Microwave-assisted synthesis of resveratrol imprinted polymers with enhanced selectivity

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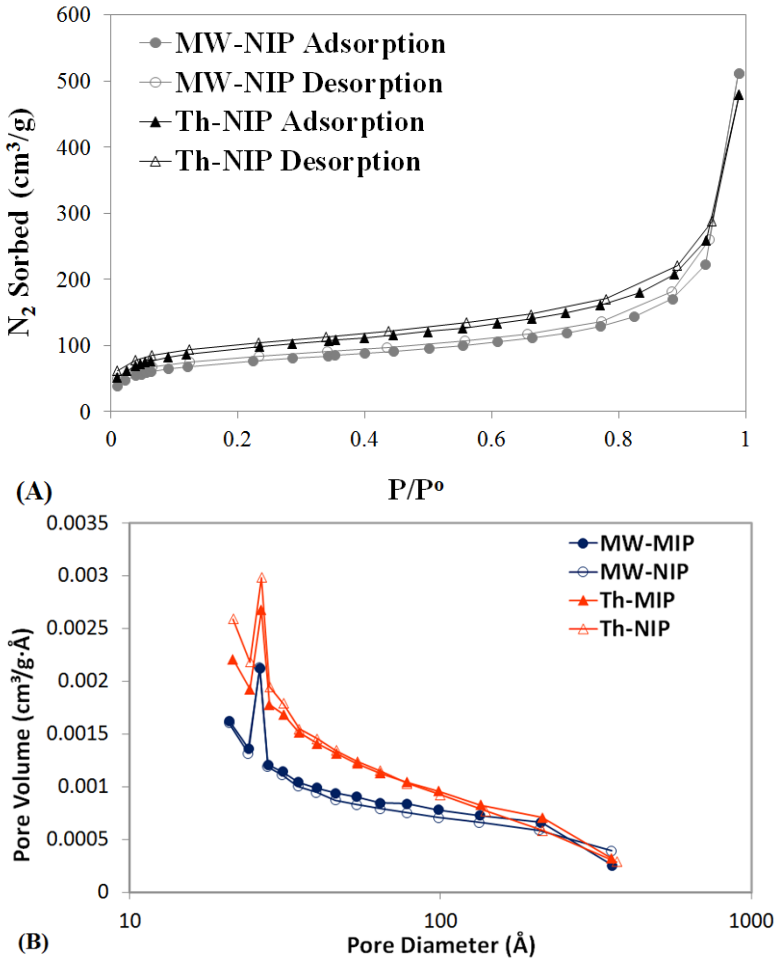
Electronic Supplementary Information

BET surface areas and porosity measurements

Nitrogen physisorption was conducted on both sets of polymers (MW- and Th-) to determine porosity characteristics of both MIP and NIP materials. N_2 sorption isotherms (adsorption and desorption) were used to determine the BET and BJH surface area and pore size information. Polymers prepared *via* thermal methods demonstrated greater N_2 sorption although pore size distribution was similar regardless of preparation.

Supp. Fig 1. Nitrogen physisorption data was collected at 77 K for both the MW- and Th- polymer materials. **(A)** Overlay of nitrogen sorption isotherms for the MW- and Th-NIP materials. **(B)** BJH pore size distribution plots were derived from the N_2 isotherm data and overlayed for each material. A clear peak was observed with a narrow distribution for each material over a pore diameter range of 24 – 28 Å.



