Supporting Information

Magnetism-Assisted Density Gradient Separation of Microplastics

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REAGENTS AND MATERIALS

Sodium hydroxide (NaOH) was purchased from Alfa Aesar (Heysham, England). Manganese (II) chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$) was purchased from Sigma-Aldrich (Beijing, China). Tween 20 was purchased from bioWORLD, Ltd. (Dublin, OH, USA). 4-(2-Pyridylazo)resorcinol monosodium salt hydrate was purchased from Sigma-Aldrich (Darmstadt, Germany). Red PE microspheres (0.98 g cm^{-3}, 180–212 μm), green PE microspheres (1.025±0.005 g cm^{-3}, 180-212 μm), blue PE microspheres (1.08±0.005 g cm^{-3}, 180-212 μm), white PE microspheres (1.35±0.05 g cm^{-3}, 180-212 μm), and borosilicate glass microspheres (2.20±0.1 g cm^{-3}, 180-212 μm) were purchased from Cospheric, LLC. (Santa Barbara, CA, USA). The soil sample was collected from the campus of the University of Tasmania. Roadside soil was collected at the East Derwent Highway in Hobart (Australia). All chemicals were used as received. All solutions were prepared in milli-Q water obtained from a Millipore (North Ryde, Australia) purification system.

Neodymium cylinder magnets (21004/N52) with dimensions of 25.4 mm (diameter)×50.8 mm (length) were purchased from AMF Magnetics, Ltd. (Rozelle, NSW, AU). The separation cell was 3D printed using SUP707 water-soluble supporting material and Veroclear-RGD810 printing material, both purchased from Stratasys, Ltd. (Eden Prairie, MN, USA). The support for the magnets was 3D printed using a polylactic acid filament purchased from Prusa Research, Ltd. (Partyzánská, Prague, Czech Republic).

INSTRUMENTATION

The magnified images of the microspheres were collected using a Nikon Eclipse E200 optical microscope (Nikon, Melville, NY, USA). A Prusa i3 MK2S 3D printer (Prusa, Partyzánská, Prague, Czech Republic) was used to fabricate the magnet housing. The quaternary HPLC pump from a Waters Alliance 2695 Separations Module (Waters, Milford, MA, USA) was used for generating the $MnCl_2$ gradient. A Metertech SP-8001 UV-visible spectrophotometer (Nankang, Taipei, Taiwan) was used to determine the Mn(II) concentration in the separation cell. FT-IR spectra were collected from the Bruker Vertex 70V FT-IR spectrometer (Bruker, Billerica, MA, USA).

A Stratasys Objet Eden 260VS (Eden Prairie, MN, USA) 3D printer was used to fabricate the separation cell. After 3D printing, the separation cell was soaked in milli-Q water for 30 min, followed by sonication in a 2% NaOH solution for 8 h to remove the water-soluble support material. Then the device was rinsed with milli-Q water (5×100 mL) to remove any residual NaOH and was air-dried overnight.

DETECTION OF Mn(II) CONCENTRATION AND SOLVENT DENSITY IN THE SEPARATION CELL

The Mn(II) concentration in the separation cell was determined by a simplified colorimetric method. Fractions of the paramagnetic solution were collected every minute from the outlet of the separation cell using a 2 mL Eppendorf tube. A volume of 5 μL of the collected fraction was diluted to 1 mL using milli-Q water. A volume of 0.25 mL of the abovementioned dilution was mixed with 4-(2-pyridylazo)resorcinol monosodium salt solution (1 mL, 0.05 M) and left for 10 min. After colour development, 50 μL of the solution of the produced metal complex was further diluted to 10 mL for quantification using UV-vis spectrophotometric detection at 496 nm (Figure S4, pink line). Linearity of this method ($y_{adsorption,\ A.U.} = 0.01466 + 0.04483x_{Mn\ concentration,\ M}$) was evaluated using Mn(II) standard solutions with concentrations from 0.5 M to 4 M, and it showed acceptable linearity (R^2>0.97).

The solvent density (ρ) in the separation cell was determined by weighing difference in mass (m_d) before and after accurately retrieving 0.1 mL solvent in selected fractions (Figure S4, violet line). The solvent density in each fraction was calculated by Equation S1.

$$\rho = \frac{m_d}{0.1\ mL} \quad (1)$$

DETERMINATION OF RESIDENCE TIME AND RESOLUTION

To evaluate the separation performance quantitatively, we defined the residence time of each type of microspheres by the weighted sum of its group (Equation S2).

$$t_r = \sum P_i t_i \quad (2)$$

in which t_r is the average residence time of each type of microspheres, P_i is the fraction of each type of microspheres present in a group (gradient samples collected every 30 s), t_i is the individual residence time of microsphere in the given group.

Therefore, we can also define the resolution (Rs) using Equation S3.

$$R_s = \frac{2(t_{ra} - t_{rb})}{w_a + W_b} \quad (3)$$

in which R_s is resolution between two group a and b, t_{ra} and t_{rb} are calculated residence times from group a and b, w_a and w_b are group widths from group a and b. Group width, w, is the time window a group occurred. The resolution from adjacent groups is calculated and shown below the corresponding plots. Because of non-integral groups, resolution calculated in this method cannot be directly compared with that in traditional chromatography. Generally, Rs≥1 means two fully separated groups. In exceptional cases (long tailing, high asymmetry), we will integrate resolution with other factors to evaluate the separation performance adequately.

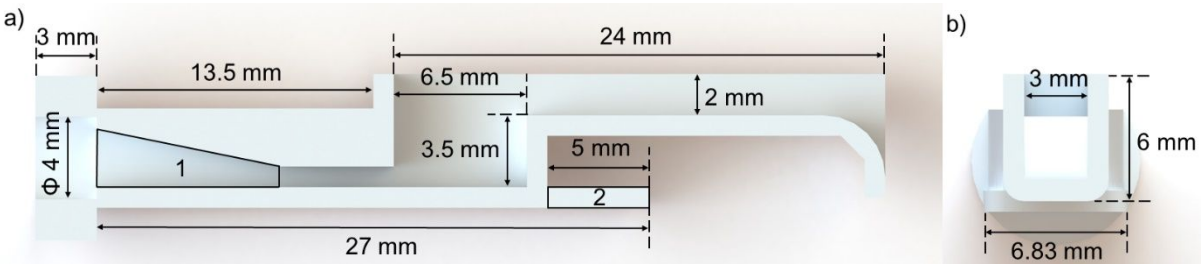


Figure S1. a) Side section and b) front views of the bespoken separation cell with annotated dimensions. Zone 1. The taper channel for the gradient flow to prevent any bubbles from accessing to the stepped container. Zone 2. Protrusion to fix the separation cell to the 3D printed anchorage shown in Figure 1.

