Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Abbreviations Lists

No	Abbreviation	Full Name	No	Abbreviation	Full Name
1	SAs	sulfonamides	19	PBE	Perdew-Burke-Ernzerhof
2	MMIPs	multi-template imprinted polymers	20	Di-MIP	double template MIP
3	BET	Brunauer–Emmett–Teller	21	MMIP	multi-template MIP
4	DFT	density functional theory	22	NIP	non-imprinted polymer
5	AMR	antimicrobial resistance	23	AM	acrylamide
6	MIPs	molecularly imprinted polymers	24	FESEM	Field emission scanning electron microscopy
7	SBSE	stir-bar sorptive extraction	25	MAA	methacrylic acid
8	SMIP	supramolecularly imprinted polymeric	26	AIBN	azobisisobutyronitrile
9	SPME	solid-phase microextraction	27	EGDMA	ethylene glycol dimethacrylate
10	SMA	sulfamethazine	28	4-VP	4-vinyl pyridine
11	SMO	sulfamethoxazole	29	CHCA	a-cyano-4-hydroxycinnamic acid
12	SCPA	sulfachloropyrazine	30	DHB	2,5-dihydroxybenzoic acid
13	CHCA	a-cyano-4-hydroxycinnamic acid	31	DHAP	2.5-dihydroxy acetophenone
14	S-Acid	sinapinic acid	32	TFA	trifluoroacetic acid
15	ACN	acetonitrile	33	SEM	Scanning electron microscopy
16	DHB	2,5-dihydroxybenzoic acid	34	FT-IR	Fourier transform-infrared

1. Design and Theoretical Calculations of the Template and Functional Monomers

The structural optimization of the SAs and functional monomers were performed by using the DMol^3 package in Materials Studio 6.0. To explore the interaction between each of the functional monomers and target molecules, we further verified the high selectivity recognition of SA molecules at 25 °C by using a theoretical calculation model. The computer windows operating systems based on the calculation module DMol^3 and the functions within the generalized gradient approximation framework using Perdew-Burke-Ernzerhof functional method were selected for DFT-D correction. All calculations were performed using the Materials Studio software package. Molecules were optimized (geometry and energy) individually prior to mixing. Optimized molecules and monomers were subsequently assembled and calculated (**Table S1**). The ground-state structure without symmetric constraints was optimized using density functional theory (DFT). According to the total DFT-D energies and binding energies, the energy value differences of total DFT-D energy (ΔT) and binding energy (ΔE) could be calculated based on the following formulas:

$$\Delta T = T(template: monomer) - T(template) - T(monomer) \quad (1)$$

$$\Delta E = E(template: monomer) - E(template) - E(monomer) \quad (2)$$

2. Synthesis of MMIP

Briefly, mixed template molecules (1mmol) and functional monomer (4 mmol) were dissolved in acetonitrile (5 mL) with the aid of ultrasonication for 5 min. After static storage for 12 h, the cross-linking agent (30 mmol) and initiating agent (50 mg) were added into the mixture, followed by ultrasonic treatment for 5 min. To exclude oxygen, the solution was purged with nitrogen for 5 min. These solutions were injected into a group of capillaries (φ 1.3-1.6 mm), which were fritted at one end. The other group of capillaries (φ 0.5 mm, also fritted at one end) were inserted into the injected ones. The interface was sealed using adhesive tape. These resulting capillaries were placed in a water bath of 60 ℃ for 24 h. After polymerization, the MMIP was immersed into hydrofluoric acid (15%) for 10 min to remove the outer capillaries. Then the MMIP adhering

to the inner capillaries was intercepted at a length of 1.8 cm. Samples without template (the non-imprinted polymer (NIP)) were processed by using the same method. The synthesized MMIP was then eluted by using the mixture of methanol/acetic acid (*v*: *v* = 9:1). Until no residual templates and impurities could be detected by LC-MS. After the template molecules had been eluted, the MMIP was used for extraction analysis. The mass of MMIP was 12.84 mg.