MIP binding evaluation: Static binding isotherms and competitive binding

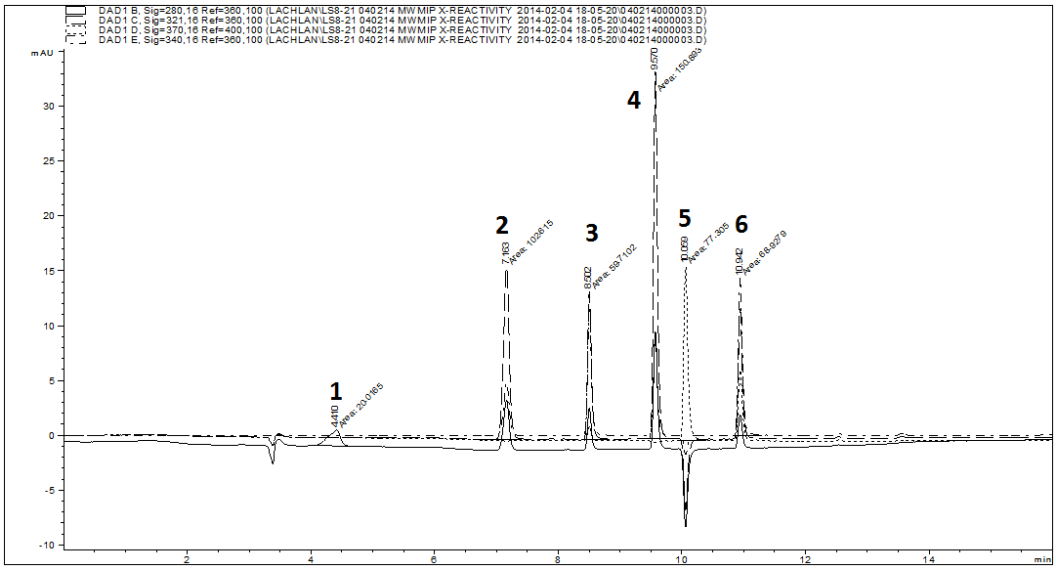
Single analyte static batch binding studies were conducted with the MW-MIP, MW-NIP, Th-MIP and Th-NIP materials (20 mg) in acetonitrile solutions (1 mL) containing (E)-resveratrol at concentrations ranging from 0-4 mM. The binding solutions were incubated for a period of 2 h and then centrifuged at 13,000 rpm (16,200 × *g*) for 15 min to pellet the polymer. Aliquots (200 □L) of the supernatants were removed for RP-HPLC analysis from which the concentration of unbound (E)-resveratrol was determined using a linear 5-point calibration

curve. Subtraction of this value from the initial total analyte concentration gave the amount of analyte bound [B], expressed as µmol/g polymer.

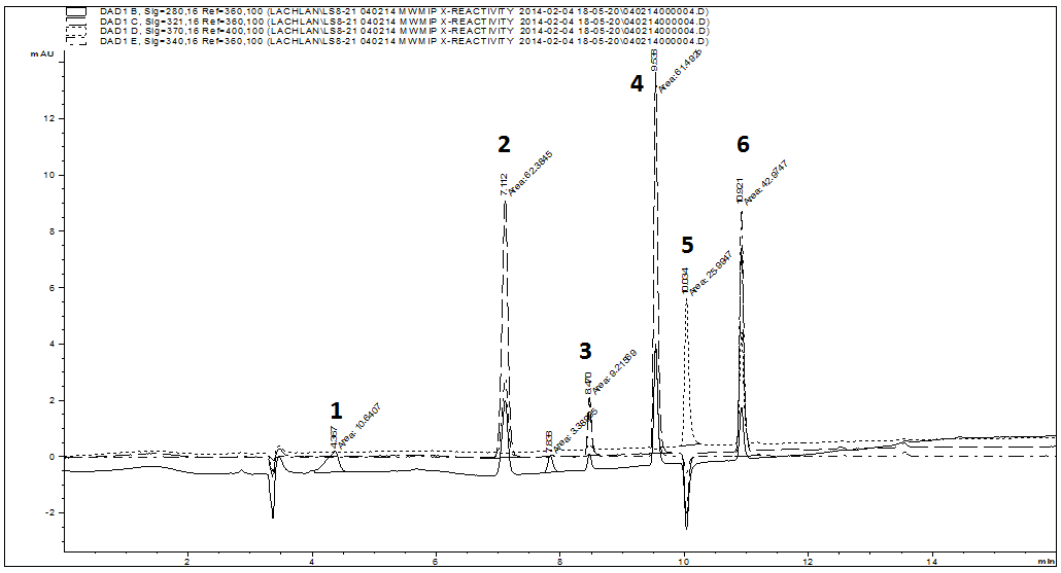
Competitive static cross reactivity binding experiments were conducted with the MW-MIP, MW-NIP, Th-MIP and Th-NIP materials (20 mg) in acetonitrile solutions (1 mL) containing a mixture of resveratrol, catechin, quercetin, piceid, rosmarinic acid and apigenin, each at a concentration of 0.05 mM. The binding solutions were incubated for a period of 2 h and then centrifuged at 13,000 rpm (16,200 × *g*) for 15 min to pellet the polymer. Aliquots (200 µL) of the supernatants were removed for RP-HPLC analysis from which the concentration of unbound analyte was determined using a linear 5-point calibration curve. Subtraction of this value from the initial total analyte concentration gave the amount of analyte bound [B], expressed as µmol/g polymer.

