Electronic Supporting Information

A Novel Platform using a Regenerable SDS-Functionalized Membrane with AF4-DAD-MALS for Online Enrichment and Detection of Polydisperse Nanoplastics in Biological Samples

Xingling Luo[ad]†, Aobo Geng[ab]†, Yingting Wu[c], Zhen Mao[ab], Xiaodan Zhang[ab], Jin Cai[ab] and Peili Huang*[ab]

[a]School of Public Health, Capital Medical University, Beijing 100069, China

[b]Beijing Key Laboratory of environment and aging, Beijing 100069, China

[c]Core facility Center, Capital Medical University, Beijing 100069, China

[d]Yundonghai Street office, Foshan, 528100, China.

†These authors contributed equally to this work and should be considered co-first authors.

*Corresponding author.

School of Public Health

Beijing Key Laboratory of environment and aging

Capital Medical University

Beijing 100069, China

E-mail: wshxjys@126.com

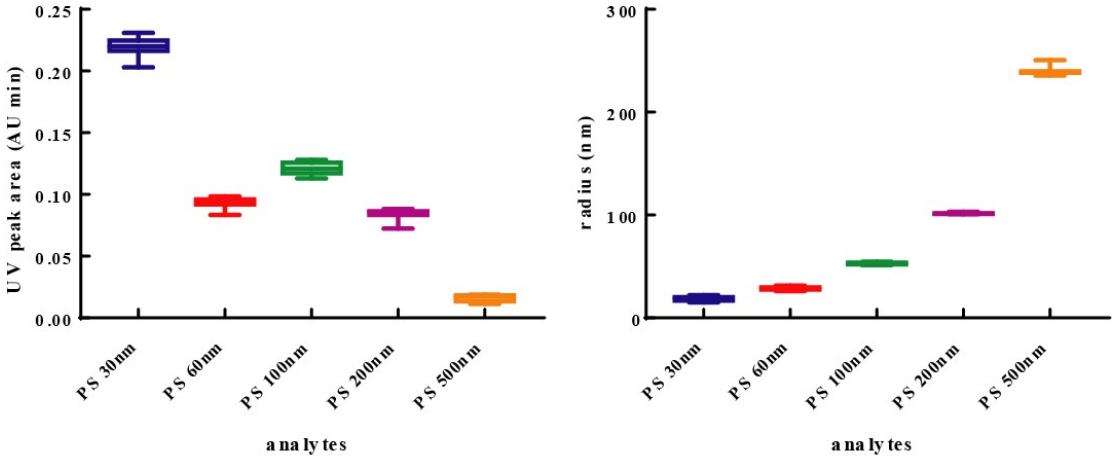
CONTENT

S1. Chemicals and Equipments

Five PS NPl with particle sizes (30 nm, 60 nm, 100 nm, 200 nm, and 500 nm) were purchased from Thermo Fischer Scientific, USA and were abbreviated as PS 30 nm, PS 60 nm, PS 100 nm, PS 200 nm and PS 500 nm according to their size were stored in a 4 ° C refrigerator. Chromatographic grade methanol was purchased from Thermo Fischer Scientific (USA); 10 KD Millipore regenerated fibrous membrane (RC 10 KD) was provided by Wyatt Technology (USA); Sodium Dodecylbenzene Sulfonate (SDS) was purchased from Sigma-Aldrich (USA); Sodium chloride was purchased from Beijing Chemical Works. 10 mM pH 7.4 Phosphate Buffered Solution (PBS) was purchased from Beijing Solaibao Technology Co., LTD. 0.8μm stream needle filter were purchased from Tianjin Jinteng Experimental Equipment Co., LTD. Asymmetric flow field flow Dual Tech separation system (Wyatt, USA); 1260 high performance liquid chromatograph (Agilent, USA); Diode array detector (Agilent, USA); DAWN EOS multi-angle laser scattering detector (Wyatt, USA); JEM-2100 transmission electron microscope (JEOL, Japan);