Table S1. Theoretically calculated values of total DFT-D energies and binding energies

	Complex	Total DFT-D Energy /Hartree	Binding (E)/Hartree	ΔT/Hartree	ΔE/Hartree
monomer	4 (4-VP)	-1301.52535	-11.20212	--[a]	--[a]
	4 (MAA)	-1224.98339	-8.30487	--[a]	--[a]
	4 (AM)	-988.41714	-6.76888	--[a]	--[a]
Tri-template	Tri-template (SMA: SMO: SCPA)	-4020.96449	-15.88601	--[a]	--[a]
Homolog	Tri-template :4 (MAA)	-5244.05645	-24.28412	-0.10857	-0.09324
	Tri-template :4 (4-VP)	-5322.59165	-27.16663	-0.10181	-0.07850
	Tri-template :4 (AM)	-5009.56777	-22.82555	-0.18614	-0.17366
	Tri-template :4 (AM) with solvation-water	-5009.62213	-22.88291	-0.24051	-0.23422
	Tri-template :4 (AM) with solvation-methanol	-5009.61583	-22.87661	-0.23421	-0.22792
Homolog-Di-template	(SMA: SMO)	-2407.58983	-11.00075	--[a]	--[a]
	(SMA: SCPA)	-2845.98818	-10.89395	--[a]	--[a]
	(SMO: SCPA)	-2788.42864	-9.95500	--[a]	--[a]
	(SMA: SMO) :4 (AM)	-3396.09762	-17.84780	-0.09066	-0.07818
	(SMA: SCPA) :4 (AM)	-3834.50926	-17.75428	-0.10394	-0.09145
	(SMO: SCPA) :4 (AM)	-3776.97500	-16.84062	-0.12923	-0.11674
Homolog-single-template	SMA	-1232.54840	-5.94356	--[a]	--[a]
	SCPA	-1613.38687	-4.89748	--[a]	--[a]
	SMO	-1175.02179	-5.03755	--[a]	--[a]
	SMA:4 (AM)	-2221.08338	-12.81779	-0.11784	-0.10535
	SCPA:4 (AM)	-2601.92394	-11.77380	-0.11993	-0.10745
	SMO:4 (AM)	-2163.53846	-11.89347	-0.09953	-0.08704

[a] No data was observed

3. Mouse Model and SA Administration

All experimental animal procedures were approved by the animal ethics committee of Hong Kong Baptist University. Balb/c mice (6-8 weeks old) were purchased from the Chinese University of Hong Kong. The mice were divided into five groups and housed separately. The mice were treated by gastric infusion with the mixed sulfanilamide solution, which was dissolved in salt solution at four dosages of 0 (the control group), 10, 50, 100, and 200 mg/kg of body weight, respectively, and administered by gavage. After five hours of sulfanilamide metabolism in the body, the mice were anesthetized and decapitated. The kidney, liver, brain, and heart tissue from each mouse were immediately dissected. After washing with normal saline, the tissue samples were quick-frozen in liquid nitrogen and stored at −80 °C before analysis.

4. SA Residue Identification and Quantification

For the chromatographic detection of the SA templates, acetonitrile:1% acetic acid (30:70, *v*:*v*) was used as mobile phase. Flow rate, column temperature, detection wavelength, and injection volume were set at 1 mL/min, 30°C, 270 nm, and 20 μL, respectively. For MMIP extraction and analysis conditions, 30 mL of the spiked solution was used, the extraction time was 120 min, 1.8 mL of methanol was added as the desorption solvent, ultrasonic desorption was performed for 10 min, the injection volume was 20 μL.

(1) Study on extraction capacity

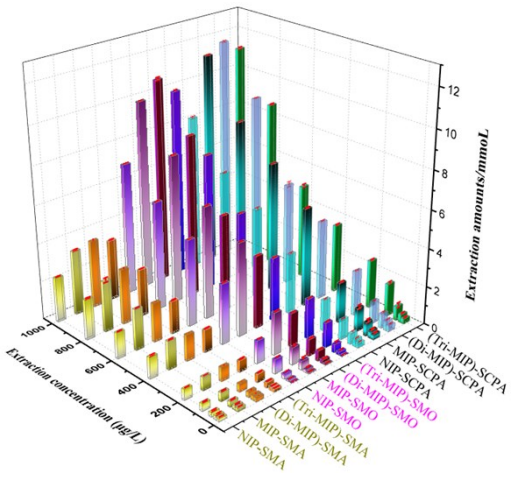

Figure S1. Study on extraction capacity of three SA mixture standards with three kinds of SA MIP (Single-template MIP: SMO-MIP; optimal Di-template MIP: (SMA+SMO)-MIP; Tri-templates MIP: (SMA+SMO+SCPA)-templates MIP) and

NIP.

