

Supplementary data for article:

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Supporting information

1. Experimental

1.1. Polymer preparation

MAA, MMA, and EDMA were purified before use by using an inhibitor remover column (Sigma-Aldrich). DVB and styrene were purified by percolation through an alumina sorbent bed. Prior to use, propranolol hydrochloride was transformed to its free base form, by means of neutralization with 0.2 M NaOH solution followed by extraction with methyl-*tert*-butyl-ether.

The polymerization mixture containing the functional monomer, crosslinker, initiator and the polymerization solvent (the “porogen”) was prepared in a glass vial prior to polymerization. The mixture was purged with argon for 5 minutes, tightly sealed with a PTFE septum cap and was placed into a water bath at 60°C for 24 hours. Two types of polymers were prepared: polymer monolith and polymer with precipitation polymerization method, respectively. The difference between the two methods is in the volume of porogen used. The solvent/ total monomer volume ratio was 1.3 for the polymer monoliths (**P1-5** and **P7-13**) and 50 for polymer prepared with precipitation polymerization (**P6**). The molar ratio of crosslinker to functional monomer was 5:1 unless otherwise mentioned. The amount of initiator was always 1.3 mol% of the total monomer amount. In the case of MIP preparation (**P13**), propranolol template (12.5 mol% of the MAA amount) was added to the polymerization mixture, according to [1]. For the detailed composition of the prepared polymers see Table 1. The formed polymer monoliths were crushed. All NIPs were thoroughly washed with methanol and then dried overnight. The MIP (**P13**) was washed several times with 0.01 M HCl solution in methanol-water 1:1, and then with methanol.

1.2. Examination of polymerization efficiency

The prepared, unwashed NIP (100 mg) was equilibrated with MeOH (1mL) for one hour. Then the sample was centrifuged and the supernatant was analyzed by HPLC or potentiometric titration as described below.

1.2.1. Supernatant analysis by HPLC

MAA content of the supernatant was determined by HPLC (column: Merck SeQuant ZIC-pHILIC, 2.1 mm x 150 mm, 5 μ m, eluent: 9:1=ACN: 10mM NH₄OAc/H₂O (pH 6.74), flow: 0.15 mL/min, wavelength: 210 nm, retention time: 6.6 min).

1.2.2. Supernatant analysis by potentiometric titration

The acidic components of the supernatant (0.5 mL aliquots) were titrated with 0.1 M NaOH. 5.0 mL of 0.1 M NaCl (in methanol-water 1:1) was added to each aliquot to define the medium and to keep the ionic strength constant. All titrations were carried out in N₂ atmosphere.

1.3. Determination of the cation exchange capacity of the polymers with potentiometric titration

1.3.1. Titration with NaOH

Carefully weighed dried polymers were equilibrated with an excess (1.2 equivalent to the carboxyl group content of the polymer) of 0.1 M NaOH in methanol-water 1:1. After half an hour (or in control experiments one day) mixing, the samples were centrifuged. The unreacted NaOH in the supernatant (0.5 mL aliquot) was backtitrated with 0.1 M HCl as described in Section 1.2.2.

1.3.2. Titration with NH₃/ Me₄NOH / Bu₄NOH

Polymer **P2** was also titrated with NH₃, Me₄NOH and Bu₄NOH. The titrations were carried out as described in Section 1.3.1. replacing NaOH with NH₃ or Me₄NOH or Bu₄NOH. Me₄NOH titration of polymer **P2** was also made in acetonitrile instead of methanol-water 1:1 by reducing the equilibration time to 5 min and the Me₄NOH amount to 1.0 equivalent to avoid hydrolysis.

1.4. Analyte binding studies

Batch binding measurements were carried out at room temperature (ca. 26 °C). Dry polymers were weighed into polypropylene microtubes and the solution of analyte was added. The solutions were prepared in ACN or in methanol-water 1:1. The amount of added analyte was equivalent compared to the theoretical MAA content of the polymers (as calculated from the polymer preparation prescription). The initial concentration of the analyte was always 10^{-2} M, unless otherwise mentioned. After one day mixing the samples were centrifuged. The supernatant was diluted hundred times with the HPLC eluent (see below) and injected (10.0 μ L) into the HPLC system to quantify the unbound analyte concentration. The chromatographic measurement was accomplished on a reversed phase column (Purospher RP18-e, 125 \times 3 mm, 5 μ m, Merck) and the eluent flow rate was 0.6 mL/min. The mobile phase consisted of phosphate buffer (pH 3.0, 10 mM NaH₂PO₄) and acetonitrile, 70:30 for propranolol ($t_R=3$ min), 90:10 for DBU ($t_R=1.9$ min), 75: 25 for (*R*)-(-)-2-benzylamino-1-phenylethanol ($t_R=2.7$ min) and 90:10 for *N*-benzylmethylamine ($t_R=2.4$ min) measurement. The detection wavelength of the UV detector was set at 215 nm.