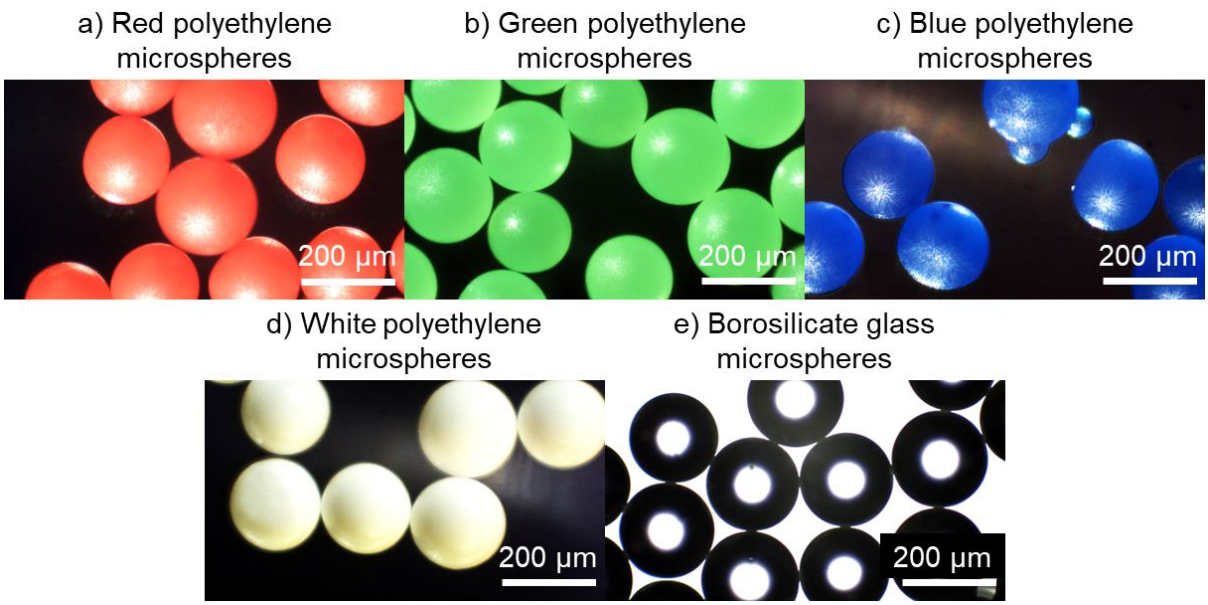


Figure S2. Optical microscope images (100X) of the a) red PE, 0.98 g cm^{-3}; b) green PE, 1.025 g cm^{-3}; c) blue PE, 1.08 g cm^{-3}; d) white PE, 1.35 g cm^{-3}, and e) borosilicate glass (2.20 g cm^{-3}) microspheres selected in this study.

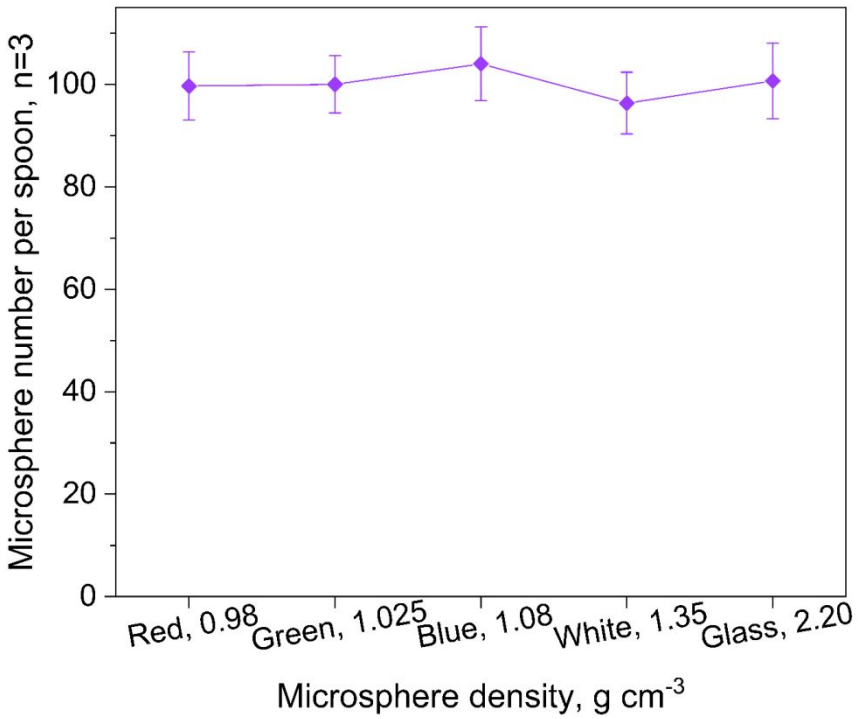


Figure S3. Average microsphere number per spoon. Red PE microspheres, 0.98 g cm^{-3}; green PE microspheres, 1.025 g cm^{-3}; blue PE microspheres, 1.08 g cm^{-3}; white PE microspheres, 1.35 g cm^{-3}; borosilicate glass microspheres, 2.20 g cm^{-3}.

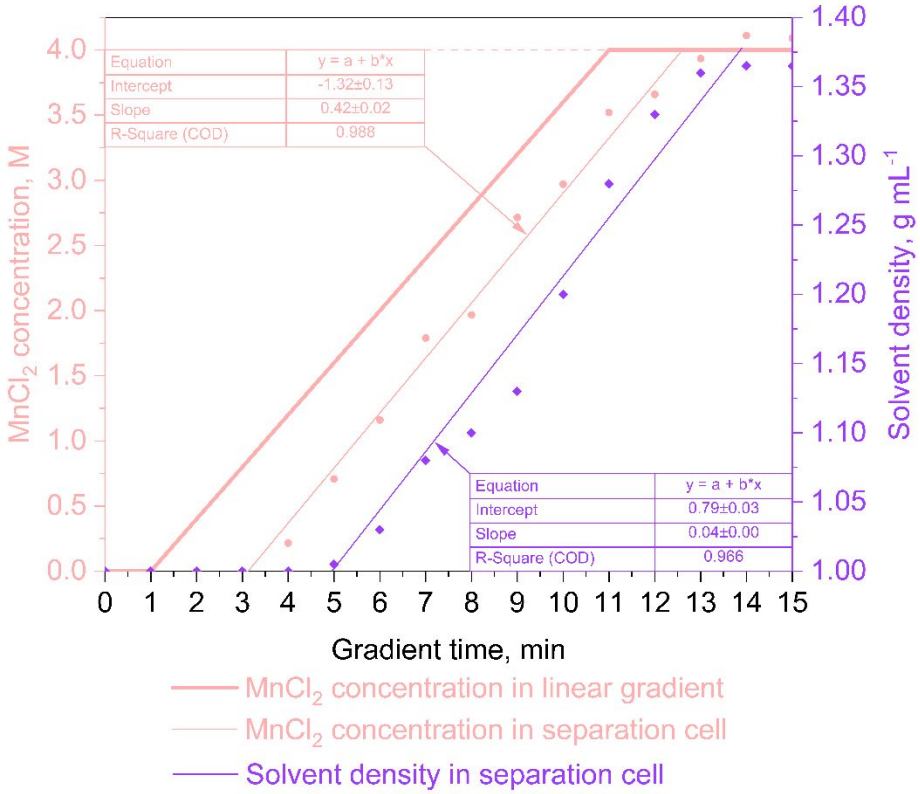
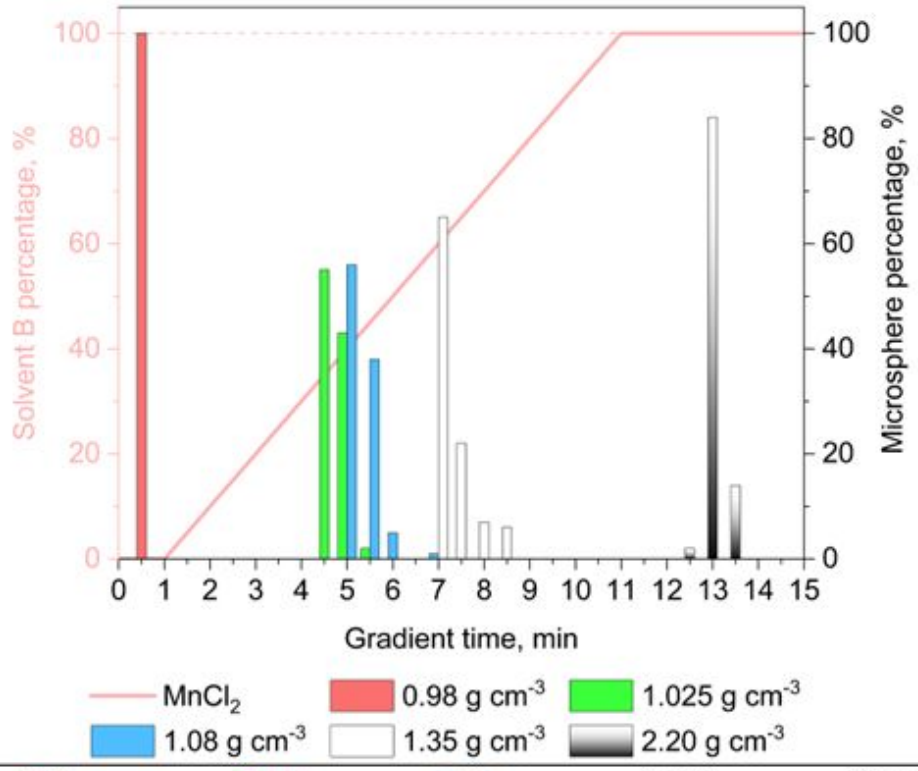
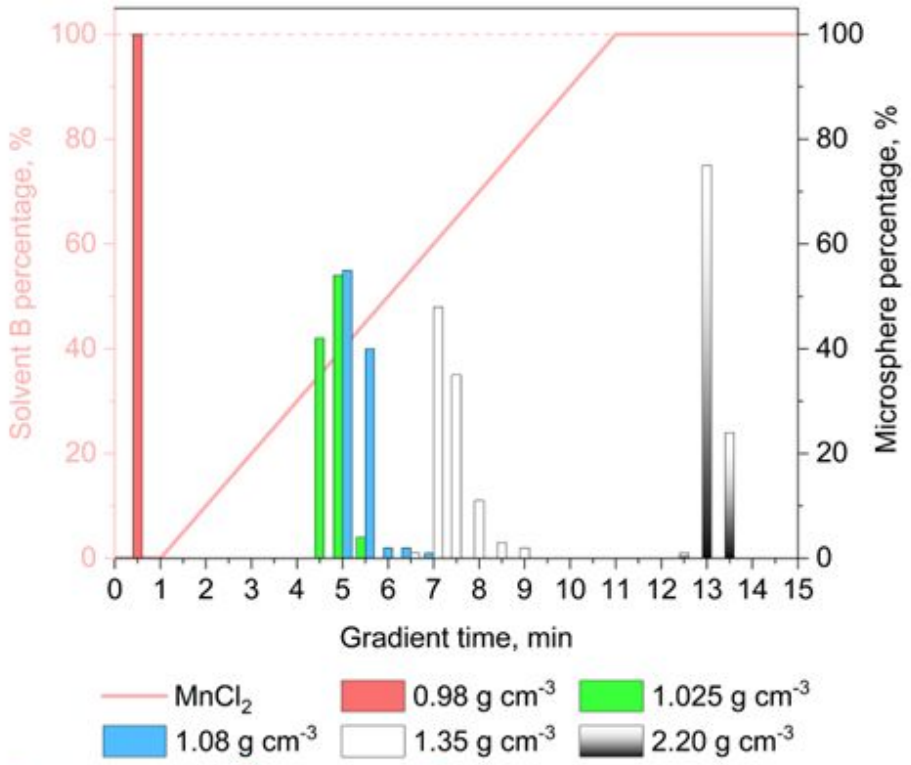