Table S2. Study on the removal capacity of 100μg/L SAs mixture with different MMIP.

Coatings	Category	Extraction amounts of analytes (nmoL)						
		(1) SMA	RSD%*	(2) SMO	RSD%	(3) SCPA	RSD%	Total
MMIP	SMA+SMO +SCPA	0.4496	4.97	1.3046	5.38	1.7359	7.08	3.4901
Di-MIP	SMA+SMO	0.4217	1.05	0.8706	4.09	1.1763	1.34	2.4685
	SMO+SCPA	0.4246	7.37	0.7766	5.67	1.0427	1.98	2.2439
	SMA+SCPA	0.3976	0.29	0.7108	1.48	1.0252	0.50	2.1336
MIP	SMA	0.4620	2.03	0.8931	7.22	1.2388	5.51	2.5939
	SMO	0.5137	5.51	1.2611	2.87	1.4930	1.23	3.2678
	SCPA	0.3864	3.78	0.7431	1.40	1.1242	0.74	2.2537
NIP	-	0.3946	2.58	0.7396	5.46	1.0452	2.28	2.1794

*n=3

（2）Selectivity adsorption experiments

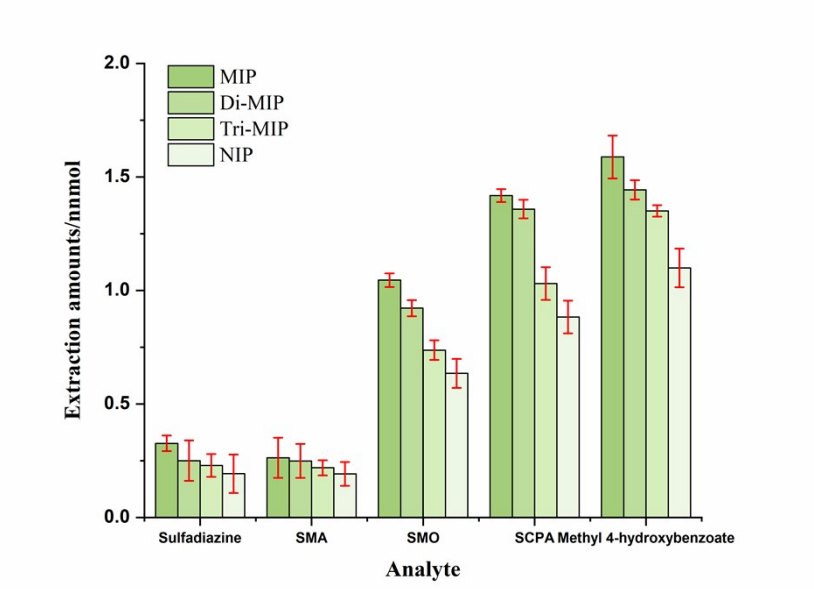

Figure S2. Study of the selectivity adsorption capacity of 100 μg/L sulfonamide mixture by different coatings.

5. *Characterization*

The morphology and structures of MMIP polymers were studied by using scanning electron microscopy (SEM, Nova Nano SEM 450FEI-IMC, USA). All the MIP polymers were studied by Fourier transform-infrared (FT-IR) spectrophotometry (TENSOR-27, Bruker, Germany). Surface area analysis of the polymer was carried out with a Micromeritics TriStar II 3020 instrument (Norcross, GA) based on nitrogen sorption perimetry. The surface area was measured by the Brunauer–Emmett–Teller (BET) method.

The MMIP has a broad infrared absorption peak at 3458cm^{-1}, which is the stretching vibration of the N-H bond in the functional monomer acrylamide. The peak at 2979 cm^{-1} corresponds to the CH stretching vibration of the methyl group of the cross-linking agent EGDMA, and a strong characteristic peak appears at 1717 cm^{-1}, which is caused by the stretching vibration of C=O in acrylamide The characteristic peaks of saturated CH bonds appear at 1450 cm^{-1}, and the characteristic peaks appearing at 1163 cm^{-1} are -COC- in the ester group (telescopic vibration). FTIR spectra of the four polymers are very similar.