RP-HPLC analysis was performed using an Agilent Technologies HPLC 1100 series (Agilent Technologies, Waldbronn, Germany) equipped with ChemStation software, a degasser, a binary gradient pump, an auto-sampler with a 900 µL sample loop, a thermostated column compartment and an UV-DAD. Separation was performed with a Zorbax Eclipse XDB C_{18} (4.6 mm × 150 mm, 5 µm particle size) (Agilent Technologies, Melbourne, Australia) double end-capped column which was operated at ambient temperature. The UV-DAD was set to 200-600 nm at a spectral acquisition rate of 2 nm scans per step with simultaneous monitoring at 280, 321, 340 and 370 nm for all compounds. The mobile phase composition was a mixture of H_2O (0.1 % formic acid) (mobile phase A) and 70:30 CH_3CN:H_2O (0.1 % formic acid) (mobile phase B) and was delivered with the following gradient elution profile: 28.5 % B, 0-1 min; 28.5-100 % B, 1-10 min, 100 % B 15 min; 28.5 % B 15.01 min; 2 min post time at a flow rate of 0.5 mL/min and injection volume of 5 µL to the RP-HPLC column. The mobile phases were filtered through a polypropylene membrane filter (0.2 µm pore size, 47 mm diameter) purchased from Pall Corp. (Melbourne, Australia) and was degassed for 20 min in an ultrasonic bath (Elmasonic, Singen, Germany) prior to use.

Supp. Fig 2. The chromatographic analysis of supernatants with RP-HPLC of a 6 component mixture of polyphenol standards (0.05 mM) after: **(A)** no pre-treatment; and incubation with 20 mg of **(B)** MW-MIP; **(C)** MW-NIP; **(D)** Th-MIP batch 1; **(E)** Th-MIP batch 2; **(F)** Th-MIP batch 3; and **(G)** Th-NIP. The 6 component mixture consisted of catechin (**1**), piceid (**2**), rosmarinic acid (**3**), resveratrol (**4**), apigenin (**5**) and quercetin (**6**). Displayed in **Supp. Fig. 2** are the chromatograms (with peak areas displayed) for the incubation standard (**Supp Fig. 2(A)**) and MIP and NIP counterparts via both microwave (MW) and thermal (Th) preparative techniques. For both MW and Th preparations, an imprinting effect was observed with the binding performances considerably different to those of the NIP materials. Of note is the improved selectivity towards resveratrol with respect to all other analytes, when employing the MW-MIP (**Supp Fig. 2(B)**) instead of the Th-MIP (**Supp Fig. 2(D-F)**). Of further interest was the variability in selectivity displayed by the Th-MIP preparation, an observation that can be attributed to the Th- imprinting technique when considering the similarity in selectivity and affinity of the MW-NIP (**Supp Fig. 2(C)**) and Th-NIP (**Supp Fig. 2(G)**) towards each of the competing analytes.



