Supplementary Information

Tailored Imprinted Polymers for Selective Recognition of Sulfonated Dyes: Extraction from Soft Drink, Water and Food Samples

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EXPERIMENTALS

Materials and Methods

Commercial Drinks

Ingredients of commercial drinks are as follows. **CD1**: carbonated water, sugar, acidity regulator, caffeine (29 mg/100 ml), allura red AC (E129) color, stabilizer, preservative, artificial sweetener, vitamins and artificial strawberry flavoring substances; **CD2**: carbonated water, sugar, acidity regulator, sunset yellow FCF (E110), stabilizer , preservative and flavors; **CD3**: carbonated water, sugar, acidity regulator, caffeine (13 mg /100 g), tartrazine (E102), stabilizer, preservative, and flavors; **CD4:** water, sugar, orange juice (10.5%) (reconstituted from orange juice concentrate and orange pulp cells), acidity regulators, antioxidant , tartrazine (E102) and sunset yellow FCF (E110), orange flavors; **CD5:** water, sugar, concentrate mango pulp (5.1%), acidity regulator, stabilizers , preservative, sweetener, antioxidant, sunset yellow FCF (E110), artificial flavors.

HPLC Instrumentation

Chromatographic analyses were performed using high-performance liquid chromatography (Agilent Infinity 1260 II HPLC)) system equipped with a quaternary solvent delivery module, autosampler, column thermostat, and diode array (DAD) detector. Data collection and processing were carried out using instrument-supplied chromatography software (OpenLab CDS).

ATR-FTIR Spectral Analysis Method

The Fourier transform infrared (FTIR) spectra were recorded using a Tensor-37 ATR-FTIR spectrometer (Bruker) equipped with a platinum attenuated total reflectance (ATR) crystal. All spectra were collected in transmittance mode over the wavenumber range of 4000–500 cm^{-1}, at a spectral resolution of 4 cm^{-1}, with 24 scans. Background correction, atmospheric compensation, and baseline correction were applied to the spectra. Data acquisition and spectral analysis were performed using OPUS software. The polymers were dried for 3 h in the oven at 50°C before analysis.

Binding tests using imprinted and nonimprinted polymers:

In a 1.5 mL microcentrifuge tube, 10 mg of (P1, P2, RP1 and P_N1, P_N2, RP_N2) polymers were separately mixed with 0 to 3 mM of TBA PSA or PSA. The analytes were mixed in 1 mL of the respective solvents or mixture of solvents. The polymer was suspended in the rebinding solvents and shaken for 100 minutes, unless otherwise specified. Next, the vials were centrifuged at 10000 rpm, and the supernatants were analyzed by C18-HPLC-UV analysis. From the supernatant solution, the unbound concentration of analytes was calculated. In the case of MeCN as a rebinding solvent, 600 µL supernatant solution was evaporated to dryness, redissolved in 600 µL distilled water, and further used for quantification of PSA in LC analysis.

The specific amount of analyte bound by the polymer particles (B) was determined by the following formula:

$B = (C_0 - C) \times v/m$..... (S1)

where C_0 is the initial solute concentration, C is the final free solute concentration in the supernatant, v is the total volume of the adsorption mixture, and m is the mass of polymer in each vial.

Binding curves were constructed by plotting B against free concentration C and were subsequently fitted by non-linear regression in the Graphpad Prism 7 curve fitting software to a Langmuir mono-site model:

$B = Bmax.c/(Kd + c)$..... (S2)

where Bmax is the maximum amount of solute bound by the polymer particles at saturation. The association constants Ka was calculated as the inverse of the dissociation constants (Kd).

C-18 HPLC-UV method: Reversed phase HPLC detection of unbound analytes: Chromatographic analysis was carried out with an HPLC 1260 Infinity II instrument (Agilent) equipped with a quaternary pump auto-injector and DAD detector. The analytical column was a C18 reversed phase Agilent (150 mm x 4.6 (i.d.) mm), particle size: 5 µm. The mobile phase consisted of methanol/water (0.1 % TFA): 20/80 (v/v) for PSA. The flow rate was 0.7 mL min^{-1} and the injection volume was 10 µL. The column was kept at room temperature. Absorbance wavelength for PSA was 205 nm. For quantification purposes, calibration standards were made using the same solvent as used in the binding experiments. The calibration curve comprised the 0-3 mM concentration range and was recorded after the incubation of the anions. The retention times of PSA were 3.1 min.

SY Dye Binding

5 mg of P2 and P_N2 particles were suspended in 4 ml of water with 0.1 mM sunset yellow FCF (E110) dye for 15 min, then centrifuged at 10000 rpm for 5 min. The particles were washed sequentially with 1 mL of 0.1N HCl, 1 ml of 90:10 MeOH: 25% NH_3 (twice), 1 mL of 80:20 Methanol: 0.1 N HCl, and 1 mL of 100% methanol. After drying, optical and fluorescent images were captured using a Carl Zeiss Axio observer A1 fluorescent microscope with a red excitation

filter at 700 ms exposure. The first three washing fractions were collected and analyzed using a C18-HPLC-UV method. The analytical method and parameters were adopted from the same procedure used for the analysis of the dye mixture.

Rebinding isotherm for TZ dye

Specifically, 2.5 mg of both P2 and PN2 polymers were suspended in 1 mL of TZ dye solutions ranging from 0 to 2 mM, and agitated gently for 100 min. Subsequently, the polymer particles were separated via centrifugation at 10000 rpm. The supernatant was then analyzed directly using a multiplate reader, with spectral scanning performed from 300 nm to 700 nm. The rebinding isotherm was plotted by quantification of TZ dye at 430 nm utilizing an external calibration curve.