Figure S4. Mn(II) concentration and solvent density in the separation cell (n=3). Gradient method, linear.



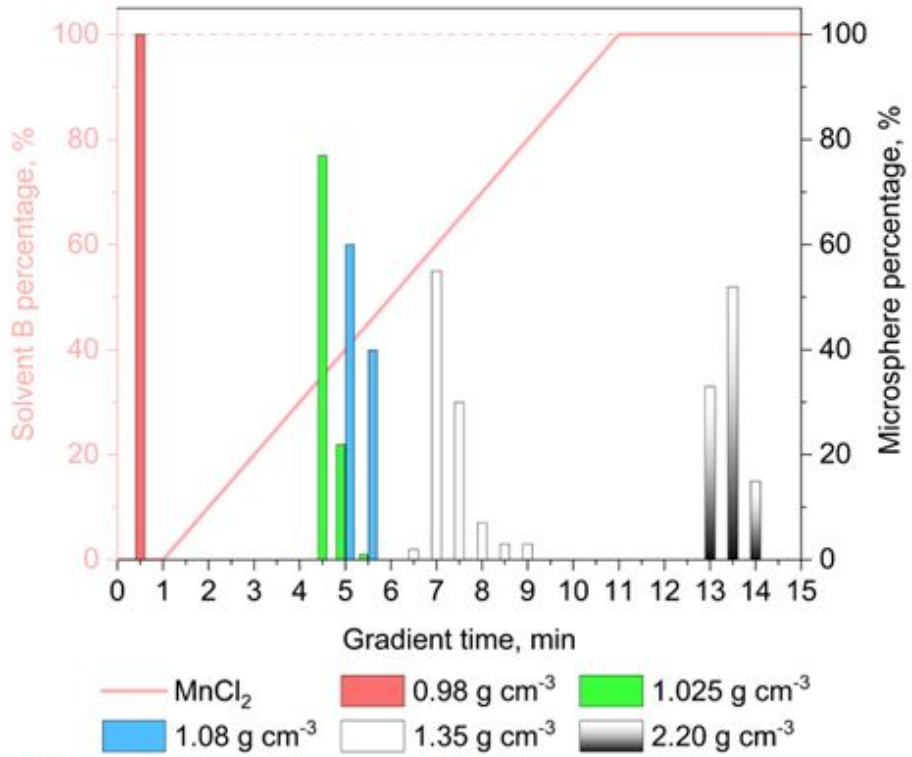
	Red	Green	Blue	White	Glass
Residence time, min	0.5	4.74	5.26	7.27	13.06
Group width, min	0.5	1.5	2.5	2.0	1.5
Resolution	4.24	0.26	0.89	3.31	

Figure S5. Linear Mag-DG-Sep for the simultaneous separation of microspheres. Replication of experiment shown in Figure 3b.



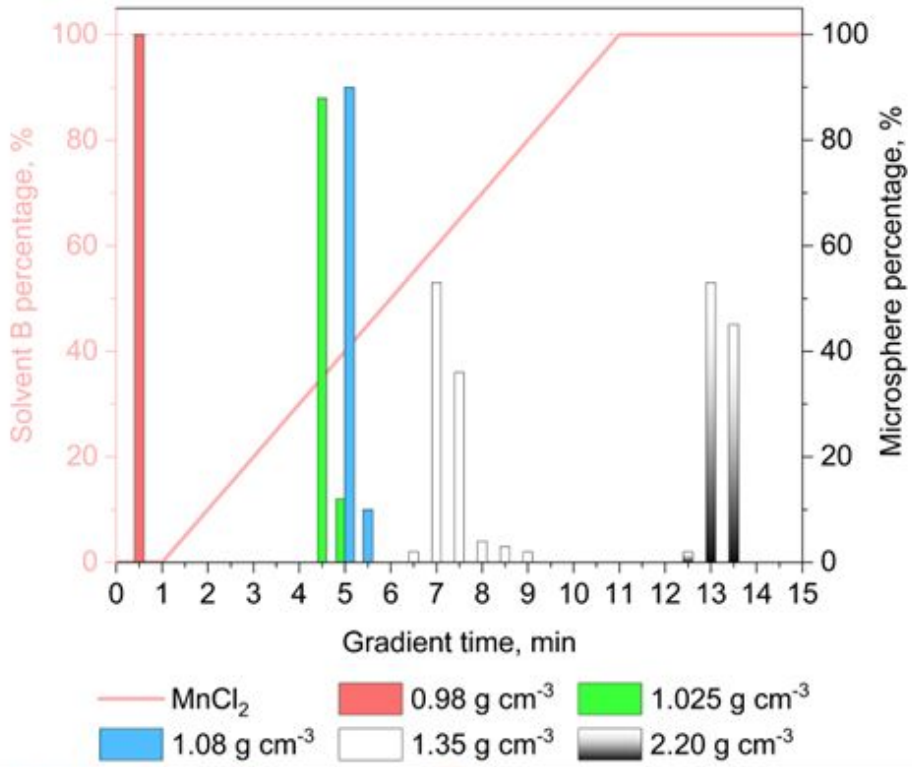
	Red	Green	Blue	White	Glass
Residence time, min	0.5	4.81	5.27	7.37	13.12
Group width, min	0.5	1.5	2.5	3.0	1.5
Resolution	4.31	0.23	0.76	2.56	

Figure S6. Linear Mag-DG-Sep for the simultaneous separation of microspheres. Additional replication of experiment shown in Figure 3b.