(A)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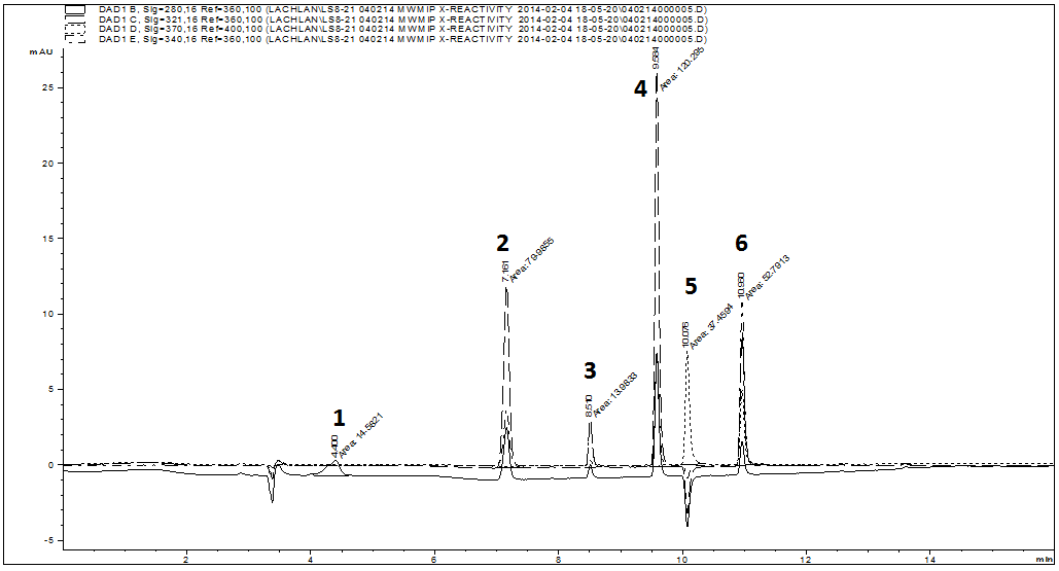
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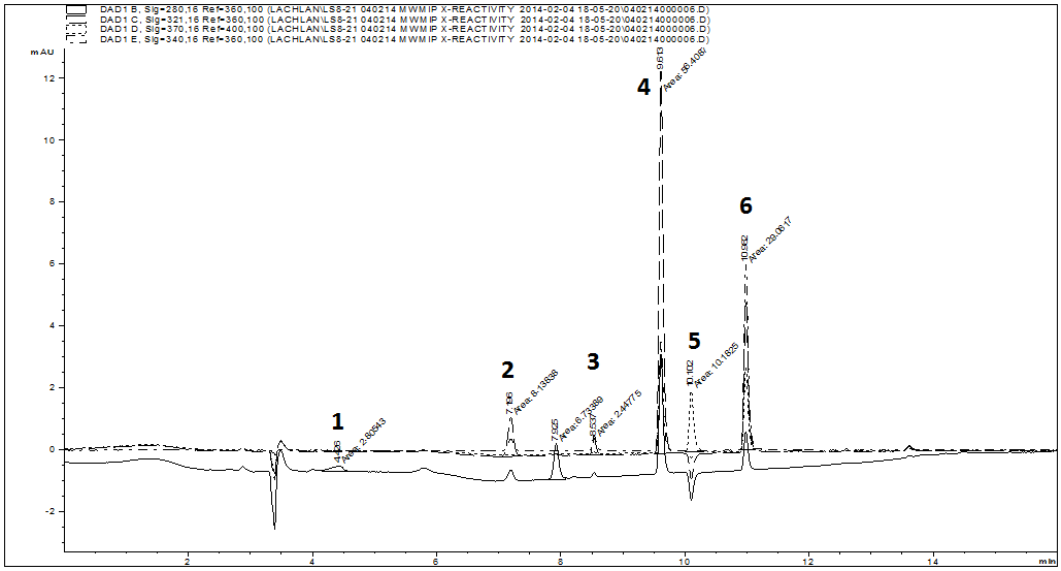
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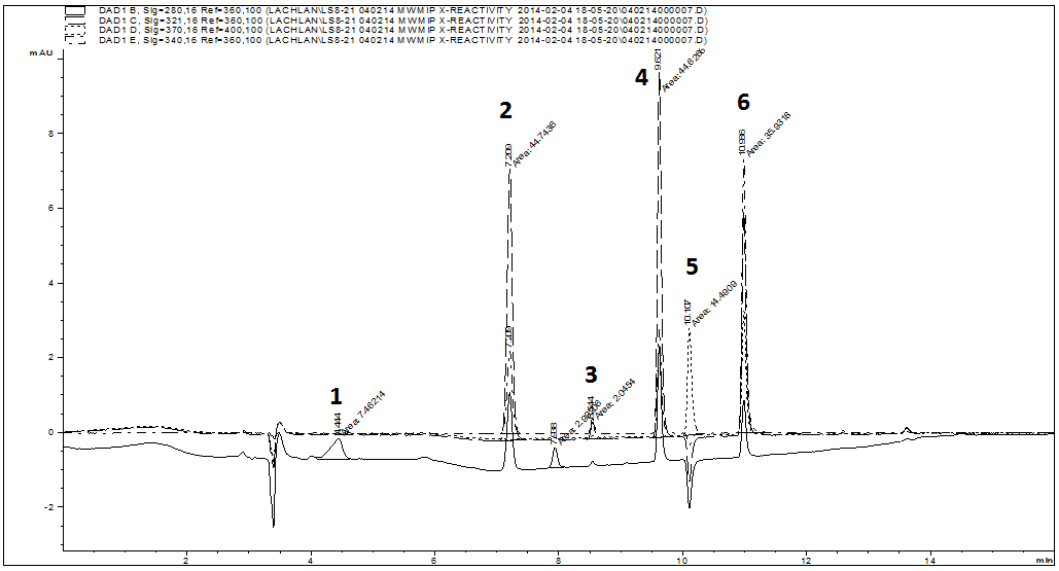
