**Supplementary material**

**Characterization of nanoprecipitated PET nanoplastics by**  **1H NMR and impact of residual ionic surfactant on viability of human primary mononuclear cells and hemolysis of erythrocytes**

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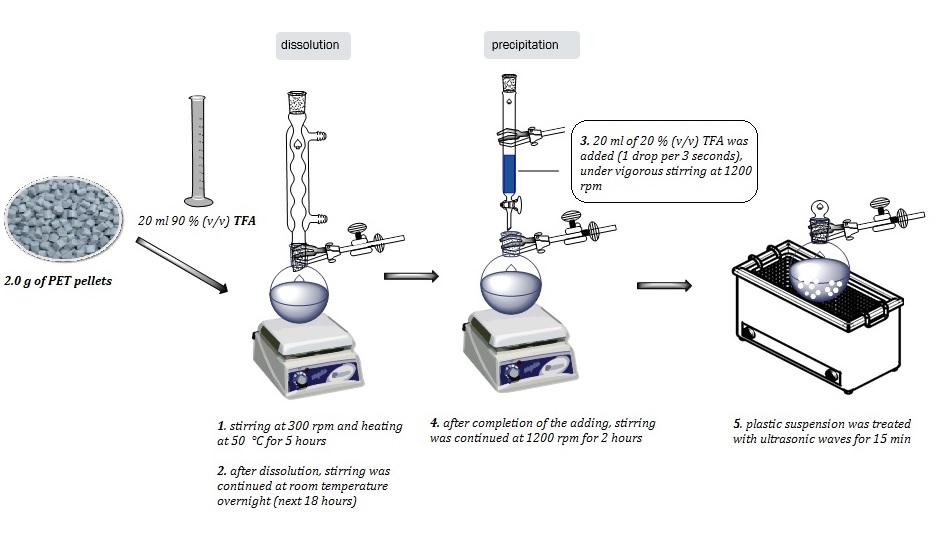
Solna, Karolinska Institutet, Stockholm, Sweden

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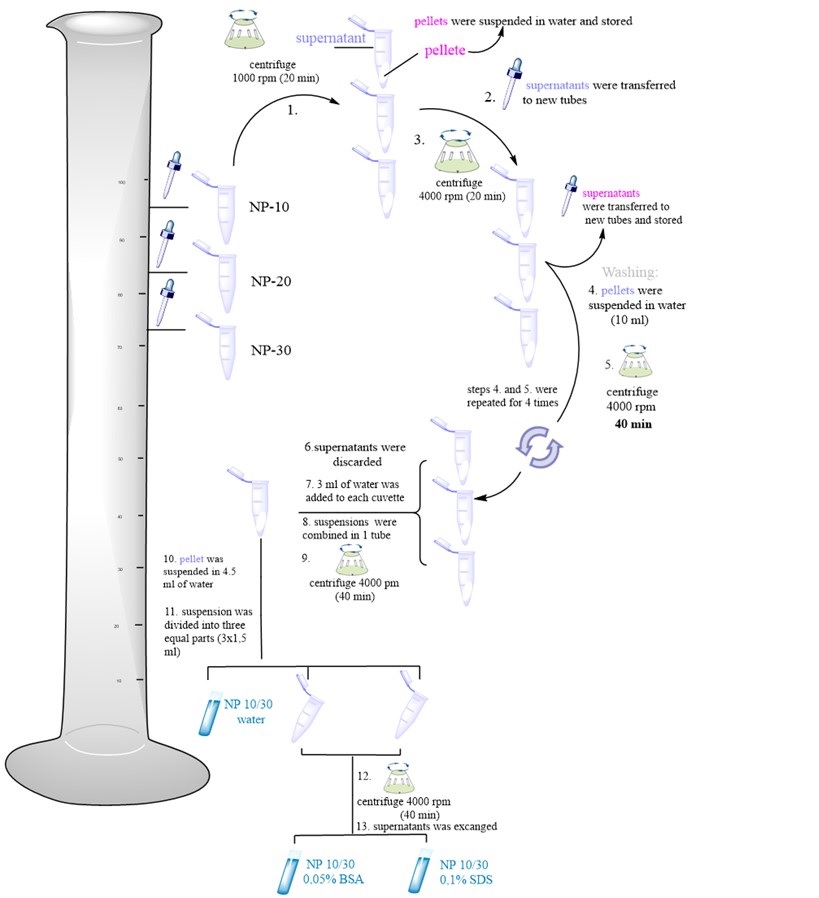
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**Preparation of PET NPs**