S2. Experiment of reproducibility

After rinsing the modified film, the SD of the UV peak area of PS NPl with each particle size was < 0.007 AU min measured 20 times under the same conditions, and the measured particle size was consistent with the theoretical value, and the difference between the results of multiple measurements was small (SD< 2.7 nm), indicating that the method has good reproducibility and high accuracy



analytes	*n*	Average (AU min)	SD
PS 30nm	20	0.219	0.007
PS 60nm	20	0.094	0.004
PS 100nm	20	0.121	0.005
PS 200nm	20	0.084	0.004
PS 500nm	20	0.016	0.003

analytes	*n*	radius (nm)	SD
PS 30nm	20	18.8	2.1
PS 60nm	20	28.8	1.6
PS 100nm	20	53.0	0.8
PS 200nm	20	101.5	0.5
PS 500nm	20	239.6	2.7

Figure UV Peak Areas and Sizes of Various PS NPl Measured 20 Times After Membrane Rinsing and Modification. (*n*=20)

S3. The recovery of PS NPl before and after rinsing and modifying the membrane

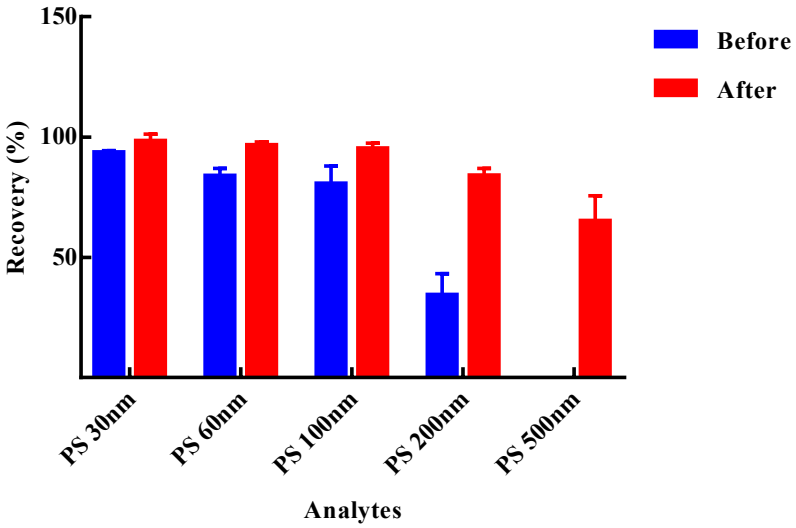


Figure The recovery of PS NPl before and after rinsing and modifying the membrane (mean±SD, n=3)

S4. The AF4-MALS fractogram of PS NPl after various pretreament methods for the whole blood sample

After centrifugation, PS NPl could not be detected. However, after centrifugation and alkali digestion, PS NPl with five particle sizes could be detected. Five kinds of PS NPl with different particle sizes could also be successfully separated and detected by alkaline digestion directly treating spiked whole blood samples. However, the non-spiked blood blank sample only had a signal at the empty peak position, and no corresponding particle signal was detected at the target peak position, indicating that the polydisperse PS NPl particles could be effectively separated and eluted from the blood matrix after alkali treatment.