Dye Binding experiments with P2 and P_N2 polymers

The binding affinity of polymers P2 and P_N2 for dyes (1-10) was evaluated through rebinding assays. Dye stock solutions were prepared in milli-Q water, with TZ, AR, and MY at 1 mM, and SY, FG, AM, BO, IC, and MO at 0.1 mM. For each dye, 5 mg of polymer (P2 and P_N2) was suspended in 4 mL of the respective dye solution and agitated for 15 min to allow binding, followed by polymer settling. Supernatant solutions were analyzed using a multiplate reader (BioTek Synergy H1) from 230 to 900 nm, using dye stock solutions as references to quantify dye bound to the polymer by recording absorbance values at dye-specific wavelengths. Finally, dye-bound P2 polymers were dried under vacuum and photographed to visually document the dye binding process. After the rebinding experiment, SY dye-absorbed particles (P2 and P_N2) were washed sequentially with 1 ml of 0.1 N HCl, twice with a 90:10 methanol:25% ammonia solution, and

then with a 20:80 mixture of 0.1 N HCl: methanol, each wash including 10 min of stirring, centrifugation, and supernatant decanting. The particles were then washed with methanol, dried thoroughly, and imaged using a Carl Zeiss fluorescent microscope (model: Axio Observer A1) with a red excitation filter and a 700 ms exposure time.

Evaluation of commercial soft drink by P2, P_N2

The binding affinity of polymers P2 and P_N2 for several commercial soft drinks (CD1 to CD5) was evaluated using rebinding assays. The soft drinks were sourced from the local market. CD1, CD2, and CD3 were carbonated drinks containing Allura Red (AR), Sunset Yellow (SY), and Tartrazine (TZ) dyes, respectively. CD4 and CD5 were juice drinks containing Sunset Yellow (SY) dye. For the carbonated drinks (CD1, CD2, and CD3), 11 mg, 5 mg, and 1 mg of polymer (P2 and PN2) were suspended in 4 mL of each drink, respectively. The juice solutions (CD4 and CD5) were first centrifuged to remove pulp, and the supernatant was used for subsequent experiments. 2.5 mg of polymers (P2 and P_N2) were then suspended in 4 mL of the respective CD4 and CD5 juice solutions. After adding the polymers to the soft drink solutions, the mixtures were agitated for 10–15 minutes to facilitate binding, followed by a 10-minute settling period. Photographs were taken to document the rebinding experiments. The supernatant solutions were then analyzed using a UV-Vis multiplate reader (BioTek Synergy H1) in the 230 to 900 nm range. The original soft drink solutions served as a reference, and absorbance values at dye-specific wavelengths were recorded to quantify the amount of dye bound to the polymers.

Dye binding from turmeric samples to the polymers (P2 and P_N2)

200 mg of (1-4) turmeric samples were taken in 25 ml conical flasks separately, and 10 mL of milli-Q water was added to them. Shake and sonicate well for 15 min, centrifuge, and then separate the supernatant solution.

In 1.5 mL Eppendorf vials, 1 mL the supernatant solutions of turmeric samples were added to the 2 mg of P2 and P_N2 polymer. The polymer was shaken for 5 min in shaker, then centrifuge at 9000 rpm for 4 min. The supernatants solution was completely removed from polymer vials. 1st elution: Bound dye to the polymer was removed by washing with number of consequential washing solvents with sonication for 15 min. The solvents were used as follows: 500 µL of MeOH:acetic acid (70:30) x 4 times; 500 µL 45:45:10 (MeOH:1 mM NaCl solution: 25% ammonia) x 5 times and 500 µL of MeOH:acetic acid (70:30) x 1 times. After each washing, supernatant was collected after centrifugation. The supernatant solutions were collected and evaporated by rotary evaporator, redissolved to 1 mL in milli-Q water. The extraction was quantified further for C18-HPLC-UV analysis. In second elution, the polymers were washed with 500 µL MeOH: acetic acid (70:30) twice with sonication for 15 min. The washing solvent were separated by centrifugation and extracts was evaporated by vacuum oven at 60°C. The bound dye was dissolved in 500 µL of milli-Q water and was used for C18-HPLC-UV analysis.

MY C-18 HPLC-UV method

The method for detecting MY dye using Reversed-phase HPLC is similar to that of PSA, with slight modifications. Specifically, the mobile phase consisted of methanol/water (0.1 % TFA) at a ratio of 25/75 (v/v). The flow rate was set to 1.1 mL min^{-1}, and the absorbance wavelength for PSA was 430 nm. The retention time for MY was observed to be 7 min.

Molecularly Imprinted Polymer - Solid Phase Extraction (MIP-SPE) ofdye mixture from water

To perform MIP-SPE on dye mixtures in water, 20 mg of P2/P_N2 polymers were packed into 3 ml cartridges. A 1 L water sample was spiked with a mixture of Tartrazine (TZ), Metanil yellow (MY), Fast green (FG), Alizarin red (AR), and Sunset yellow (SY) dyes (1.5 ml of 0.03 mM of each). This spiked water was passed through the P2/P_N2 cartridges at a flow rate of 2.5 mL/min under a vacuum of approximately 30 mmHg. After drying the polymers, the adsorbed dyes were eluted in two steps: Elution 1 (E1) used 1 mL of 0.1 N HNO_3: methanol (80:20), 3 mL of 0.1 N HNO_3: methanol (60:40), and 2 mL of 0.1 N HNO_3: methanol (50:50). Elution 2 (E2) used 4 mL of 0.25 N HNO_3: methanol (50:50). To neutralize the elution, 100 µL of 25% ammonia was added to E1 and 280 µL to E2. For HPLC analysis, 500 µL of the combined elution fractions were mixed with 500 µL of milli-Q water.