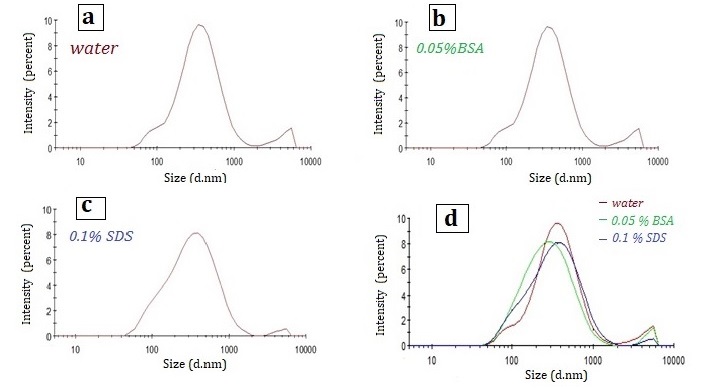
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**Figure S1.** Overall scheme of nPET production procedure. First, 2.0 g of PET pellets was dissolved in 20.0 mL of TFA in Mili-Q water (*v/v)* with stirring at 300 rpm for five hours at 50 °C and next 18h at room temperature. Then, 20 mL of 20% TFA in Milli-Q water (*v/v)* was added dropwise during 110 min (1 drop of 10 μL per 3 s), under vigorous stirring at 1200 rpm using a dropping funnel. Stirring of suspension was continued for an additional 2 hours, before being sonicated in an ultrasonic bath for 15 min.

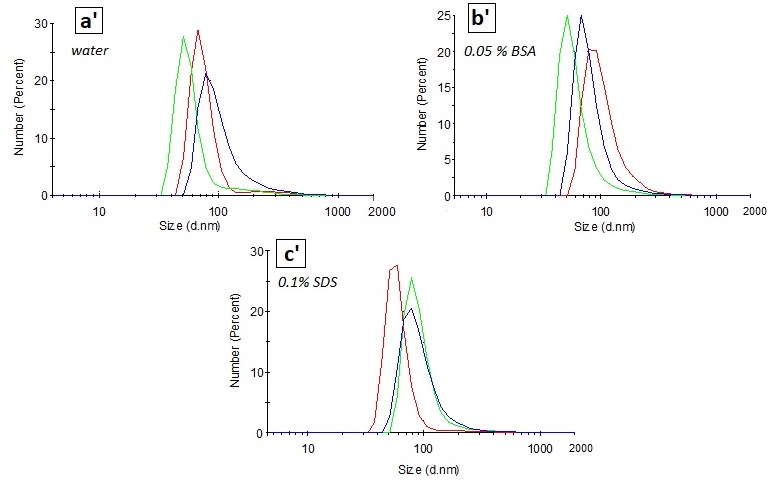


**Figure S2.** Schematic illustration of washing and size separation processes of the first three fractions (each 10 mL), denoted NP-10, NP-20 and NP-30.

**Characterization of PET NPs**



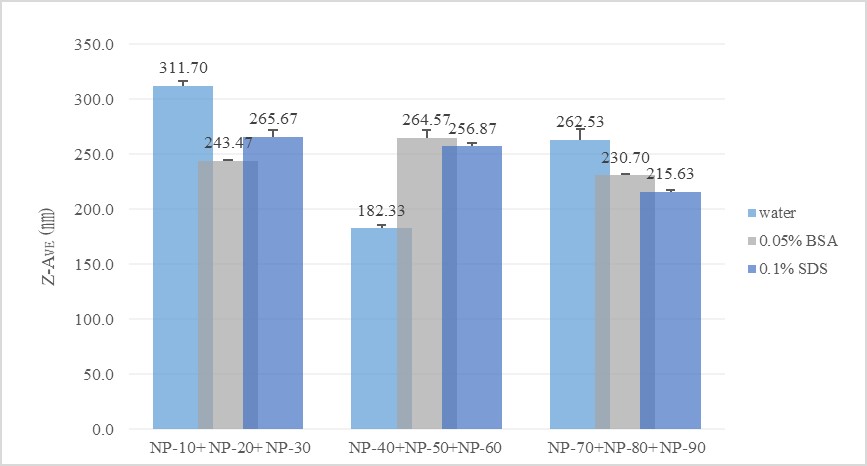
**Figure S3.** Size distributions (by **intensity percentage**) of **NPs washed** suspended in different dispersants: **water, BSA (0.05%) and SDS (0.1 %)** are presented. Size distributions are shown for NPs obtained from combinated fractions: **NP-10, NP-20 and NP-30**.