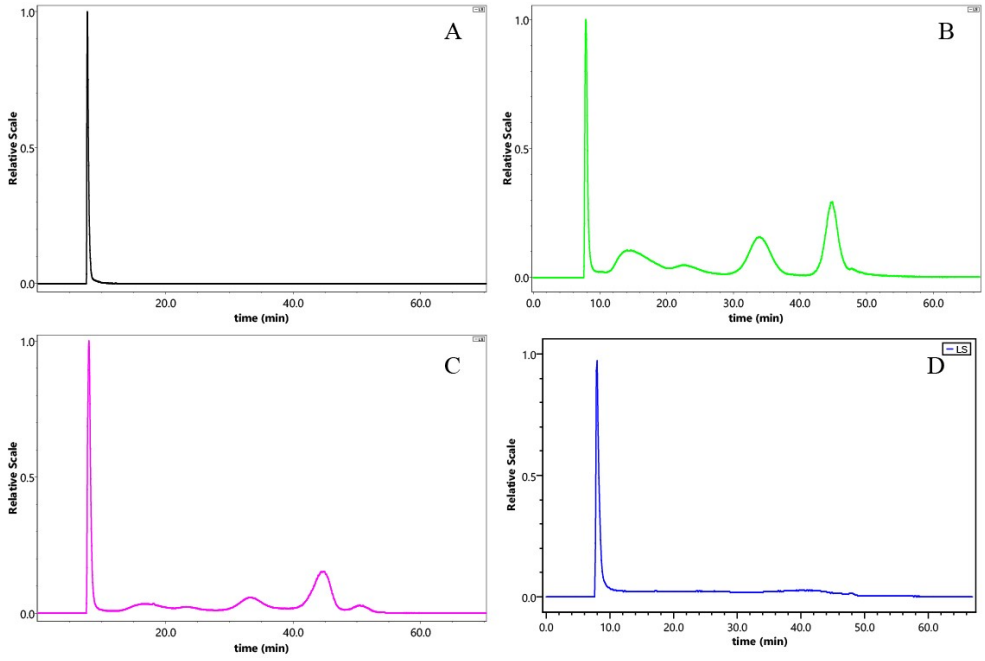


Figure The AF4-MALS fractogram of PS NPl after various pretreament methods for the whole blood sample （A: Centrifugation; B: Centrifugation+Alkali digestion; C: Alkali digestion; D: Blood Blank sample）

S5. The alkaline digestion protocol

For the alkaline digestion protocol, 0.2 mL whole blood was transferred to a centrifuge tube. A 10% (m/v) KOH solution was added at a 1:10 (v/v) ratio and the mixture was vortexed thoroughly. Following a 2 h incubation at 60 °C, the mixture was diluted with ultrapure water to achieve a final KOH concentration of 1% (m/v). Subsequently, 30% H_2O_2 was added dropwise slowly while maintaining the water bath conditions. The addition was stopped when the solution color changed from brown to colorless. The solution was then cooled to room temperature, filtered through a 0.8 μm syringe filter.

S6. The eluted program of AF4-DAD-MALS for polydisperse PS NPl

Table The eluted program of AF4-DAD-MALS for polydisperse PS NPl

Steps	Mode	Time (min)	Initial cross flow (mL/min)	End cross flow (mL/min)	Flow velocity pattern
1	Elution	1	1.0	1.0	Constant
2	Focus	1	1.0	1.0	Constant
3	Focused injection	5	1.0	1.0	Constant
4	Elution	40	1.0	0.1	Linear
5	Elution	30	0.1	0.1	Constant
6	Elution injection	5	0	0	Constant
7	Focused injection	5	1.0	1.0	Constant
8	Elution injection	10	0	0	Constant
9	Focused injection	3	1.0	1.0	Constant
10	Elution injection	20	0	0	Constant

S7. Comparison of other reported methods with this methodfor analysis of NPl

Table Comparison of other reported methods with this methodfor analysis of NPl

Method	Target object	Matrix	Linear range	Pre-processing duration	Limit of detection (μg/L)	Enrichment factor / Extraction rate	References
AF4- MALS	PS 100 nm	Fish meat	-	16-17 h	52 μg/g	-/-	[1]
Transverse flow filtration - AF4-MALS	PS 50 nm、PS 100 nm、PS 200 nm、PS 500 nm	Water	0.1-100mg/L	-	5-50 μg/L	200/ 12.7-54.0 %	[2]
AF4-DAD-MALS	PS 30 nm、PS 60 nm、PS 100 nm、PS 200 nm、PS 500 nm	Serum, whole blood, urine, tissue	1.0-4000 mg/L	2 h	0.25-5 mg/L	-/-	This article
Online enrichment /AF4-DAD-MALS	Same as above	Same as above	1.6-1000μg/L	2.25 h	0.5-2.0 μg/L	27.0[a]/ 91.0-100%	This article

Notes

a Suggested maximum enrichment factor for samples, considering the enrichment duration

References

[1] Correia M, Loeschner K. Analytical and Bioanalytical Chemistry, 2018, 410 (22): 5603-5615

[2] Mintenig SM, Bäuerlein PS, Koelmans AA, Dekker SC, van Wezel AP. Science: Nano, 2018, 5 (7): 1640-1649