HPLC method for separation of Dye mixture

Separations were conducted on a reversed-phase Agilent ZORBAX Eclipse Plus C18 column (150 mm × 4.6 mm i.d., 5 µm particle size). The column temperature was maintained at room temperature during analysis. The mobile phase consisted of two eluents: eluent A, water containing 0.1% (v/v) trifluoroacetic acid, and eluent B, methanol containing 0.1% (v/v) trifluoroacetic acid. The flow rate was set to 1.1 mL min^{-1}, and the system pressure was limited to a maximum of 400 bar. The total analysis time for each run was 15 min. A gradient elution strategy was applied to achieve efficient separation. The initial mobile phase composition was 75% A and 25% B and was held for 3 min. The proportion of eluent B was increased to 70% at 4 min and further raised to 100% at 6 min. At 10.2 min, the composition was adjusted to 85% B and 15% A. Finally, the mobile phase was returned to 75% B and 25% A at 15 min to allow column

re-equilibration before subsequent injections. Detection was carried out using a diode array detector. Chromatograms were recorded at 430nm wavelength.

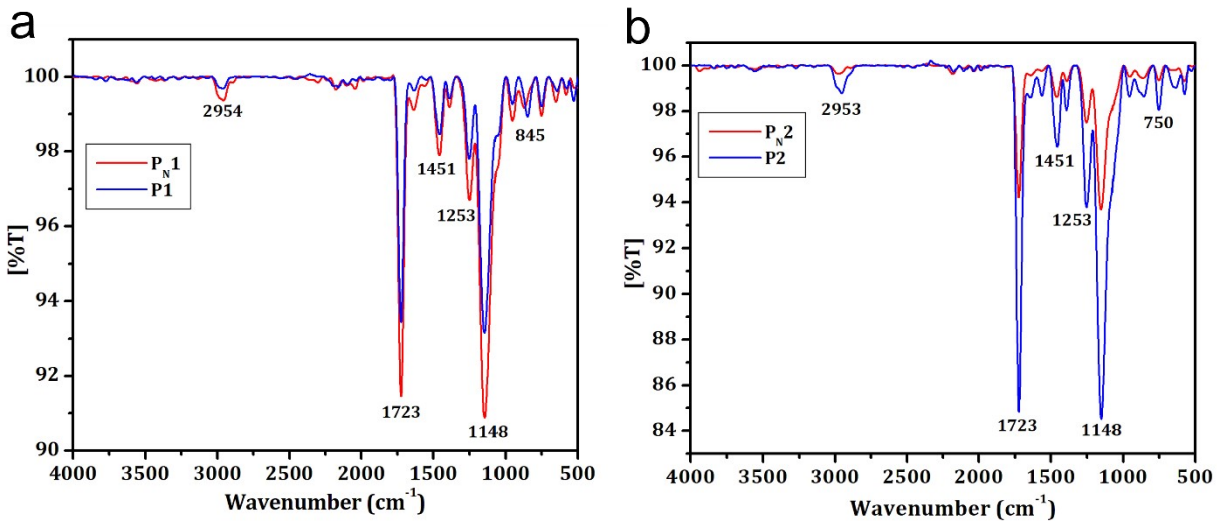
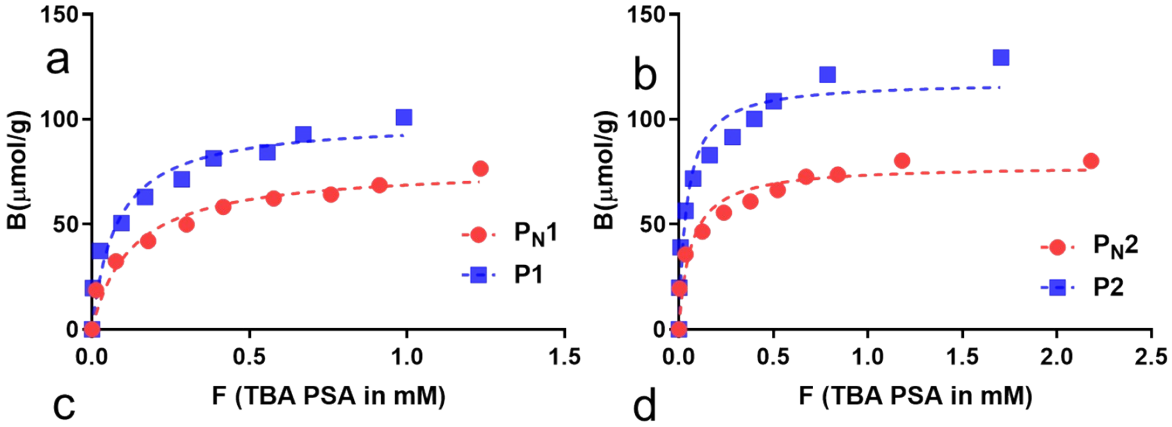


