonin [8].

Therefore, detection of serotonin in biological samples is an important task for analytical chemistry. Chemically, serotonin is an indolic compound that is synthesized from the essential amino acid tryptophan. A wide variety of analytical methods has been used for the qualitative and quantitative determination of serotonin in blood. For example, fluorometric methods, enzyme immuno-

assay, gas chromatography, gas chromatography-mass spectrometry, and high-performance liquid chromatography (HPLC) with ultraviolet, fluorometric, electrochemical detectors are well adapted techniques for the measurement of either a specific indole or a group of indoles. Concerning the determination of serotonin, other indolic compounds are often interferences [9-18]. The use of specific binders for serotonin in a first extraction step before HPLC may allow to circumvent this problem. One type of (synthetic) selective binders is molecularly imprinted polymers (MIPs). These are tailor-made materials with selective binding sites for a target molecule, similar to the antigen binding site of an antibody. The principle of the molecular imprinting is: target molecules (template) and functional monomers are polymerized with a cross-linking reagent. After removal of the template, the functional groups in the resulting binding sites should be arranged in suitable positions for interaction with the template molecule [19]. MIPs have been employed in a wide area, such as liquid chromatography, solid-phase extraction, membranes, sensors, artificial antibodies, catalysis, biotransformation process and diagnostic tools for drug assays [20-21].

Here, we report the development of a new MIP for serotonin, for use in solid-phase extraction of serotonin from whole blood.

## MATERIALS AND METHODS

### Materials

2-(Trifluoromethyl)acrylic acid (TFMAA), methacryl-

amide (MetAM), methacrylic acid (MAA), itaconic acid (IAA), 2-hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), DMSO, methanol and L-tryptophan methyl ester hydrochloride were from Sigma. 2,2-azobis-(2,4-dimethylvaleronitrile) (ABDV) was from Wako. Serotonin hydrochloride was purchased from Molekula Ltd.

### Preparation of imprinted polymers

The imprinted polymers were prepared as shown in Table 1. The reaction mixtures were purged with nitrogen for 5 min and thermally polymerized at 45°C for 24 h. The resulting polymer was ground into particles of < 50 µm diameter using a mortar. The imprinting template was removed from the particles by incubation 3 x 1 h in methanol/acetic acid (4:1); and 2 x 1 h in methanol, followed each time by centrifugation steps. The polymers were finally dried in vacuum. Non-imprinted control polymers without the addition of the template molecule were prepared in the same manner.

### Binding analysis

The binding capacities of the polymers were determined using equilibrium binding analyses. Varying amounts of polymers (0.1- 10 mg/mL) were incubated for 4 h at room temperature methanol/acetic acid (MeOH/AcA) (99:1) in polypropylene microcentrifuge tubes on a rocking table with 0.5 mmol/mL of serotonin. The polymers were then separated by centrifugation at 10000 rpm for 5 min, and supernatant was measured in a spectrofluorometer (Cary Eclipse, Varian), with an excitation wavelength of 294 nm and an emission wavelength of 342 nm.

Table 1. Composition of serotonin imprinted polymers.

Polymer	Monomer(s)	Serotonin, mmol	Monomer(s), mmol	EGDMA, mmol	ABDV, mmol	DMSO, ml
I	TFMAA/MetAM	1	2/2	20	0.44	5
II	MAA/MetAM	1	2/2	20	0.44	5
III	IAA/MetAM	1	2/2	20	0.44	5
IV	TFMAA/HEMA	1	2/2	20	0.44	5

### Liquid chromatography

HPLC analysis was performed on an Agilent 1100 Series chromatograph equipped with a quaternary pump, degasser, autosampler, and a diode-array detector. The diode array detector was set at 280 nm. Spectra were recorded in the 190-400 nm range. A Kromasil C<sub>18</sub> RP column (150 x 4.6 mm i.d., 5 µm) was used as the analytical column, and was thermostated at 30°C for analysis. The flow rate was 1.5 mL/min. The mobile phase consisted of DMSO (Solvent A), acetonitrile-TFA (0.05%) (Solvent B) and gradient elution was used from 20% A to 30% A in 2 min and from 30% A to 20% A in another 3 min, then lasting for 1 min. The injected volume was 20 µL.