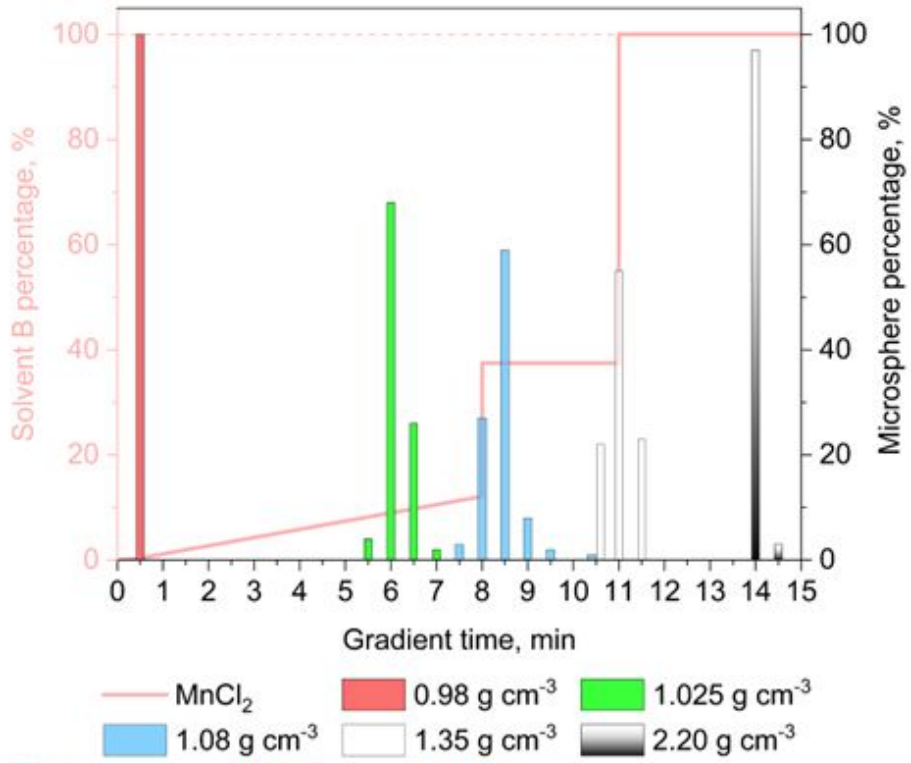
	Red	Green	Blue	White	Glass
Residence time, min	0.5	4.62	5.20	7.32	13.41
Group width, min	0.5	1.5	1.0	3.0	1.5
Resolution	4.12	0.46	1.06	2.71	

Figure S7. Overlaid plots for the Mag-DG-Sep of individual types of microspheres. Replication of experiment shown in Figure 3c.



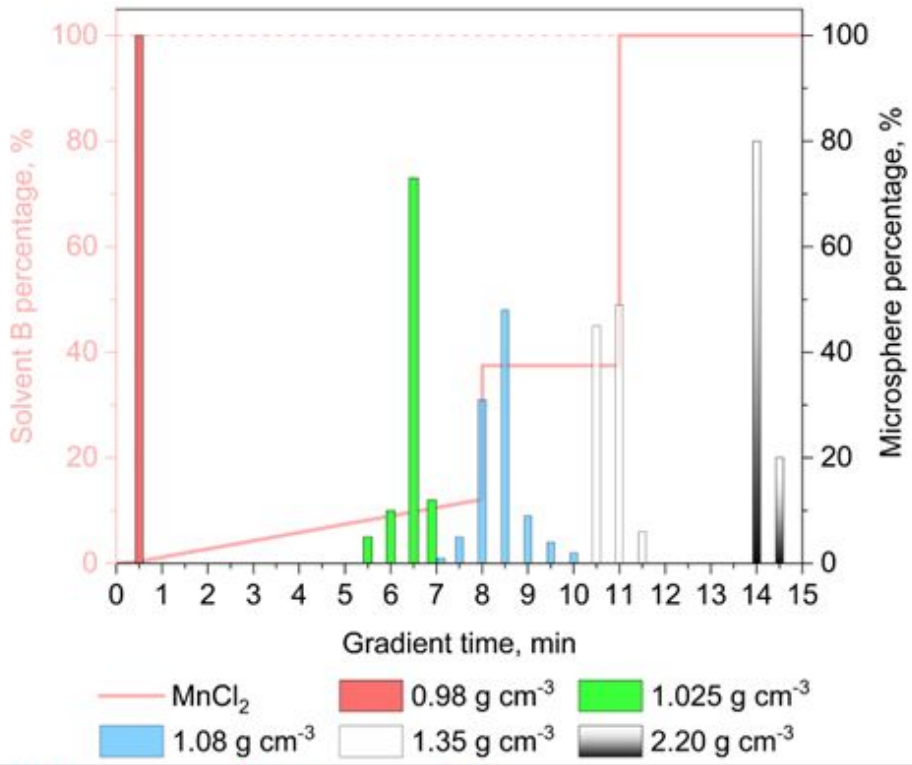
	Red	Green	Blue	White	Glass
Residence time, min	0.5	4.56	5.05	7.30	13.22
Group width, min	0.5	1.0	1.0	3.0	1.5
Resolution	5.41	0.49	1.12	2.63	

Figure S8. Overlaid plots for the Mag-DG-Sep of individual types of microspheres. Additional replication of experiment shown in Figure 3c.



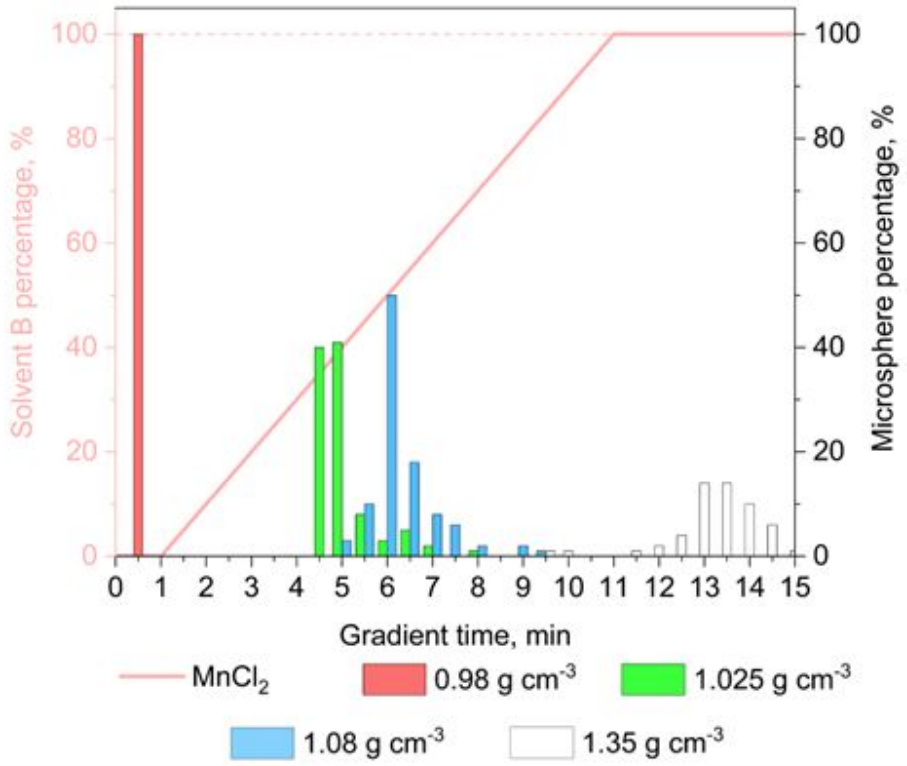
	Red	Green	Blue	White	Glass
Residence time, min	0.5	6.13	8.42	11.01	14.02
Group width, min	0.5	2.0	3.5	1.5	1.0
Resolution	4.50	0.83	1.04	2.41	

Figure S9. Step Mag-DG-Sep for the simultaneous separation of microspheres. Replication of experiment shown in Figure 4b.