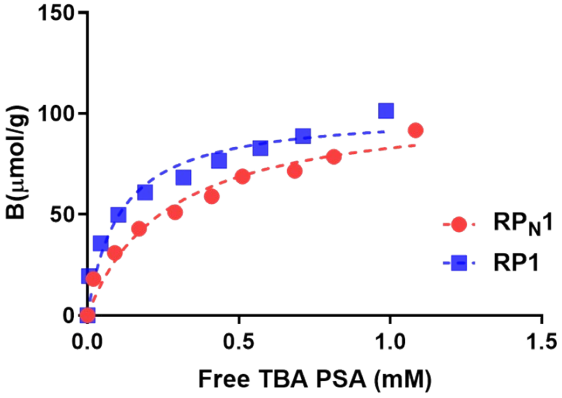
Figure S1. Overlay ATR-IR spectra of a) P1/P_N1, b) P2/P_N2.MIP and NIP spectra are in blue and red colored respectively.



	P_N1	P1
One site -- Specific binding [2]		
Best-fit values		
Bmax	77,37	99,69
Kd	0,1262	0,07828
95% CI (profile likelihood)		
Bmax	66,56 to 92,46	83,42 to 123,7
Kd	0,05784 to 0,2499	0,02767 to 0,1873
Goodness of Fit		
Degrees of Freedom	8	8
R squared	0,9568	0,9383
Sum of Squares	227,0	603,3
Sy.x	5,327	8,684
Number of points		
# of X values	10	22
# Y values analyzed	10	10

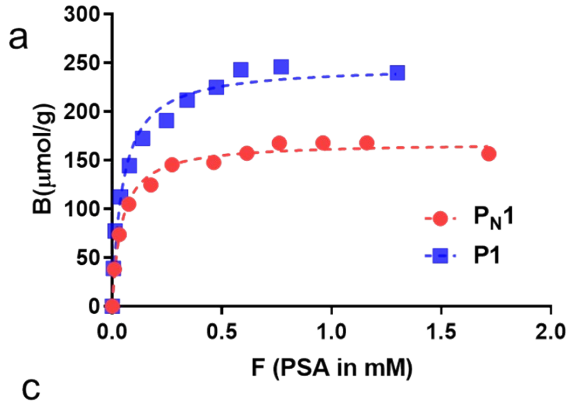
	P_N2	P2
One site -- Specific binding		
Best-fit values		
Bmax	78,08	118,1
Kd	0,06365	0,04200
95% CI (profile likelihood)		
Bmax	68,37 to 90,69	102,0 to 138,3
Kd	0,02223 to 0,1483	0,01560 to 0,09794
Goodness of Fit		
Degrees of Freedom	9	9
R squared	0,9343	0,9327
Sum of Squares	448,2	1178
Sy.x	7,057	11,44
Number of points		
# of X values	11	23
# Y values analyzed	11	11

Figure S2. Equilibrium binding isotherms for the uptake of the phenyl sulfonic acid tetrabutylammonium (TBA PSA) by (**a**) P1, P_N1 and (**b**) P2 P_N2 (right) in 100% MeCN. F = concentration of the free TBA PSA (in mM), B = specific amount of bound TBA PSA (µmol g^{-1}). (**c**, **d**) The binding constants (Ka) and specific binding capacities (Bmax) were obtained from by non-linear curve fitting of the data assuming a Langmuir monosite binding model.



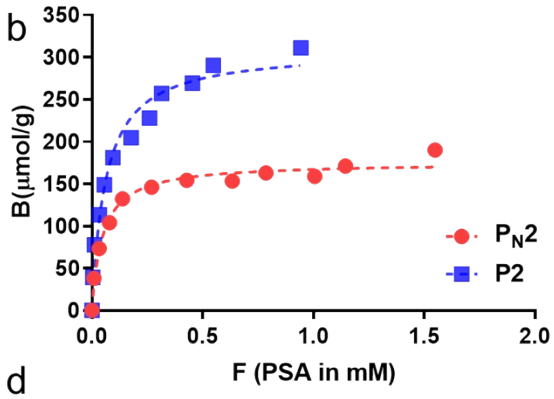
	RP_N1	RP1
One site -- Specific binding [2]		
Best-fit values		
Bmax	104,5	100,9
Kd	0,2617	0,1068
95% CI (profile likelihood)		
Bmax	86,39 to 133,6	85,51 to 123,4
Kd	0,1386 to 0,4925	0,04822 to 0,2225
Goodness of Fit		
Degrees of Freedom	8	8
R squared	0,9663	0,9516
Sum of Squares	248,1	451,0
Sy.x	5,569	7,508
Number of points		
# of X values	10	21
# Y values analyzed	10	10

Figure S3. Equilibrium binding isotherms for the uptake of the phenyl sulfonic acid tetrabutylammonium (TBA PSA) by RP1, RP_N1 in 100% MeCN. F = concentration of the free TBA PSA (in mM), B = specific amount of bound TBA PSA (µmol g−1). The binding constants (Ka) RP1 = 9.36 x 10^3 M^{-1}; RP_N1 = 3.82 x 10^3 M^{-1} and specific binding capacities (Bmax) RP1 =100.9 ± 18.95 µmol g^{-1}, RP_N1 = 104.5 ± 23.61 µmol g^{-1} were obtained from by non-linear curve fitting of the data assuming a Langmuir monosite binding model. RP1 and RP_N1 polymers previously reported share an identical composition, differing only an initiator as AIBN and polymerized at 65°C.[1]