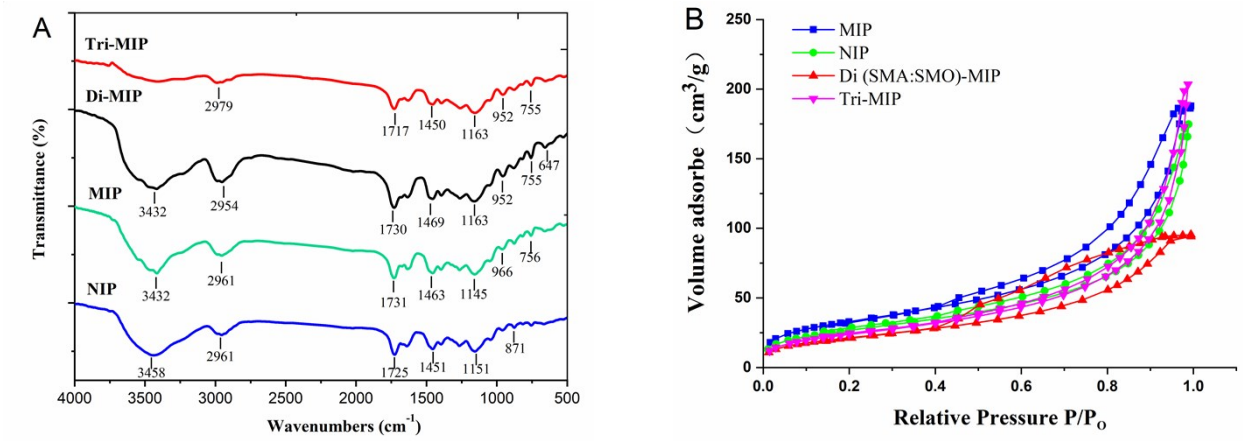

Figure S3. Comparison of infrared characterization results (A) and nitrogen adsorption and desorption (B).

Figure S3B shows that the three MIPs and NIP have class IV isotherms, similar to the second class, which is due to multi-molecular layer adsorption processes on the macroporous adsorbent. In addition, an adsorption hysteresis loop appears in the middle segment, indicating that the system is porous and has capillary condensation, which may lead to multi-layer adsorption.

Table S3. Surface area and pore analysis of MMIP.

Polymers	BET Surface area (m^2/g)	Average pore diameter (nm)	Total pore volume (cm^3/g)
NIP	98.18	11.72	0.28
MIP	120.14	9.92	0.31
Di-MIP	78.41	6.89	0.25
MMIP	87.99	14.19	0.33

The morphologies of MIP and NIP were investigated by field emission scanning electron microscopy (FESEM). The porous structure in MIP indicated that they could provide special cavities, which would be beneficial for the adsorption and desorption of the target analytes. However, there are fewer cavities in the NIP polymer. As can be seen from the average pore diameter, compared with the other two polymers, it is evident that the double-templated MIP possesses more significant and greater numbers of cavities conferring improved extraction performance.

6. Study of MMIP using Adsorption Models

Batch rebinding studies are key methods to characterize and compare MMIP adsorption formulations. The distribution of binding sites and the affinity of the polymer for the templates can be evaluated by binding isotherm experiments using different binding models. The binding isotherms were investigated over various initial concentrations ranging from 50 to 1000 μg/L of mixed adsorbate solution. The corresponding binding parameters calculated by the Scatchard diagram are the binding affinity (*K*) and the number of binding sites (*Q*). The experimental binding isotherm is plotted q/C versus q format：

$$Q/C_e = (Q_{max} - Q)/K_d \quad (3)$$

where Q is the concentration of the analytes bound to the coatings in μmol/g, and C_e is the concentration of free analytes remaining in the solution in 50 μmol/L. The Scatchard plots for most MIPs are curved, without exception for the synthesized MIP in this paper. This curvature has been considered as binding heterogeneity, shown as two separate straight lines, which represent two classes of sites.

Table S4. The results of the Scatchard curve analysis.