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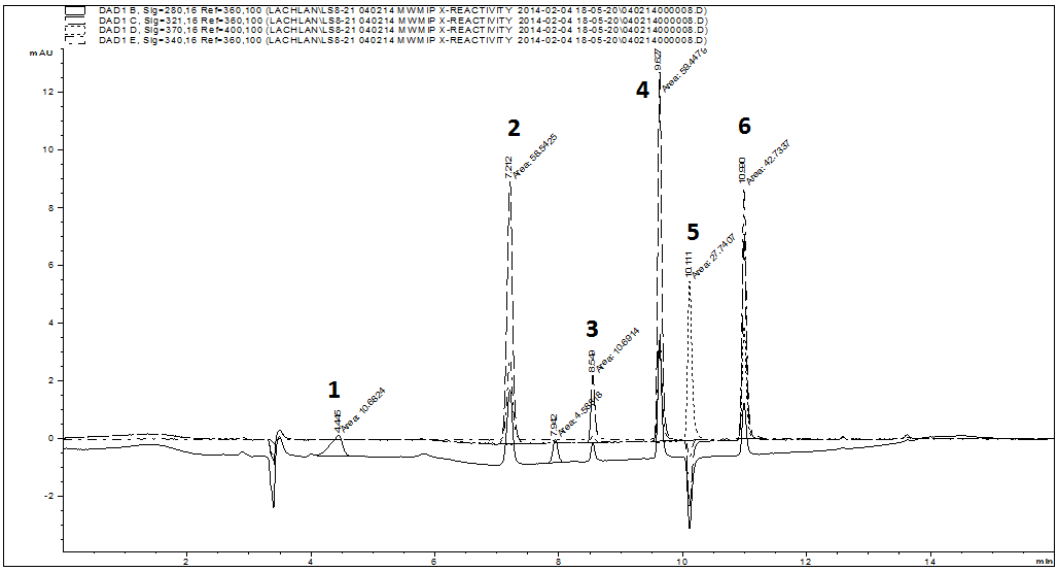
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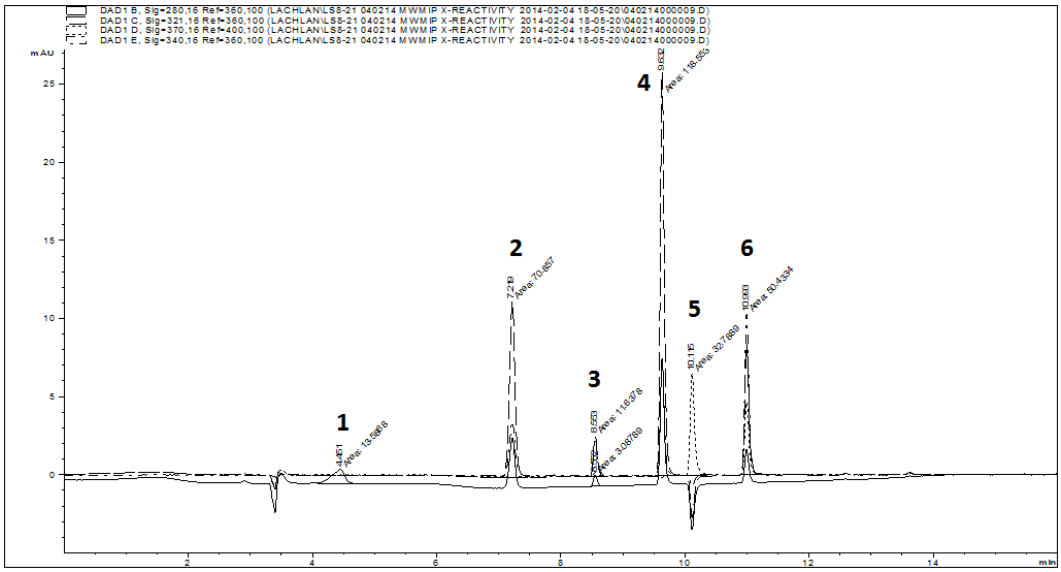
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