c

	P_N1	P1
One site -- Specific binding [2]		
Best-fit values		
Bmax	168,1	247,1
Kd	0,04280	0,04714
95% CI (profile likelihood)		
Bmax	160,8 to 175,8	230,0 to 266,2
Kd	0,03196 to 0,05679	0,03149 to 0,06937
Goodness of Fit		
Degrees of Freedom	10	10
R squared	0,9869	0,9783
Sum of Squares	451,6	1693
Sy.x	6,720	13,01
Number of points		
# of X values	12	24
# Y values analyzed	12	12



d

	P_N2	P2
One site -- Specific binding [2]		
Best-fit values		
Bmax	175,1	309,4
Kd	0,04831	0,06295
95% CI (profile likelihood)		
Bmax	165,8 to 185,0	281,0 to 343,1
Kd	0,03445 to 0,06679	0,04072 to 0,09587
Goodness of Fit		
Degrees of Freedom	10	10
R squared	0,9813	0,9740
Sum of Squares	697,1	2964
Sy.x	8,349	17,22
Number of points		
# of X values	12	24
# Y values analyzed	12	12

Figure S4. Equilibrium binding isotherms for the uptake of the PSA by (**a**) P1, P_N1 and (**b**) P2, P_N2 (right) in 100% water. F = concentration of the free PSA (mM), B = specific amount of bound PSA (µmol g^{-1}). (**c, d**) The binding constants (Ka) and specific binding capacities (Bmax) were obtained from by non-linear curve fitting of the data assuming a Langmuir monosite binding model.

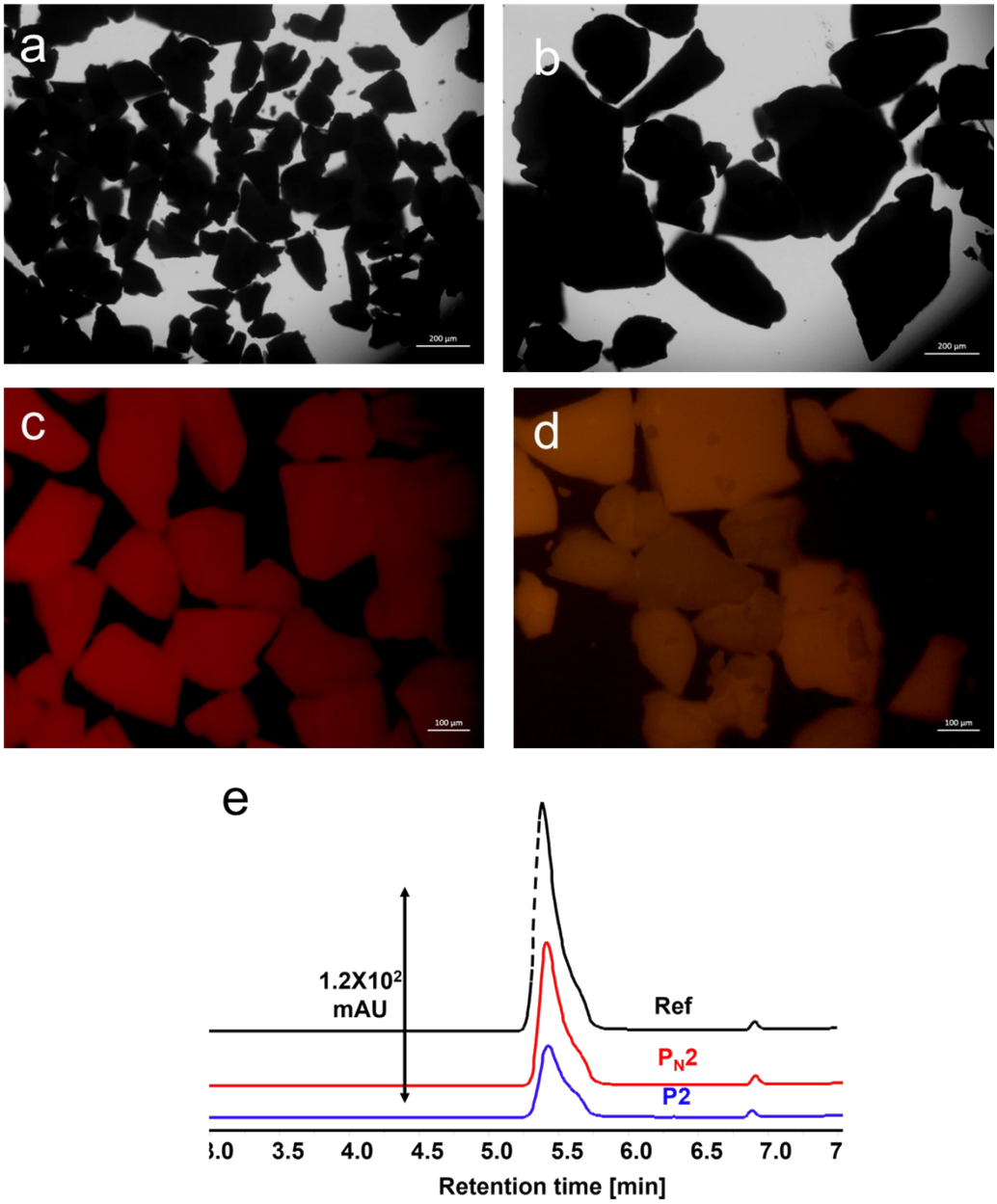


Figure S5. Optical microscope images of P2 (a), P_N2 (b); Fluorescence images of bound sunset yellow dye to P2 (c) and P_N2 (d) particles after washing with acidic and basic solvents. Fluorescent images were captured using a Carl Zeiss Axio observer A1 fluorescent microscope with a red excitation filter at 700 ms exposure. (e) C18-HPLC-UV chromatograms of the collected washing fractions of SY obtained from P2/P_N2 polymers.