Category	MIP		Di-MIP		MMIP		MIP		Di-MIP		MMIP	
	Q_M-high	Q_M-low	Q_M-high	Q_M-low	Q_M-high	Q_M-low	K-high	K-low	K-high	K-low	K-high	K-low
SMA	0.031	1.08	0.017	1.34	0.027	1.01	0.68	5.52	0.75	7.12	0.59	4.56
SMO	0.046	1.13	0.042	1.56	0.10	1.72	1.35	14.36	1.70	22.18	4.34	23.24
SCPA	0.071	1.52	0.058	1.78	0.25	1.22	3.57	31.31	4.05	34.32	9.06	23.56
Total	0.148	3.73	0.117	4.68	0.377	3.95	5.6	51.19	6.5	63.62	13.99	51.36

We also established Scatchard analysis curves to study the adsorption mechanism model. It can be seen from **Table S4** that the specific adsorption quantity at the high-affinity site is low and that the total *Q* of MIP is smaller than that of Di-MIP and MMIP. The comparison results show that both binding affinity and the number of binding sites of MMIP are more significant than that of MIP, which further proves that the force of MMIP binding sites is conducive to analyte adsorption.

7. Preparation of Mouse Tissue Samples

Removal and quantification of the accumulative SA were carried out by using MMIP adsorption combined with UHPLC-MS/MS analysis. After five hours of metabolism, the mouse tissue (brain, heart, kidney, and liver) samples from each exposure group were accurately weighed (accurate to 0.0001 g). The samples were then homogenized according to the method. Finally, the remaining antibiotics in the solution were adsorbed by MMIP and further desorbed with the optimal desorption solvent. The SA residues were further quantified by using a Thermo Scientific UHPLC coupled to a TSQ Quantiva™ Triple Quadrupole Mass Spectrometer (UHPLC-MS/MS). Positive ionization mode and multiple reaction monitoring were carried out for the analysis. In order to quantify the template, the extraction amount by MMIP was calculated using the following formulas:

$$N\ (ng) = 200(A-b)/a \qquad (4)$$

$$n\ (pmol) = 1000 \times N\ (ng)/M \qquad (5)$$

In the above formula, N (ng) and n (pmol) are the extraction amounts of the analyte. A is the chromatographic peak area, a and b are the slope and intercept of the standard curve of the analytes obtained by direct injection chromatography. M is the relative molecular mass of the analyte.

The removal and quantification of the accumulated SA drugs were carried out by using MMIP after five hours of mouse metabolism. The mouse tissue (brain, heart, kidney, and liver) samples from each exposure group were accurately weighed (accurate to 0.0001 g). Each tissue was chopped with a small amount of acetonitrile and crushed twice in a ball mill for 5 min. Samples of each tissue (20 mg approx.) were placed individually in a crushing tube. After mixing the crushed solution in a 50 mL stoppered centrifuge tube, 5 g of anhydrous sodium sulfate and 10 mL of acetonitrile were added, followed by freezing and ultrasonic extraction for 15 min, then centrifugation at 5000 rpm for 5 min. The supernatant (5 mL) was placed in a brown glass bottle, 5 mL of acetonitrile was added to the residue. The mixture was decompressed underwater at 40 °C and dried with nitrogen. The residue was transferred to a centrifuge tube, then 0.5 mL of the initial

mobile phase of 0.1 % formic acid: acetonitrile solution (3: 7, *v*: *v*) was added followed by centrifugation at 10000 rpm for 5 min. The initial mobile phase was pre-conditioned with 1 mL (0.5 mg/mL of isotope-labeled standards of sulfadimethxine-d_4 and sulfadoxine-d_4). The lower layer was filtered through a 0.2 μm filter membrane, and the remaining samples were diluted to 30 mL with deionized water. The pH value of the sample solution was 7.0. Each sample solution was sealed and stored in the refrigerator for the next extraction. Furthermore, for the MMIP extraction, 180 μL of methanol was added as the desorption solvent and ultrasonic desorption was performed for 10 min, the injection volume was 20 μL.

8. Conditions for UHPLC-MS/MS

The SA residues were analyzed using a Thermo Scientific UHPLC coupled to a TSQ Quantiva™ Triple Quadrupole Mass Spectrometer (UHPLC–MS/MS). A reverse-phase column Luna C18 (2.1 mm × 100 mm × 1.6 μm, Phenomenex) was applied for chromatographic separation.

Table S5. Instrumental method for the analysis of SAs by UHPLC–MS/MS.

