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Extreme Vertices Mixture Design Approach to Optimisation of 1,2,3-trichlorobenzene Specific Molecularly Imprinted Polymer

Dougal Cleland and Adam McCluskey

Supplementary Data

Materials and Methods

Reagents

Methanol (MeOH) was of bulk grade and re-distilled from glass. Acetonitrile (CAN) was of spectroscopic grade (Sigma Aldrich). 1,2,3-Trichlorobenzene (**7**) (Sigma Aldrich) was recrystallised from methanol-water and dried under vacuum at 40 °C. 4-Vinylpyridine (4VP), styrene (STY), 2,3,4,5,6-pentafluorostyrene (PFS), 2,4,6-trimethylstyrene (TMS), and ethyleneglycol dimethacrylate (EGDMA) (Sigma Aldrich) were distilled under reduced pressure. Azobisisobutyronitrile (AIBN) (Sigma Aldrich) was recrystallised from methanol and dried under vacuum at 40 °C.

Synthesis of MIPs

MIPs were prepared by adding the appropriate quantities of **7**, FM (STY. 4VP, PFS or TMS), AIBN (10 mg) and EGDMA to 0.25 mL of MeOH; the quantities for MIP_{STY} corresponding to each of the experimental points (*A*-*P*) are shown in Table 1 and the quantities for MIP_{4VP}, MIP_{TMS}, and MIP_{PFS} are detailed in Table 2. In all instances to ensure complete dissolution of **7**, EGDMA was added first followed by **7** and FM. AIBN (5 mg) was then added and the mixture sonicated for 5 minutes to ensure the complete dissolution of **7** and AIBN. The resulting solution was purged with nitrogen to remove dissolved oxygen. Reaction vials were placed in an ice-water bath where they were allowed 30 minutes to equilibrate with the surrounding temperature. Photochemical polymerisation was then initiated by UV-radiation emitted from a 450 watt quartz mercury vapour lamp (Ace Glass) over a period of 24 hours. NIPs were prepared in an identical manner as the MIPs, excepting for the addition of **7**.

The bulk polymers were ground in MeOH by mortar and pestle and wet sieved (< 45 μM). Smaller particles were removed by repeated mixing in acetone and, after 5 minutes, decanting the supernatant containing suspended fines. Removal of the template, **7**, was achieved by exhaustive soxhlet extraction in MeOH.

Table 1. Quantities of **T**, **FM** and **XL** added to **7**, **STY** MIPs.

Experimental Point	7	FM	EGDMA
	Quantity (mmol)		
A	0.25	0.17	1.25
B	0.10	0.58	1.25
C	0.19	0.48	1.25
D	0.07	0.15	1.25
E	0.08	0.33	1.25
F	0.27	0.27	1.25
G	0.29	0.38	1.25
H	0.16	0.16	1.25
I	0.50	0.75	1.25
J	0.63	0.21	1.25
K	0.94	0.94	1.25
L	1.00	0.25	1.25
M	1.25	0.63	1.25
N	0.42	0.42	1.25
O	0.75	0.50	1.25
P	0.86	0.04	1.25

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Table 2. Quantities of T, FM and XL added to **7**, 4VP, TMS and PFS MIPs.

Experimental Point	7	FM	EGDMA
	Quantity (mmol)		
A	0.86	0.04	1.25
B	1.04	0.31	1.25
C	1.32	0.72	1.25
D	0.65	0.26	1.25
E	0.78	0.57	1.25
F	0.43	0.47	1.25

Batch Rebinding Assays

A measured quantity (20 mg) of the polymers was suspended in 1 mL of 0.1mM **7** solution (1 mL, MeOH : H_2O 7 :3) with shaking at 180-200 rpm for 24 h and then centrifuged at 10 000 rpm for 5 min. An aliquot of the supernatant was then removed for analysis. To insure reliability, all rebinding assays were conducted in triplicate involving the preparation of 3 samples of MIPs and 3 samples of NIPs.