From the equilibrium concentration of the solution phase the bound amount of the analyte was calculated according to the following equation:

$$\text{bound} = V(c_0 - c_e) \quad (2)$$

where c_0 and c_e are the initial and equilibrium solution concentrations [M] of the analyte, respectively.

1.5. Swelling studies

Dried polymer (approx. 150 mg) was weighed in an NMR tube (i.d.4 mm) and sonicated to remove air bubbles. Acetonitrile or methanol-water (1:1) was added to the tube in excess and sonicated again. The volume of the swollen polymers was determined after 24 h equilibration and the volume swelling ratio was calculated as volume of the swollen polymer per volume of the dry polymer.

2. Results and discussion

2.1. Loadability of NIPs by adsorption onto their functional groups

It is interesting to know how high loadings can be achieved by adsorption on the NIPs when only sites with functional groups are used for target binding. The adsorption experiments of propranolol from acetonitrile may give some idea about this. The loading depends on the equilibrium solution concentration as shown in the theoretical part. This concentration cannot be increased arbitrarily due to solubility limits. For propranolol in acetonitrile this limit is slightly above 0.1M at room temperature. The batch adsorption measurement, used in this work, sets the practically achievable maximum concentration somewhat lower, since the concentration drop against the starting solution has to be measured with sufficient accuracy and precision. In this work the highest equilibrium solution concentration observed with polymer **P2** was 0.067M. The adsorbed concentration at this level was 0.30 mmol/g or 33% of the theoretical -COOH content. It is unclear if this is the saturation level of the adsorption isotherm (if such exists below the theoretical capacity). The isotherm is already quite flat here, since at 0.055M solution concentration the adsorbed concentration is found already to be 0.27 mmol/g. Further measurements (not shown) with polymer **P8** (four times less carboxyl concentration than in **P2**) only confirm the results with polymer **P2** (with ca. four times less binding) but do not give new information. Note in any case that the saturation level of the isotherm need not be the same as the maximum available binding capacity, as will be pointed out immediately.

It was seen above that the adsorbed amounts from solutions equimolar with the polymer increase as the strength of the base increases. The highest loading (from 0.1 M solution) with a neutral base in acetonitrile could be achieved with DBU on **P5** (prepared in DMF) with 89% load compared to the -COOH content. This may show that the limit of propranolol binding on **P2** (33% from 0.1 M solution) is due partly to its lower base strength compared with DBU and partly to the less elastic structure of **P2** compared to **P5**. Therefore the “saturation” level of the propranolol isotherm cannot be simply identified with the maximum binding capacity of the polymer.

It is very interesting that the total binding capacities estimated in the literature from isotherms which were measured well below the loading levels achieved here, usually predict much lower, eventually by

orders of magnitude lower total binding capacities than the actual loadings measured here. For example Andersson [1] estimated merely 4% of the -COOH content as the total binding capacity for propranolol from toluene on a similar polymer to ours.

2.2. Adsorption on neutral control polymers

Propranolol adsorption was measured on neutral control polymers, the results with MMA-EDMA NIP (**P9**) were shown in the manuscript. Another control polymer, styrene-DVB (**P12**) was also prepared. As there was no propranolol adsorption on this polymer, the MAA-DVB polymer (**P7**) also binds the propranolol only with its -COOH groups.

3. List of pKa values

Compounds	pKa values in water
Propranolol	9.53 [2]
NH ₃	9.21 [3]
DBU	12.4 [4]