Instrument	Thermo Scientific UHPLC coupled to a TSQ Quantiva™ Triple Quadrupole Mass Spectrometer (UHPLC–MS/MS).
Program	Sample plate temperature: 4 °C Column temperature: 35℃ Mobile phase A: B 0.1 % formic acid: acetonitrile solution The injection volume: 10μL Gradient program: 0.0−0.3 min, 10 % B; 1.0-8.0 min, from 10 % B to 100 % B; 8.0−11.0 min, 100 % B and kept for 3.0 min; 11.0−11.2 min, from 100 % B to 10 % B; 11.2−13.0 min, 10 % B
MS/MS Parameters	MRM in positive ionization mode Capillary voltage (kV) = 2.5; Sheath gas (arbitrary units) = 40; Auxiliary gas (arbitrary units) = 10; Ion transfer tube temperature (°C) = 350; Vaporizer temperature (°C) = 300.

Table S6. MS conditions for the target analytes.

Analytes	Molecular formula	[M+H]+	R.T. (min)	Ion mode	Quantitative transition	Qualitative transition	Collision energy (eV)
SMA	$C_{12}H_{14}N_4O_2S$	279.09	4.91	P	→186.0	→124.0	20
SMO	$C_{10}H_{11}N_3O_3S$	254.05	5.77	P	→156.0	→108.0	20
SCPA	$C_{10}H_9ClN_4O_2S$	285.02	6.30	P	→130.0	→108.2	25
sulfadimethxine-d_4	$C_{12}H_{10}D_4N_4O_4S$	315		P	→156.0	-[a]	20
sulfadoxine-d_4	$C_{12}H_{10}D_4N_4O_4S$	315		P	→160.0	-[a]	20

[a] No data was observed.

Table S7. The established method and method validation in mouse sample.

Analytes	Linear range（μg/L）	Equation	Correlation coefficients	LOD(μg/L)	LOQ(μg/L)	Added 100μg/L*	
						Recovery rate (%)	RSD (%)
SMA	0.1-500	Y = 0.11+0.093*X	0.9994	0.03	0.1	110.54	2.56
SMO	10-500	Y = -0.05+0.012*X	0.9990	5	10	97.14	2.28
SCPA	10-500	Y = 0.001+0.0015*X	0.9987	5	10	95.76	3.62

*The spiked was performed in the mouse kidney tissue sample, n=3.

Table S8. Comparison of the published methods for multi-template imprinted polymer analysis with the proposed method in this work.

Sample preparation	Samples	Mass	Detection method	Analytes	Linear rang	LOD	LOQ	Recovery (%)	Ref.	Available times	Maximum
VTTS-MGO@mSiO2@MIP	Well water Lake water Pond water	0.02g	DLLME-HPLC-PDA	bisphenol A 4-tert-OP 4-nonylphenol	0.05-10μg/L	0.013, 0.01μg/L	-	81.5–104.1	1	-	-
MIP-SPE	Wastewater	0.05g	HPLC	ibuprofen, naproxen and diclofenac	5-50μg/L	0.15, 1.00 and 0.63 μg/L	0.5-3.3μg/L	82-103	2	-	11.4μg/L
MIP particles-SPE	Milk	0.03g	HPLC-DAD	six sulfonamide	50–500 mg/kg	1.9-13.3 μg/kg	5.6-42.2 μg/kg	85.8-115.7	3	-	101.9-113.5 μg/kg
DMIP-SPE	Maize, wheat and cottonseed samples	0.05g+3 ml ACN	LC–MS/MS	atrazine and prometryn	10-200 μg/kg	0.5–8.8 μg/kg	-	61.3–105.9	4	5 times	-
MMIP	Mouse brain Liver Kidney heart	0.012g	UHPLC-MS/MS	SMA SMO SCPA	0.1-500 μg/L	0.03μg/L (0.27μg/kg)	0.1μg/L (0.81μg/kg)	95-110	This work	More than 100 tims	598.12mg/g

References

[1] X. Xie, X. Ma, L. Guo, Y. Fan, G. Zeng, M. Zhang, J. Li, *Chem. Eng. J.*, 2019, **357**, 56-65.

[2] L. M. Madikizela, L. Chimuka, *J. Pharm. Biomed. Anal.*, 2016, **128**, 210-215.

[3] M. Kechagia, V. Samanidou, A. Kabir, K. G. Furton, *J. Sep. Sci.*, 2018, **41**, 723-731.

[4] S. Wang, Y. She, S. Hong, X. Du, M. Yan, Y. Wang, Y. Qi, M. Wang, W. Jiang, J. Wang, *J. Hazard. Mater*., 2019, **367**, 686-693.