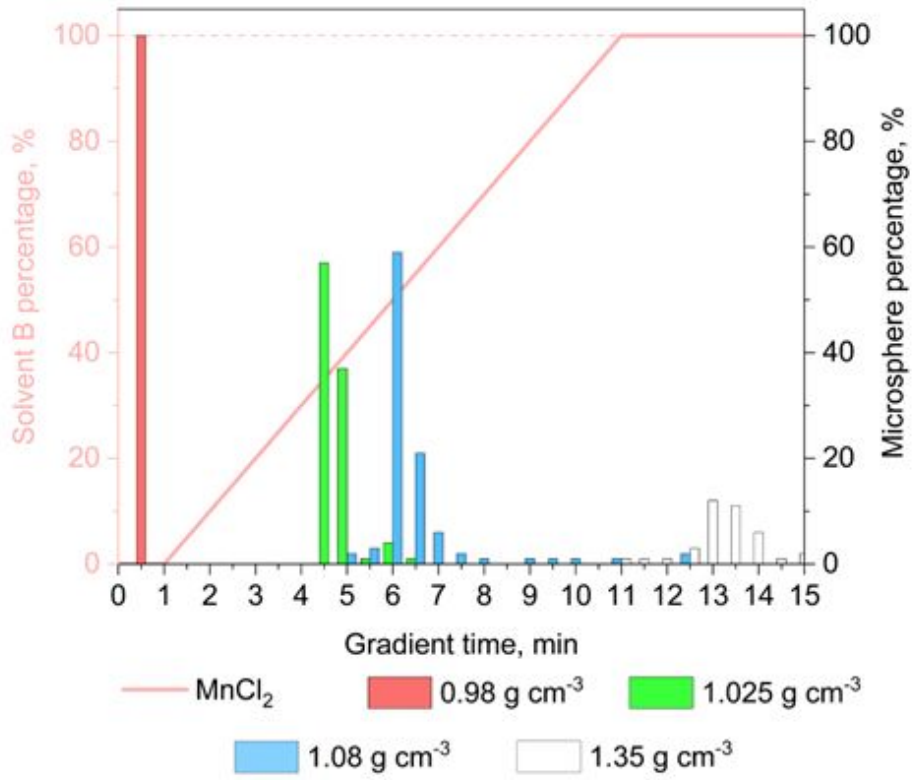
	Red	Green	Blue	White	Glass
Residence time, min	0.5	6.46	8.40	10.81	14.10
Group width, min	0.5	2.0	3.5	1.5	1.0
Resolution	4.77	0.70	0.96	2.64	

Figure S10. Step Mag-DG-Sep for the simultaneous separation of microspheres. Additional replication of experiment shown in Figure 4b.



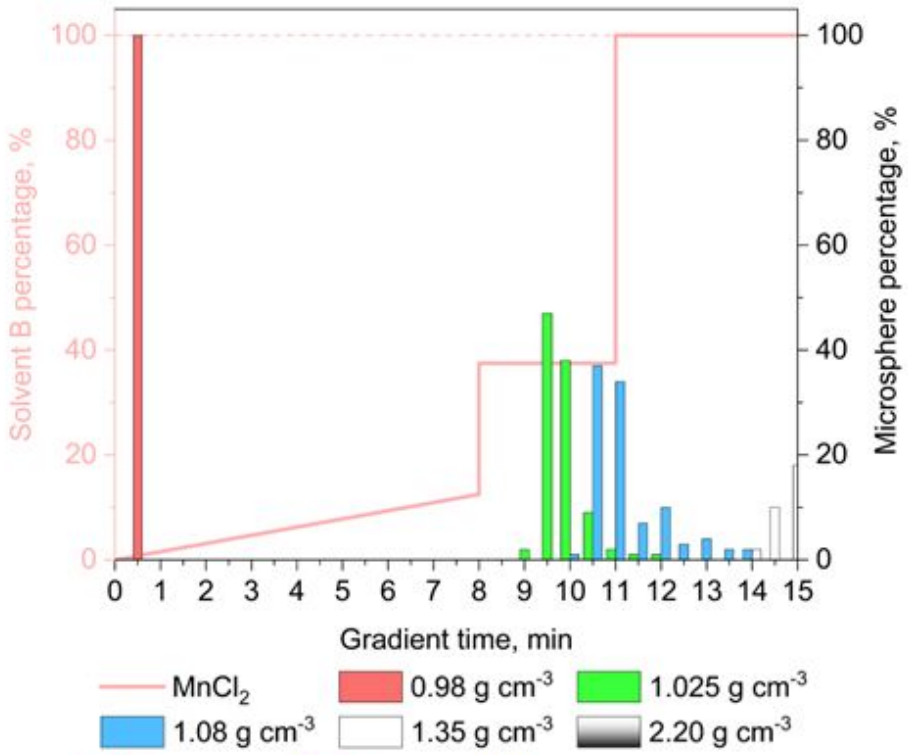
	Red	Green	Blue	White	Glass
Residence time, min	0.5	5.02	6.32	N/A	N/A
Group width, min	0.5	4.0	5.0	N/A	N/A
Resolution	2.01	0.29	N/A	N/A	

Figure S11. Linear Mag-DG-Sep (in the absence of magnets) for the simultaneous separation of microspheres. Replication of experiment shown in Figure 5b.



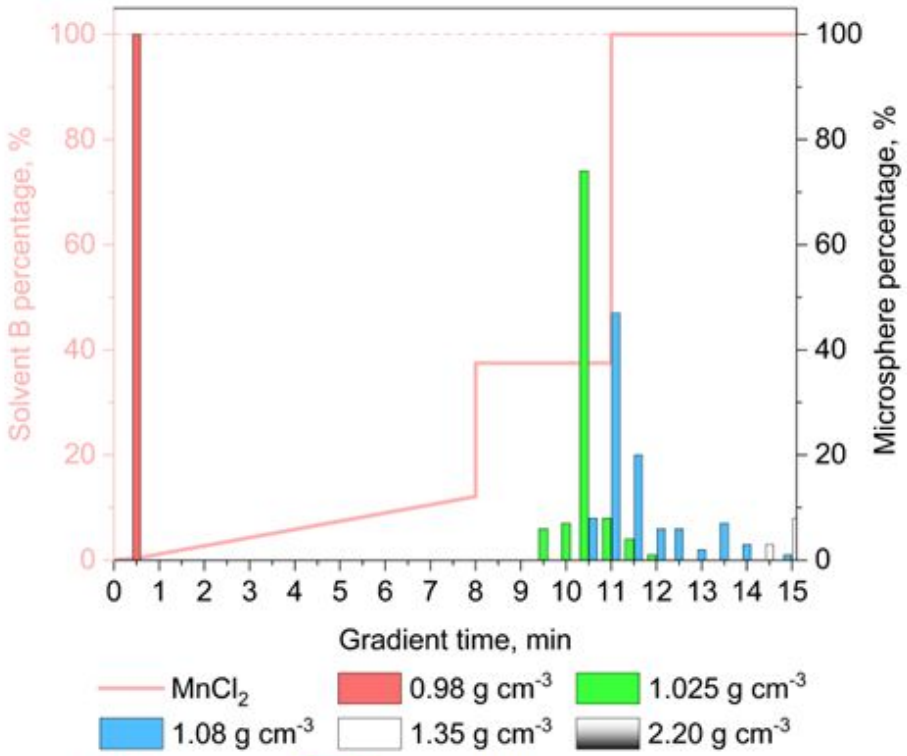
	Red	Green	Blue	White	Glass
Residence time, min	0.5	4.78	6.46	N/A	N/A
Group width, min	0.5	2.5	8.0	N/A	N/A
Resolution	2.85	0.32	N/A	N/A	

Figure S12. Linear Mag-DG-Sep (in the absence of magnets) for the simultaneous separation of microspheres. Additional replication of experiment shown in Figure 5b.