Solid Phase Extraction Rebinding Assays

A measured quantity (20mg) of the polymers was packed into 1 mL solid phase extraction cartridges between porous polyethylene frits. Rebinding solutions with a concentration of 0.1 mM were then prepared by adding the appropriate target to a mixture of MeOH and water (7: 3). The cartridges were first conditioned by eluting 1 mL of MeOH and water (7: 3) under manual pressure applied by a 3 mL syringe. 1 mL of rebinding solution was then eluted under manual pressure (20 seconds) and collected in a 1.7 mL chromatography vial for analysis. All rebinding assays were conducted in triplicate involving preparation of 3 samples of MIPs and 3 samples of NIPs.

Rebinding Analysis

The concentration of target remaining in a solution after a rebinding experiment was analysed by high performance liquid chromatography (Shimadzu LC-20AD) conducted using a 5 µm C_{18} column (Grace Econosphere). The mobile phase for the different targets consisted of 70% MeCN in water. A 20 µL injection volume was used with a run time of 15 minutes and a flow rate of 1.0 mL / minute. Detection was conducted by a photodiode array detector and analysed at 230 nm using Shimadzu LC Solution software. The response of the detector to the target concentration was calibrated using a series of target solutions over a concentration range of 1 to 100 nM.

After the analysis of the rebinding solution, the amount of target bound to a polymer (T_b) was calculated from the difference between the amount of target in the bulk rebinding solution (C_i) and the amount of target remaining in solution after a rebinding experiment (C_f) using Equation 1.

$$T_b = C_i - C_f \qquad \text{(Equation 1)}$$

T he imprinting factor (IF) was then calculated from the amount of target bound to the MIP (T_bMIP) and NIP (T_bNIP) using Equation 8.2.

$$IF = \frac{T_bMIP}{T_bNIP} \qquad \text{(Equation 1)}$$

Chemometric Study

Development and analysis of the extreme vertices mixture designs was conducted using JMP version 08 software (SAS Institute Inc).

Development of an extreme vertices mixture design began by the selection of the upper and lower proportions of T, FM and XL to be evaluated. JMP software then used the XVERT algorithm to calculate experimental points based on extreme vertices and the midpoints of these limits. The MIPs were then synthesised and evaluated as described above.

A mixture design model was then fitted which most closely related the IF of the MIPs to the proportions of T, FM and XL added to the pre-polymerisation mixtures (Equation 3).

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$$y = a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3$$ (Equation 83)

Equation 3 includes the main terms, which are the proportion of T (x_1), FM (x_2), and XL (x_3), and crossed terms, in which the proportions of T and FM (x_1x_2), T and XL (x_1x_3), and FM and XL (x_2x_3), are multiplied. Fitting, which was conducted by JMP software, involved the calculation of coefficients for the terms in Equation 3 (a_1, a_2, a_3, a_{12}, a_{13} and a_{23}) which minimised the sum of squared differences between y and the experimentally measured IFs.

An analysis of variance was then calculated to determine the R^2 for the model. An R^2 is scaled from '0 to 1' where '0' would indicate that the model had no explanatory power and a '1' would indicate that the model exactly fit the experimentally measured IFs. The significance of each coefficient to the fit of the model was then evaluated by its *p*-value, which was calculated from the ratio of the coefficient to its standard deviation. Coefficients with a *p*-value less than 0.05 were considered to have a significant influence on the fitting of the model. If a crossed term had a large *p*-value it was removed and the model was recalculated. The new model was then evaluated and accepted only if the removal of the term did not have a significant effect on R^2. The main terms are, however, required for a mixture design model and were retained irrespective of their *p*-value.

After the fitting of the model it was plotted as a series of contours over a ternary plot of the experimental design. The contours, indicating the level of IF, were then inspected to identify the location on the plot, and the corresponding proportion of T, FM and XL, where the level of IF was the highest.