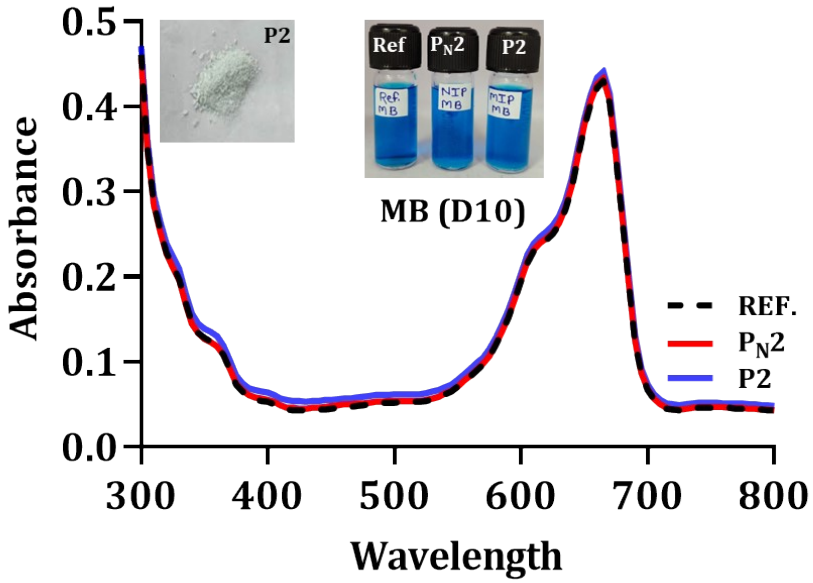


Figure S6. Binding of Methylene Blue (MB) dyes containing sulfonate moiety by P2, P_N2 in 100% water. Absorption spectra of MB, after binding by P2, P_N2 in comparison to reference dye solutions. Inset: In glass vials, photographs of MB dye binding experiment after 20 min and polymers with respective MB capture by P2 polymers.

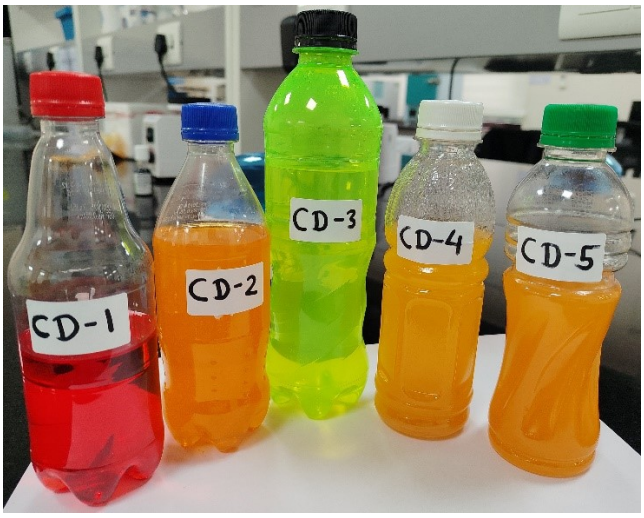


Figure S7. Commercial soft drinks (CD1- CD5) were utilized for binding experiments. The sulfonated dyes from CD1 to CD5 were extracted using imprinted polymer P2/P_N2.

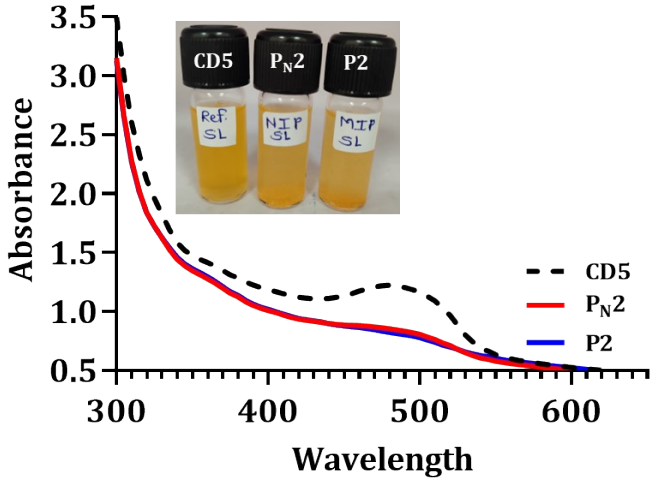


Figure S8. Binding of sulfonate dye by P2, P_N2 in soft drink CD5. Absorption spectra of CD5, after binding by P2, PN2 in comparison to CD5. Inset: In glass vials, photographs of dye binding