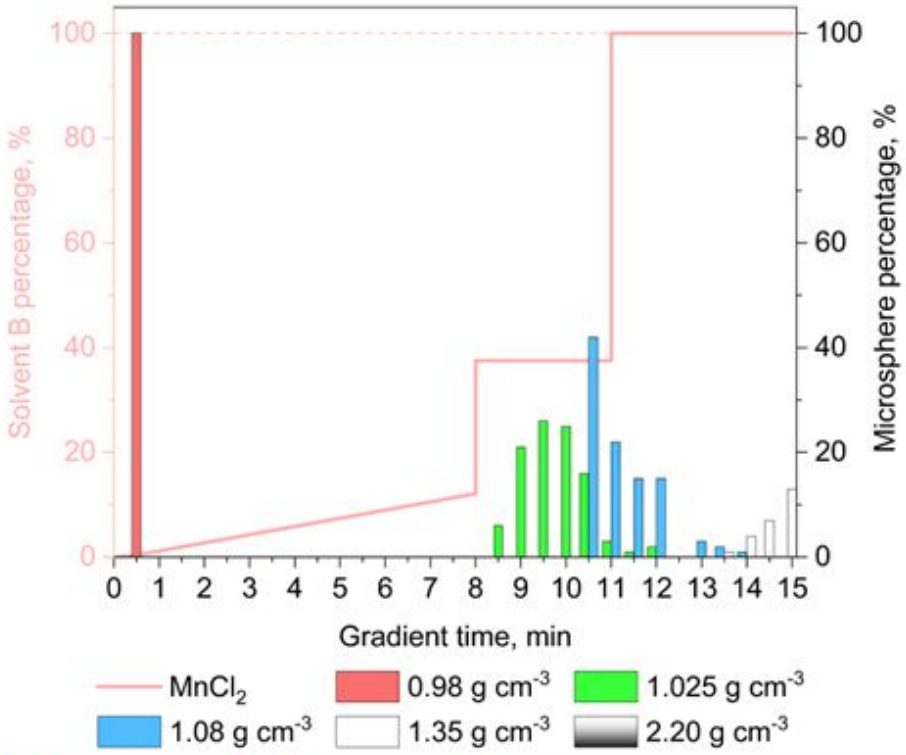
	Red	Green	Blue	White	Glass
Residence time, min	0.5	9.84	11.18	N/A	N/A
Group width, min	0.5	3.5	4.5	N/A	N/A
Resolution	4.67	0.33	N/A	N/A	

Figure S13. Step Mag-DG-Sep (in the absence of magnets) for the simultaneous separation of microspheres.



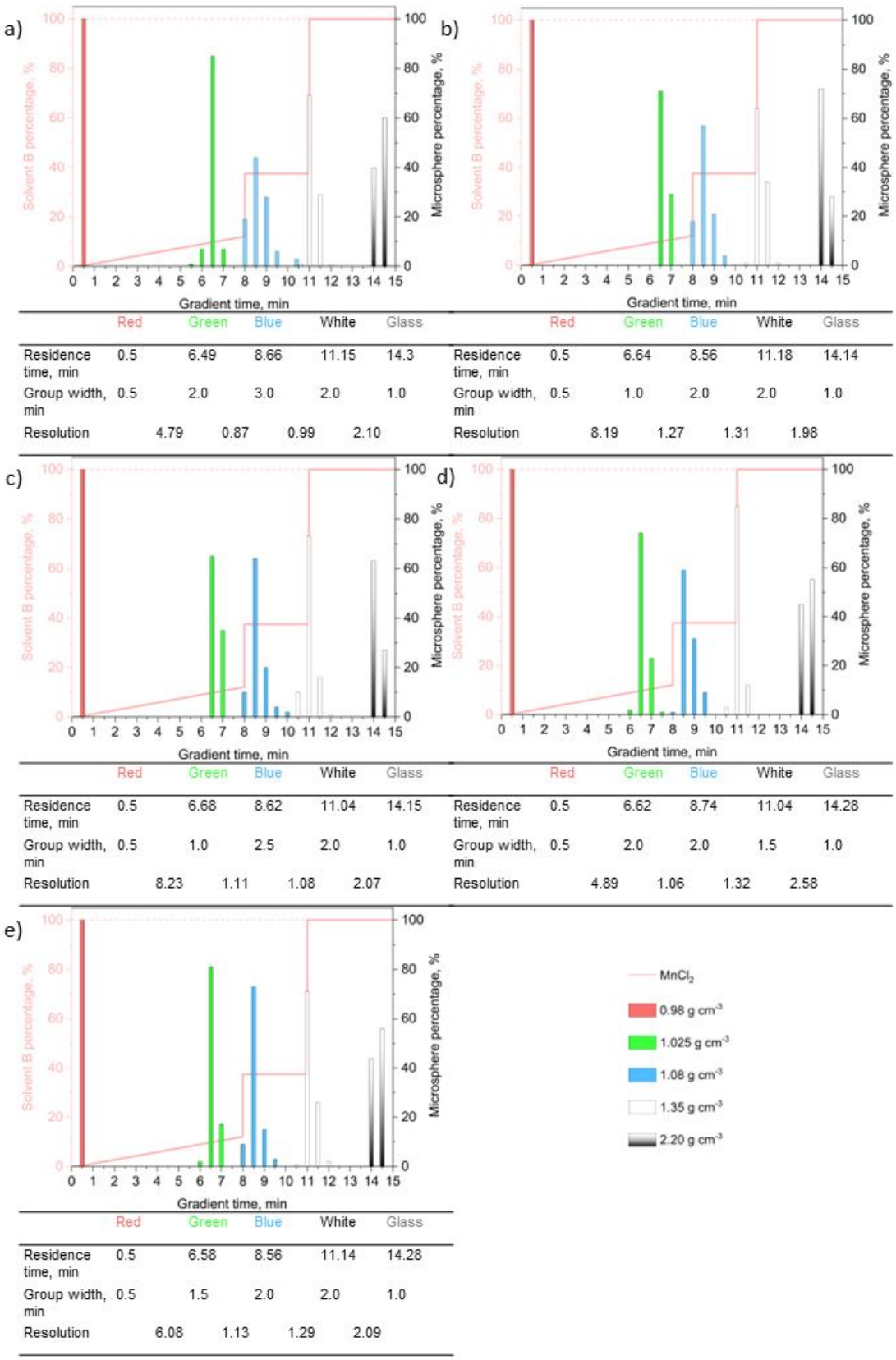
	Red	Green	Blue	White	Glass
Residence time, min	0.5	10.5	11.56	N/A	N/A
Group width, min	0.5	3.0	5.0	N/A	N/A
Resolution	5.71	0.26	N/A	N/A	

Figure S14. Step Mag-DG-Sep (in the absence of magnets) for the simultaneous separation of microspheres. Replication of experiment shown in Figure S13.