### Blood sample preparation and analysis

Whole blood was used for serotonin analysis. 250 µL whole blood and 50 µL ascorbic acid (1.5 M) were mixed, and the sample was spiked with 75 µL of a 1 mg/mL stock serotonin solution prepared in 170 mM perchloric acid. The samples were deproteinized by adding 50 µL of cold perchloric acid (3.4 M) under mixing. After 10 min incubation, samples were centrifuged at 14000 g for 10 min. Supernatants were again centrifuged at 6000 g for 5 min. 20 µL of the supernatant was used for HPLC. The injected amount of serotonin spiked blood sample was 1.66 mg/mL.

### SPE experiments on the MIP

10 mg each of serotonin-imprinted polymer and the corresponding non-imprinted control polymer were suspended in 500 mL MeOH/AcA (99:1) in 1.5 mL polypropylene microcentrifuge tubes. Treated plasma sample (250 µL) were then added and allowed to equilibrate for 4 h at room temperature under gentle mixing on a rocking table. The polymers were then separated by centrifugation at 10000 rpm for 5 min. Polymers were resuspended in 500 mL of MeOH:AcA (4:1) and incubated for 4 h to elute bound serotonin. The serotonin in all

supernatants was quantified by HPLC.

## RESULTS AND DISCUSSION

In preliminary experiments we found that a combination of an acid monomer and a neutral hydrogen bonding monomer yielded the best results in terms of binding capacity of the MIP for serotonin, compared to the acid or hydrogen bonding monomers alone. Three different acid monomers and two neutral hydrogen bonding monomers were tested (Table 1). The best monomer couple was found to be TFMAA/MetAM. As can be seen from Figure 1, this combination yields the highest binding of serotonin to the imprinted polymer and the lowest non-specific binding to the corresponding non-imprinted control polymer. This is probably due to the fact that for interactions with the amino groups and the ring nitrogen, an acid monomer is necessary (TFMAA is the most acidic of the three tested), whereas for the interaction with the phenolic OH, a good hydrogen bond is required, and there the amide monomer is the best. Polymer 1 was used for further experiments.

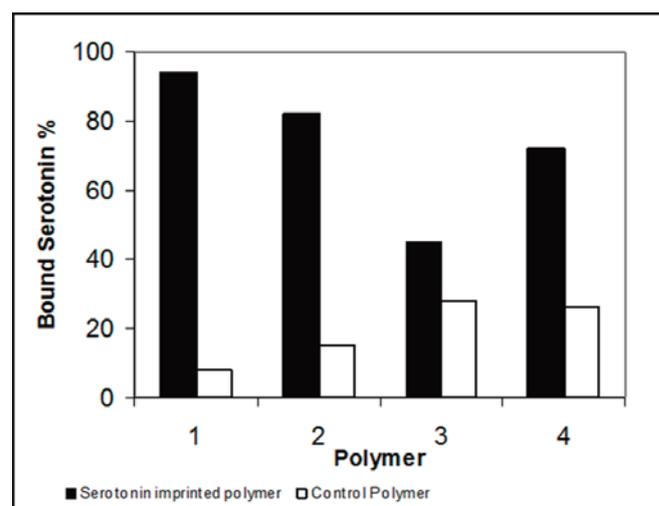


Figure 1. Binding characteristics of different MIPs.

The binding capacities of the MIP were tested in different solvents: acetonitrile, DMSO/acetic acid (99:1), methanol, methanol/acetic acid (99:1), water, water/TritonX100 (0.01%), water/methanol (9:1)

using equilibrium binding studies (Figure 2). The best imprinting effect (binding to MIP/binding to NIP) was found in methanol:acetic acid (99:1), also other solvents such as methanol or DMSO/acetic acid (99:1) yielded good results. The non-protic solvent acetonitrile resulted in considerably weaker binding, whereas aqueous solvent mixtures (water/methanol 9:1, water containing 0.01% Triton X-100) yielded good binding but also a high non-specific binding to the NIP.