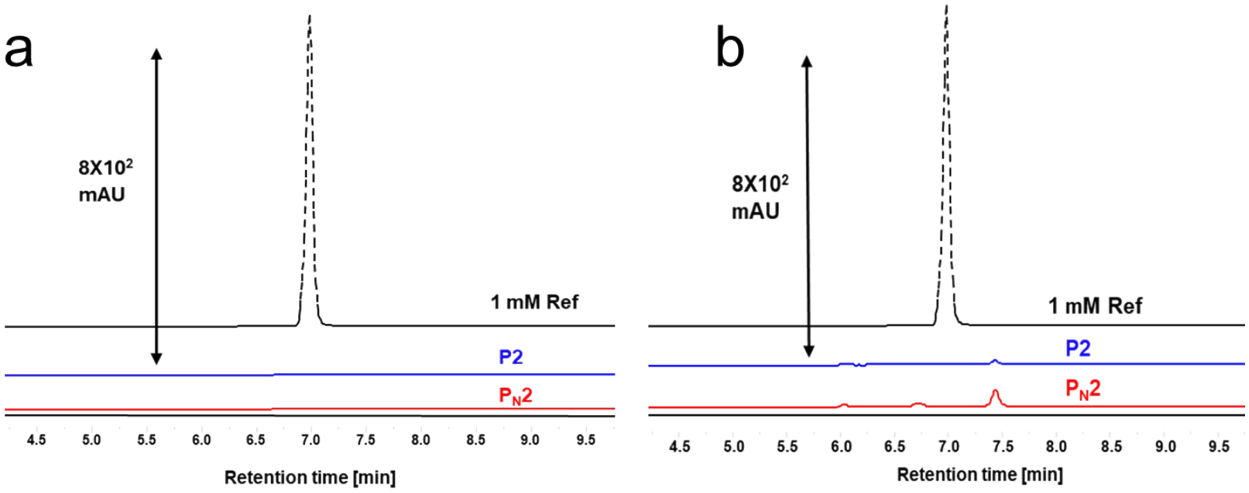


Figure S9. Overlay spectra of C18-HPLC-UV of eluted fractions of dyes from turmeric sample 4. a) Elution fraction – 1, b) elution fraction – 2.

experiment after 20 min.

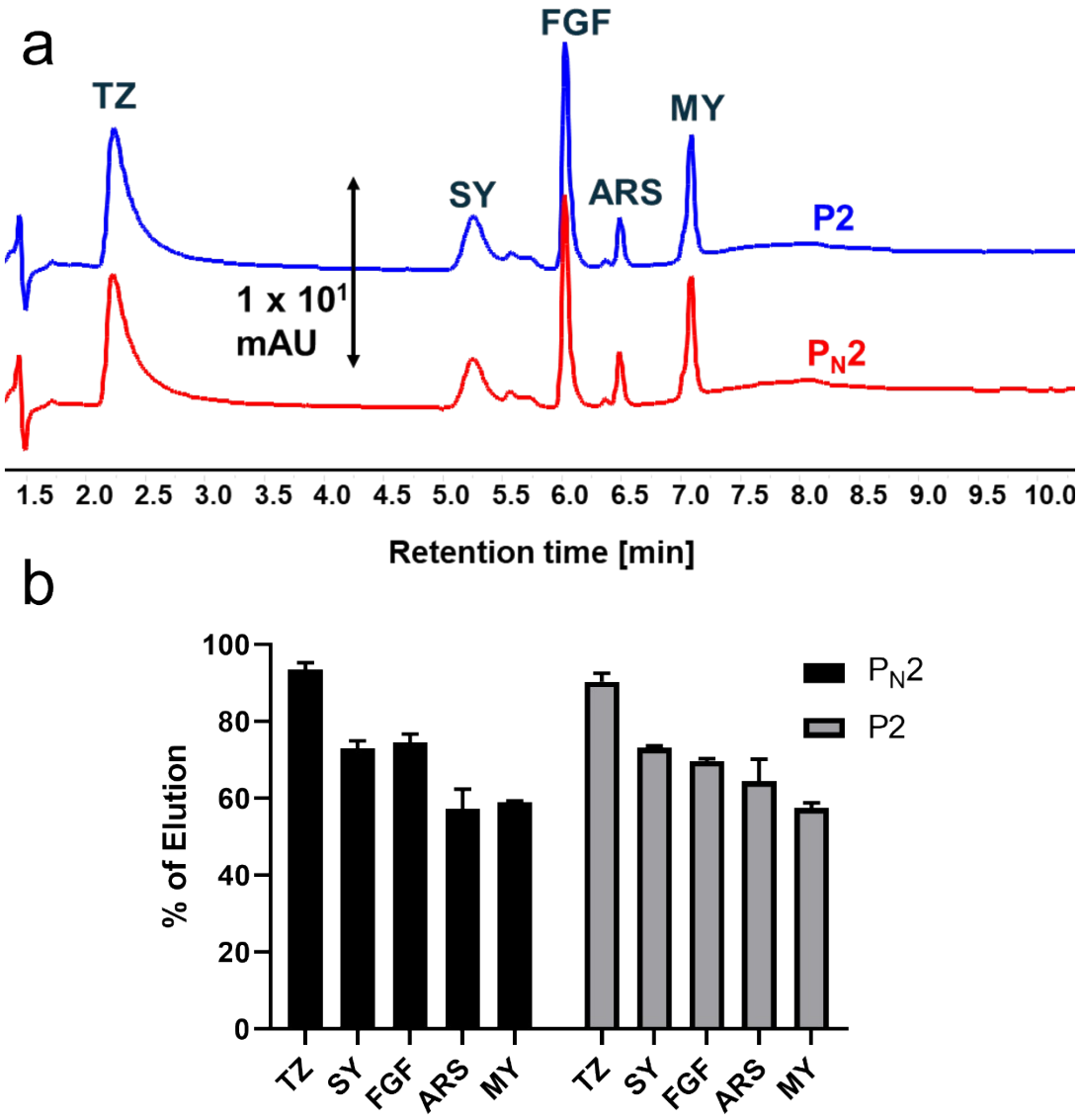


Figure S10. MIP-SPE experiments of mixture of dyes spiked in from water. (a) Overlay spectra of C18-HPLC-UV of combined eluted fractions (E1+E2) of mixture of dyes, (b) % of eluted dyes from P2/P_N2 polymers.