	Red	Green	Blue	White	Glass
Residence time, min	0.5	9.74	11.16	N/A	N/A
Group width, min	0.5	4.0	4.0	N/A	N/A
Resolution	4.10	0.36	N/A	N/A	

Figure S15. Step Mag-DG-Sep (in the absence of magnets) for the simultaneous separation of microspheres. Additional replication of experiment shown in Figure S13**.**



a)

	Red	Green	Blue	White	Glass
Residence time, min	0.5	6.49	8.66	11.15	14.3
Group width, min	0.5	2.0	3.0	2.0	1.0
Resolution		4.79	0.87	0.99	2.10

b)

	Red	Green	Blue	White	Glass
Residence time, min	0.5	6.64	8.56	11.18	14.14
Group width, min	0.5	1.0	2.0	2.0	1.0
Resolution		8.19	1.27	1.31	1.98

c)

	Red	Green	Blue	White	Glass
Residence time, min	0.5	6.68	8.62	11.04	14.15
Group width, min	0.5	1.0	2.5	2.0	1.0
Resolution		8.23	1.11	1.08	2.07

d)

	Red	Green	Blue	White	Glass
Residence time, min	0.5	6.62	8.74	11.04	14.28
Group width, min	0.5	2.0	2.0	1.5	1.0
Resolution		4.89	1.06	1.32	2.58

e)

	Red	Green	Blue	White	Glass
Residence time, min	0.5	6.58	8.56	11.14	14.28
Group width, min	0.5	1.5	2.0	2.0	1.0
Resolution		6.08	1.13	1.29	2.09

Figure S16. Step Mag-DG-Sep for the simultaneous separation of microspheres. Five additional replicates of the experiment shown in Figure 4b.

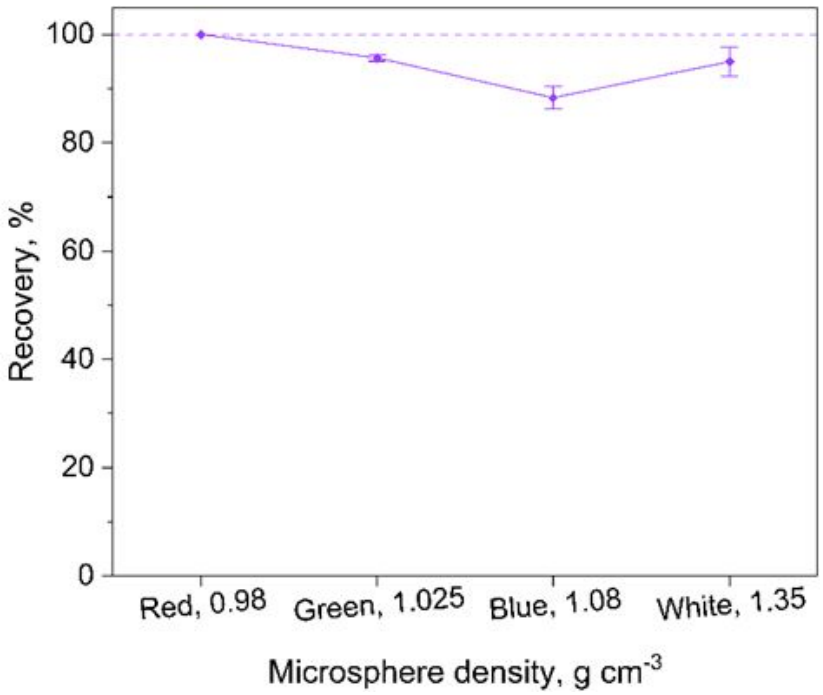
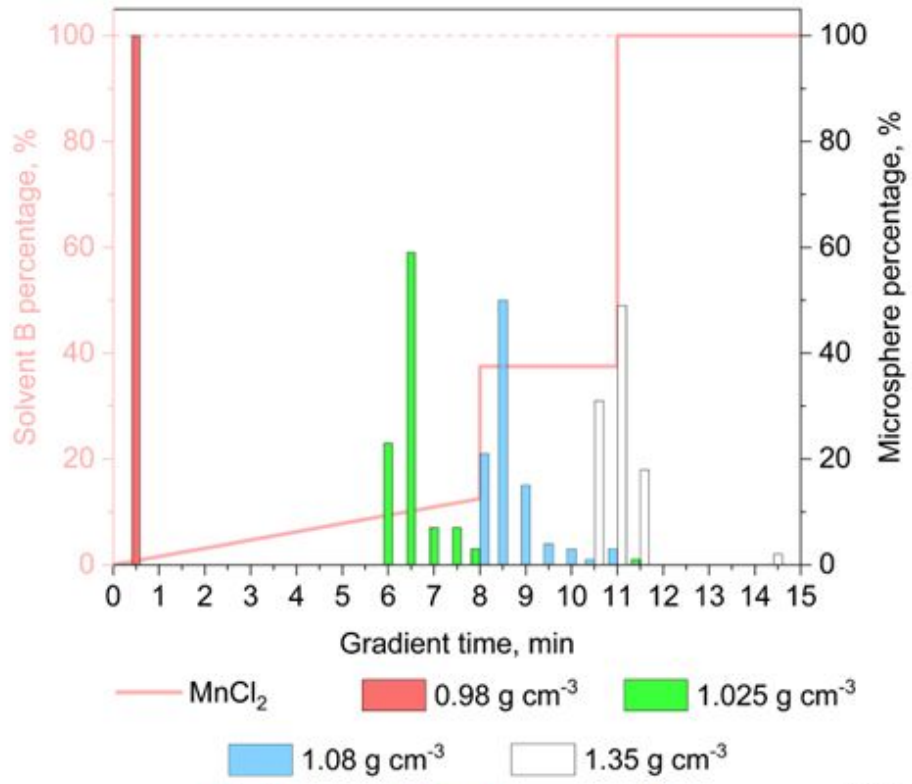
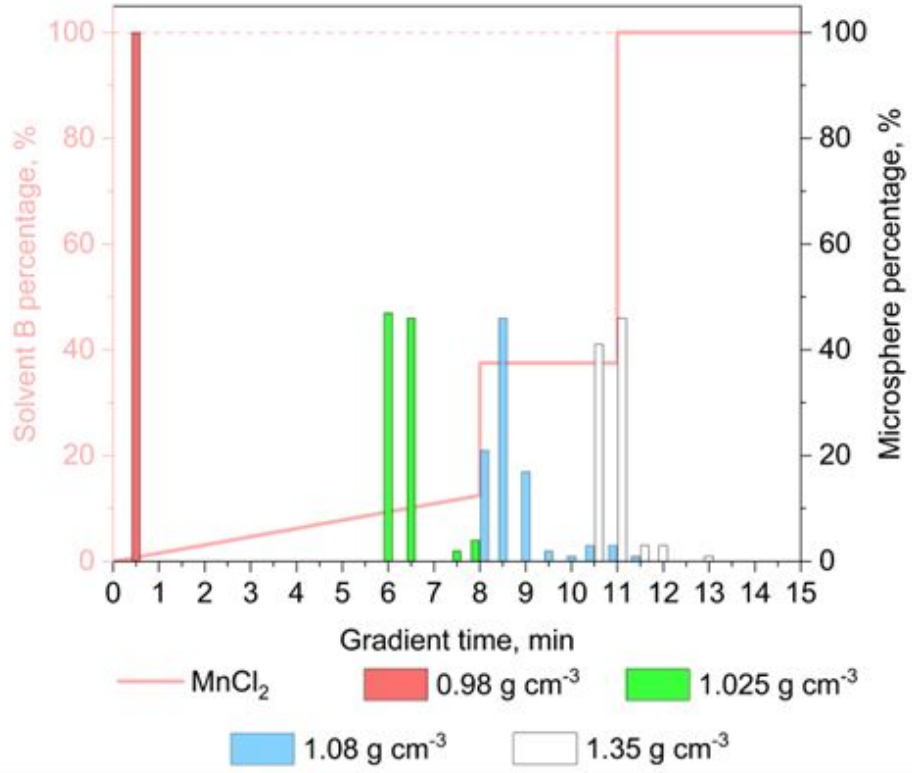


Figure S17. Recovery of selected microspheres (except glass microspheres) separated from a soil sample matrix (n=3).