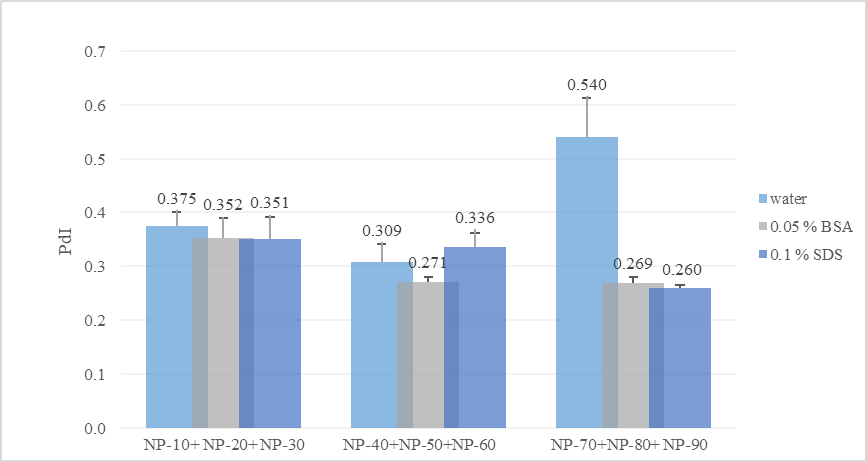


**Figure S4.** Size distributions (by **number percentage**) of **NPs washed** suspended in different dispersants: **water, BSA (0.05%) and SDS (0.1 %)** are presented. Distributions are shown for NPs obtained from combinated fractions: **NP-10, NP-20 and NP-30**. Additionally multiple consecutive measurements (lines with different colors) are presented.

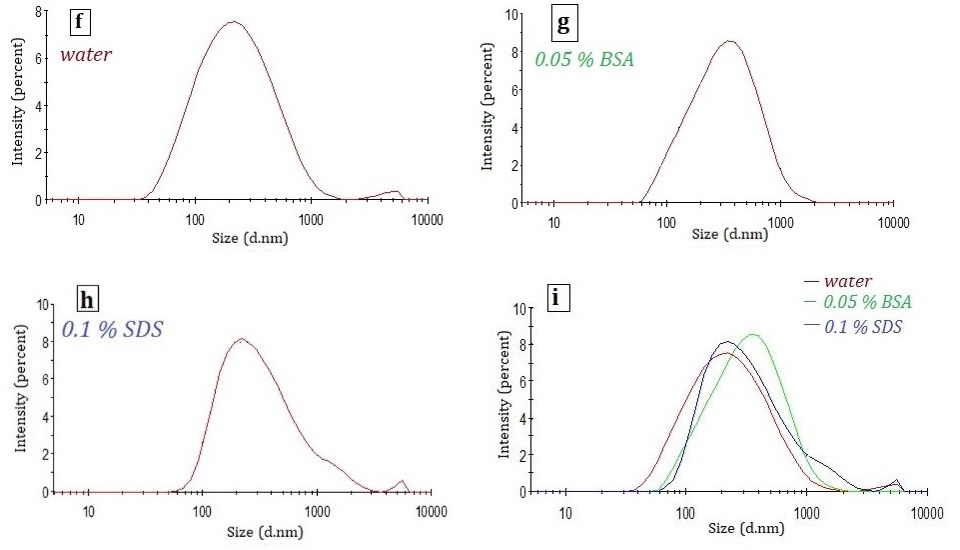
**Table S1.** Hydrodynamic diameter (d.nm) and polydispersity index of **NPs washed** obtained from combined fractions: NP-10, NP-20 and NP-30 (denoted **NP-10**+**NP-20+NP-30**); NP-40, NP-50 and NP-60 (denoted **NP-40+NP-50+NP-60**); NP-70, NP-80 and NP-90 (denoted **NP-70+NP-80+NP-90**) dispersed in different dispersants: water, 0.05% BSA and 0.1% SDS.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | dispersant | **NPS washed** | | |
|  | NP-10+NP-20+NP+  NP-30 | NP-40+NP-50+  NP-60 | NP-70+NP-80+  NP-90 |
| Z-Average  (d.nm) | water | 311.7 ± 4.4 | 182.3 ± 3.4 | 262.5 ± 9.6 |
| 0.05% BSA | 243.5 ± 1.3 | 264.6 ± 7.2 | 230.7 ± 1.1 |
| 0.1 % SDS | 265.7 ± 5.9 | 256.8 ± 3.3 | 215.6 ± 1.6 |
| Polydispersity index | water | 0.375 ± 0.026 | 0.309 ± 0.034 | 0.540 ± 0.073 |
| 0.05% BSA | 0.352 ± 0.037 | 0.271 ± 0.010 | 0.269 ± 0.011 |
| 0.1 % SDS | 0.351 ± 0.041 | 0.336 ± 0.026 | 0.260 ± 0.050 |