Table S1: Examples of sulfonated dyes imprinted polymer using sulfonated dyes as a template and their polymer compositions and reported applications.

Sr. No.	Template	Polymer Compositions	Polymer format	Applications	Ref
1.	Acid Black 1 Acid Black 210 Acid Brown 703	MAA: EGDMA AIBN (Thermal) T:FM:CL/1:10:44	Bulk Format	Selective recognition Acid Black 1, Acid Black 210, Acid Brown 703 to MIPs and separation of dyes from environmental samples.	[2]
2.	Acid Green 16 (AG16) dye	1-VI: EGDMA (FM:T:CL)/ 0.08: 0.24: 16) AIBN (Thermal)	Bulk Format	Selective recognition and extraction of AG16 dye in water samples	[3]
3.	SY dye	SY: DAC: MBA (APS) 35°C (Thermal)	MIPs grafted @SiO_2	Removal of sunset yellow, acid red 14, acid red 18 from water	[4]
4	Cibacorn Red Dye	MAA: EGDMA, AIBN (Thermal) T:FM:CL/ 2.5/0.8/40	Bulk Format	Dyes removed from tap and wastewater	[5]
5	Acid Violet 19 (AV19) dye	1-VI: EGDMA (FM: T: CL)/ 0.2: 0.8: 4) AIBN (Thermal)	BulkFormat	Removal of AV19 from river dye	[6]
6	SY dye	Titanium iso-propoxide [$Ti(OiPr)_4$] gelling by HCl and thermal treatment	Sol–gel process.	Selective extraction SY dye from mixture of dyes.	[7]
7	SY dye	DMC: EGDMA: AIBN in MeOH: H_2O (T:FM:CL/ 1:4:10)	Polymer NPs	Separation of SY from Water	[8]
8	Tratarzine	1-MA-3MI-Br (T:M/ 1:4) AIBN	Inverse Emulsions on Mag imprinted NPs	Removal of water-soluble dyes such as TZ, Sunset Yellow, Orange G, Brillant ponceau 5R, Amarnath from contaminated water	[9]
9	Direct Red 23	AA: EGDMA in DMSO (T:FM.CL /1 :79:371)	Bulk format	Dyes removed from wastewater	[10]

		AIBN (Thermal)			
10	Congo Red (CR)	MAA: EGDMA: (AIBN in DMSO + MeCN) T:FM:CL/ 0.1:4:20	Polymer particles	Removal of Congo red dye from industrial effluents and contaminated natural waters	[11]

Bulk formats refer to bulk imprinted polymer, and the particles obtained were crushed and sieved.

Following abbreviations are for table as follows:

T: template; FM: functional monomer; CL: Crosslinker; CM: Comonomer. AIBN : 2,2'-Azobisisobutyronitrile; DMSO: Dimethyl Sulfoxide; MeCN: Acetonitrile; MeOH: Methanol; MAA: Methacrylic Acid; EGDMA : Ethylene Glycol Dimethacrylate; mMA**:** methyl methacrylate; VI: vinyl imidazole, SY Dye: Sunset Yellow Dye; CR Dye: Congo Red; DMC: 2-(Methacryloyloxy)ethyl] trimethylammonium chloride); CR P-4B : Cibacron Red P-4B; CO: Cibacron Orange P-4R; CB: Cibacron Black PSG; DAC: acryloyloxyethyl trimethyl ammonium chloride; AMPS: 3-aminopropyltrimethoxysilane; MBA: N,N'-Methylenebisacrylamide; APS: Ammonium persulphate; 1-MA-3MI-Br: 1-(α-methyl acrylate)-3-methylimidazolium bromide:

Table S2 Elution and quantifications of MY dyes from turmeric samples using HPLC-UV data

	Area (E1)	**Area (E2)**	**mM (E1)**	**mM (E2)**	**mg E1**	**mg E2**	**total mg MY**	**mg MY /200 mg turmeric**	mg MY/ 1 g turmeric
N2 (S1)	149.7	1355.4	0.04	0.36	0.015	0.07	0.08	0.82	4.12
M2 (S1)	96.8	3313.4	0.03	0.88	0.010	0.17	0.17	1.75	**8.73**
N2 (S2)	73.4	2007.1	0.02	0.53	0.007	0.10	0.11	1.07	5.36
M2 (S2)	107.6	2537.6	0.03	0.67	0.011	0.13	0.14	1.37	**6.85**
N2 (S3)	11.8	295.7	0.00	0.08	0.001	0.01	0.02	0.16	0.80

M2 (S3)	11.4	276.3	0.00	0.07	0.001	0.01	0.01	0.15	**0.75**
N2 (S4)	-	-							**-**
M2 (S4)	-	-							**-**

References

1. Thorave, R.; Celentano, M.; Bankar, K.; Sellergren, B.; Manesiotis, P.; Shinde, S. A., Synthesis of an Imprinted Receptor Targeted for Sulfonated Aromatic Pollutants by a Stoichiometric Substructure Imprinting Approach. *Polym. Sci. Technol.* **2025,** *1* (5), 488-499.
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