4. Figures

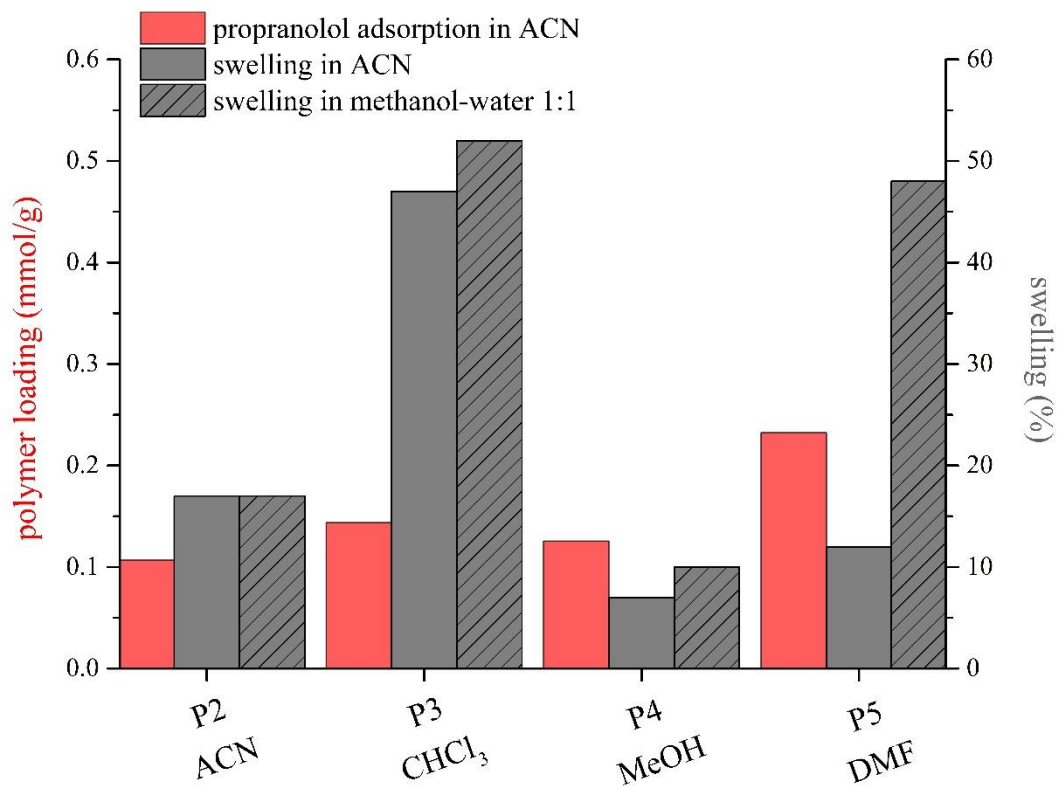


Figure S1

Adsorption from 0.01 M propranolol solution on different polymers compared to their swelling behavior

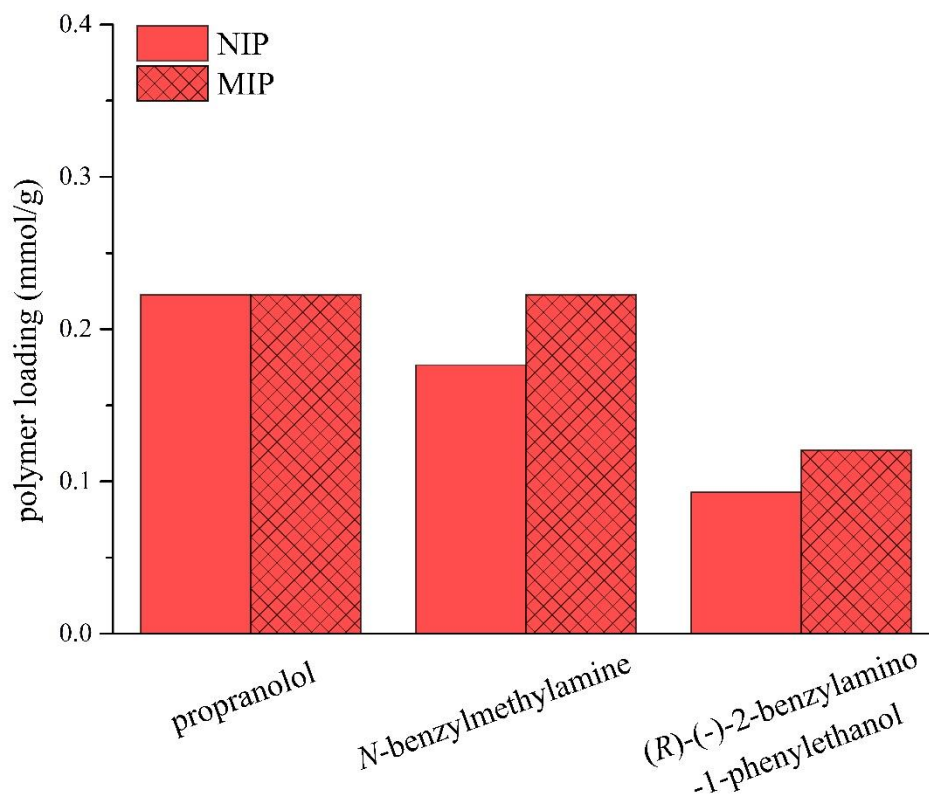


Figure S2

Adsorption of different amines from 0.01 M solution (in ACN) on NIP **P2** and propranolol MIP **P13**, respectively

5. References

- [1] Andersson, L.I., Anal. Chem. 1996, 68, 111-117.
- [2] Caron, G., Steyaert, G., Pagliara, A., Reymond, F., Crivori, P., Gaillard, P., Carrupt, P.A., Avdeef, A., Comer, J., Box, K.J., Girault, H.H., Testa, B., Helv. Chim. Acta 1999, 82, 1211-1222.
- [3] Everett, D.H., Pinsent, B.R.W., Proc. Roy. Soc. 1952, 215, 416-429.
- [4] Hofle, G., Steglich, W., Vorbruggen, H., Angew. Chem. Int. Ed. 1978, 17, 569-583.