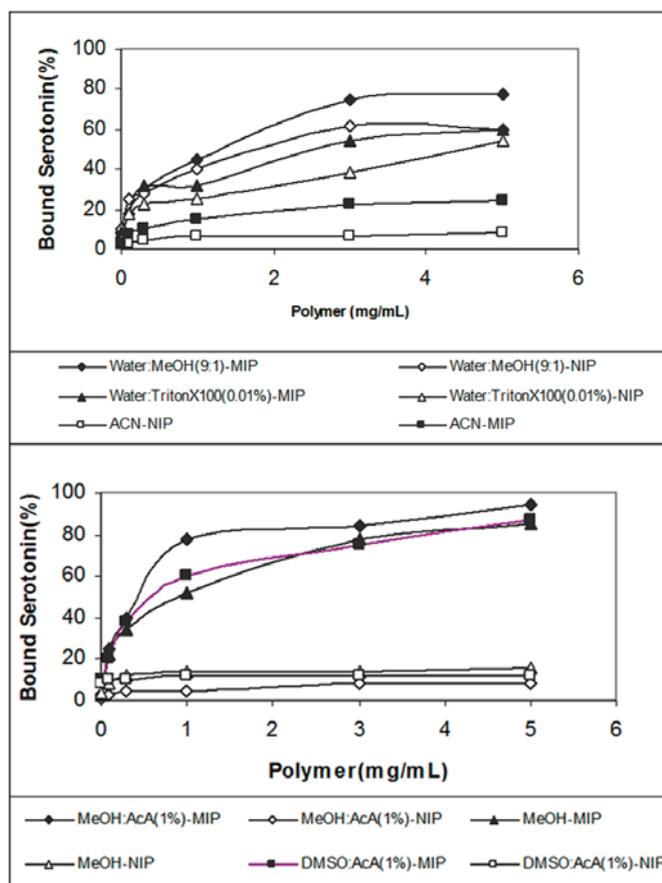


Figure 2. Equilibrium binding of serotonin to MIP I in different solvents.

In order to quantify the affinity of MIP for serotonin, a binding isotherm was recorded in methanol/acetic acid (99:1). The data were best fit by a single-site Langmuir isotherm model (Figure 3). The dissociation constant was determined as  $K_D$  10  $\mu\text{mol/L}$  and the maximum binding capacity as  $Q_{\text{max}}$  as 225.18  $\mu\text{mol/g}$ .

MIP I was then used to determine the serotonin level in whole blood. Blood samples were spiked with

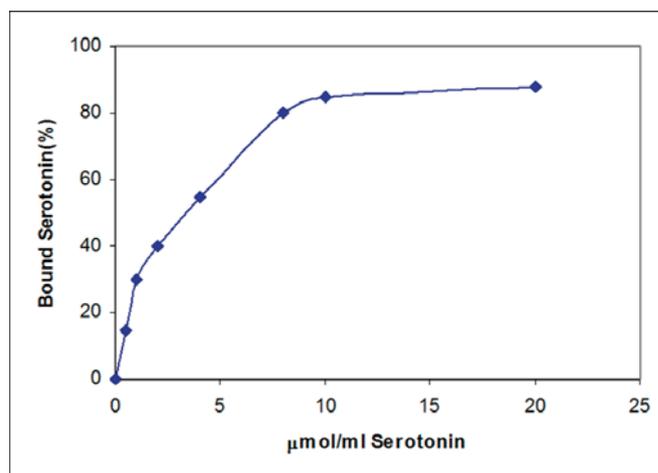


Figure 3. Binding isotherm of serotonin to MIP I (MeOH:AcA 99:1).

serotonin and treated as outlined in the Experimental part. The samples were then extracted with MIP I and with the corresponding control polymer, and the remaining serotonin in the supernatant after centrifugation was quantified by HPLC. As can be seen, treatment with NIP I does not remove serotonin from the blood (7% only is bound), whereas the extraction with MIP I removes 93% of the serotonin from the blood.

In conclusion, this study reports for the first time an imprinted polymer selective for serotonin, which is capable of binding serotonin in polar solvents and in whole blood. It was shown that serotonin could be quantified in whole blood samples after extraction onto the MIP. Although the spiked concentrations used in our experiments were higher than the serotonin levels in plasma of healthy subjects (40-400 ng/mL) or carcinoid tumor patients (650-900 ng/mL) [22], we believe that this method has a strong potential for bioanalytic chemistry.