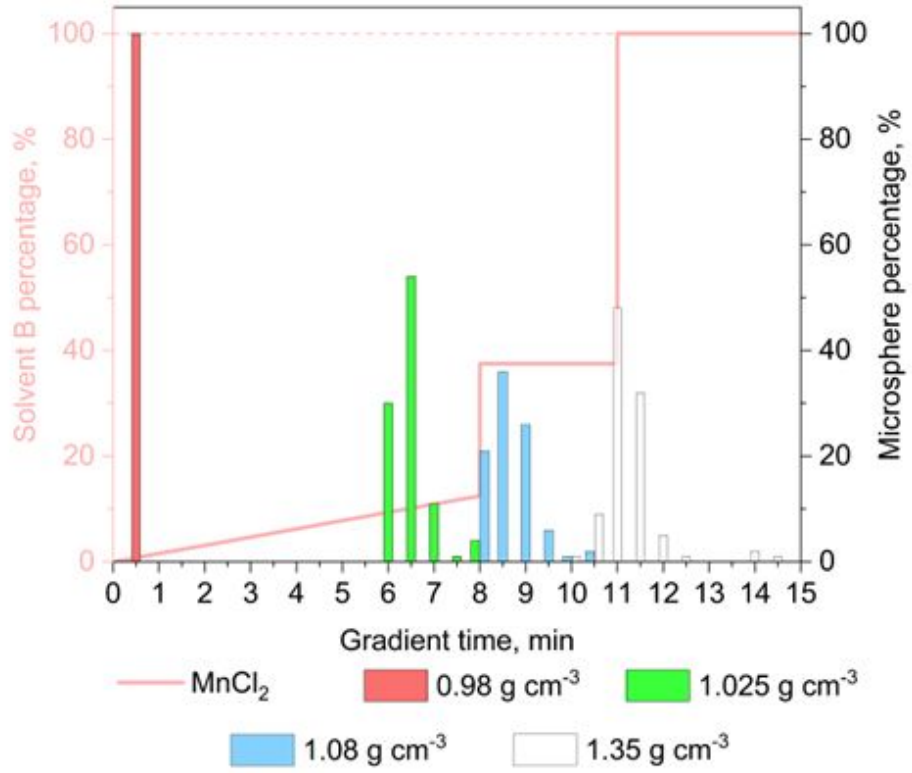
	Red	Green	Blue	White
Residence time, min	0.5	6.58	8.65	10.93
Group width, min	0.5	6.0	3.5	4.5
Resolution	1.87	0.44	0.57	

Figure S18. Recovery of PE microspheres separated from a soil sample matrix.



	Red	Green	Blue	White
Residence time, min	0.5	6.34	8.69	10.85
Group width, min	0.5	2.5	4.0	3.0
Resolution	3.90	0.72	0.62	

Figure S19. Replicate for the recovery of PE microspheres separated from a soil sample matrix.



	Red	Green	Blue	White
Residence time, min	0.5	6.48	8.65	11.27
Group width, min	0.5	2.5	3.0	5.0
Resolution	3.98	0.79	0.65	

Figure S20. Additional replicate for the recovery of PE microspheres separated from a soil sample matrix.

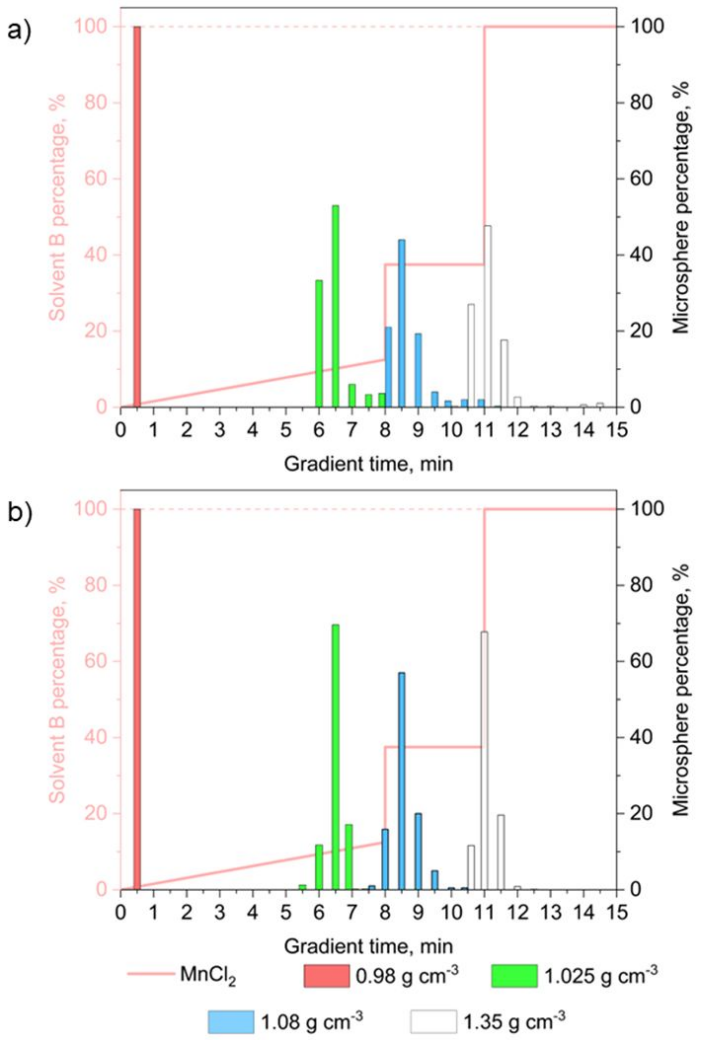


Figure S21. The average fraction distribution a) from three replicates (Figure S18, S19, and S20) of the step gradient in the presence of soil and b) eight replicates (Figure 4b, S9, S10, and S16) of the step gradient in the absence of soil.

Table S1. Summary of residence time and resolution in tests of reproducibility (n=8, Figure 4b, S9, S10, and S16).

	Red	Green	Blue	White	Glass
Residence time, min	0.50±0	6.52±0.17	8.58±0.12	11.05±0.12	14.18±0.10
Residence time RSD, %	0	2.6	1.4	1.1	0.7
Average resolution	5.68	1.00	1.13	2.20	

Table S2. Average fractions from three replicates (Figure S18, S19, and S20) of the step gradient with soil and eight replicates (Figure 4b, S9, S10, and S16) of the step gradient without soil.

	Red, %		Green, %		Blue, %		White, %	
t_r, min	With soil	No soil	With soil	No Soil	With soil	No soil	With soil	No soil
0.5	100	100						
1.0								
1.5								
2.0								
2.5								
3.0								
3.5								
4.0								
4.5								
5.0								
5.5			0	1.25				
6.0			33.33	11.75				
6.5			53.00	69.625				
7.0			6.00	17.125	0	0.125		
7.5			3.33	0.25	0	1.00		
8.0			4.00	0	21.00	15.875		
8.5			0	0	44.00	57.00		
9.0			0	0	19.33	20.00		
9.5			0	0	4.00	5.00		
10.0			0	0	1.67	0.50	0.33	0
10.5			0	0	2.00	0.50	27.00	11.625
11.0			0	0	2.00	0	47.67	67.75
11.5			0.33	0	0.33	0	17.67	19.625
12.0							2.67	0.875
12.5							0.33	0.125
13.0							0.33	0
13.5							0	0
14.0							0.67	0
14.5							1.00	0
15.0								

Table S3. Comparison of average residence times (t_r) in tests with and without the soil.

		Red	Green	Blue	White
t_r, min	With soil	0.50±0	6.47±0.12	8.65±0.02	11.65±0.64
	Without soil	0.50±0	6.52±0.17	8.58±0.12	11.05±0.12
RSD, %	With soil	0	1.9	0.2	5.5
	Without soil	0	2.6	1.4	1.1