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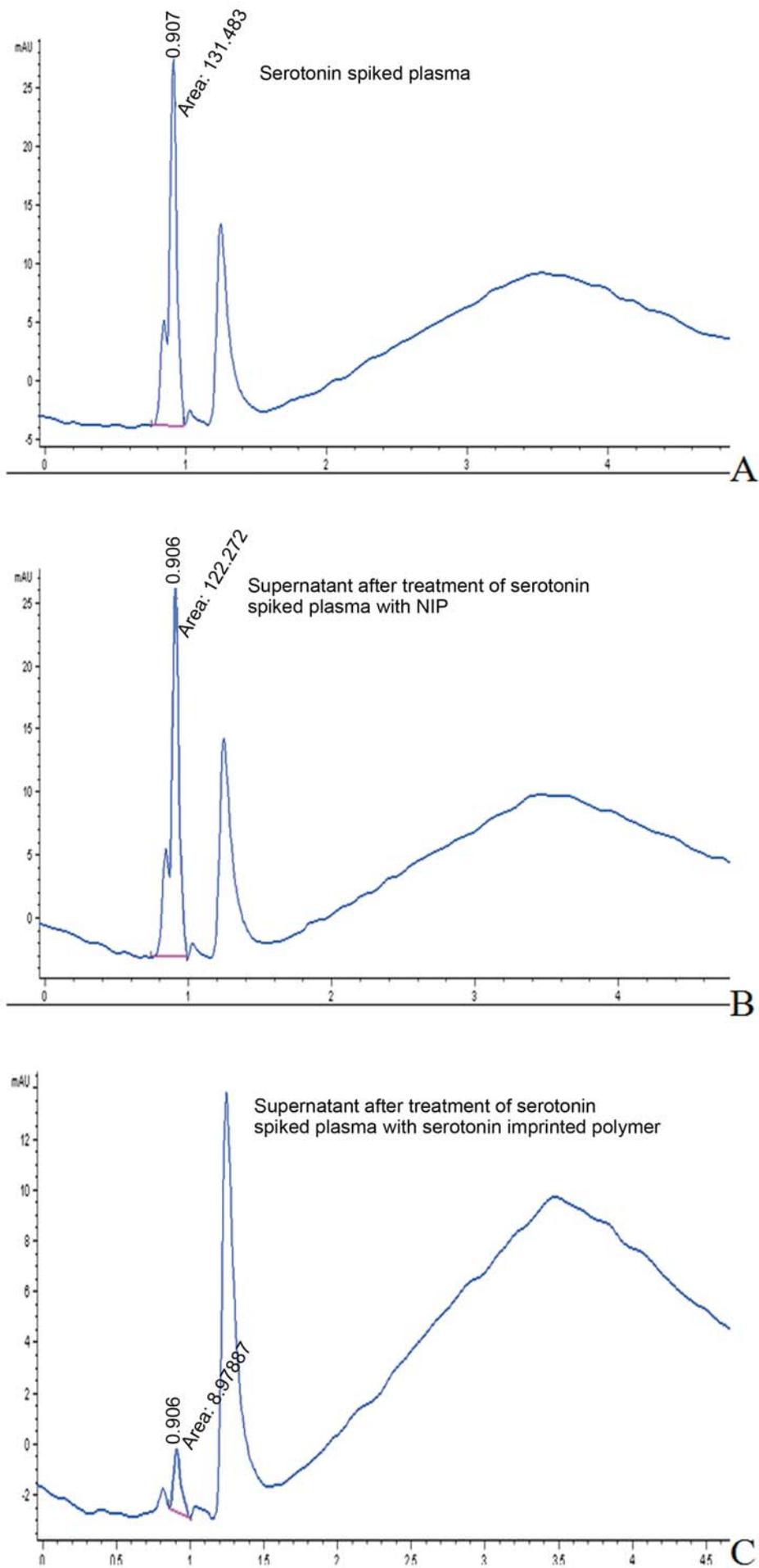


Figure 4. Chromatograms of serotonin in whole blood sample.

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