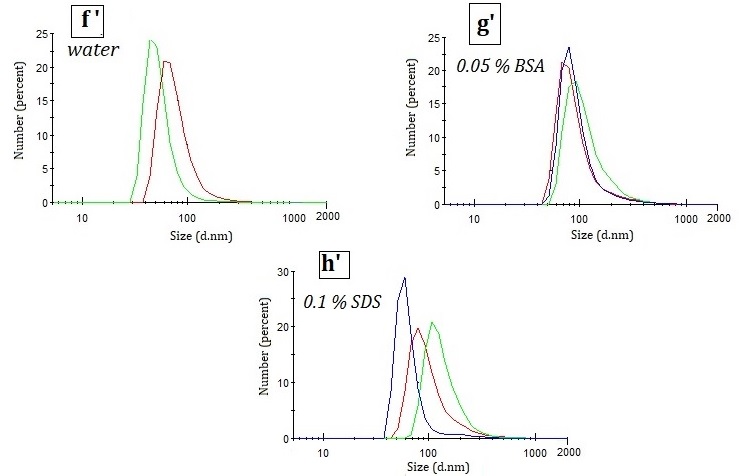
**Figure S5.** Hydrodynamic diameter (d.nm) of **NPs washed** obtained from combined fractions: NP-10, NP-20 and NP- 30 (denoted **NP-10**+**NP-20+NP-30**); NP-40, NP-50 and NP-60 (denoted **NP-40+NP-50+NP-60**); NP-70, NP-80 and NP-90 (denoted **NP-70+NP-80+NP-90**) dispersed in different dispersants: water, 0.05% BSA and 0.1% SDS.



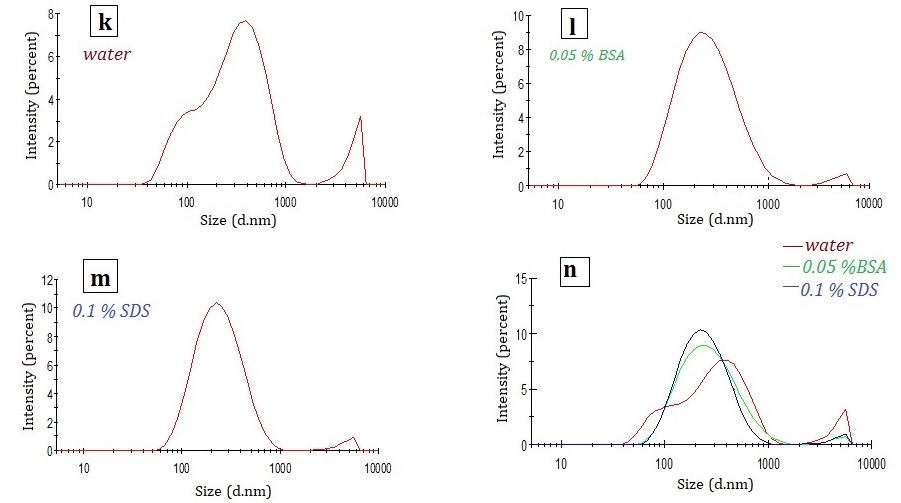
**Figure S6.** Polydispersity index of **NPs washed** obtained from combined fractions: NP-10, NP-20 and NP- 30 (denoted **NP-10**+**NP-20+NP-30**); NP-40, NP-50 and NP-60 (denoted **NP-40+NP-50+NP-60**); NP-70, NP-80 and NP-90 (denoted **NP-70+NP-80+NP-90**) dispersed in different dispersants: water, 0.05% BSA and 0.1% SDS.



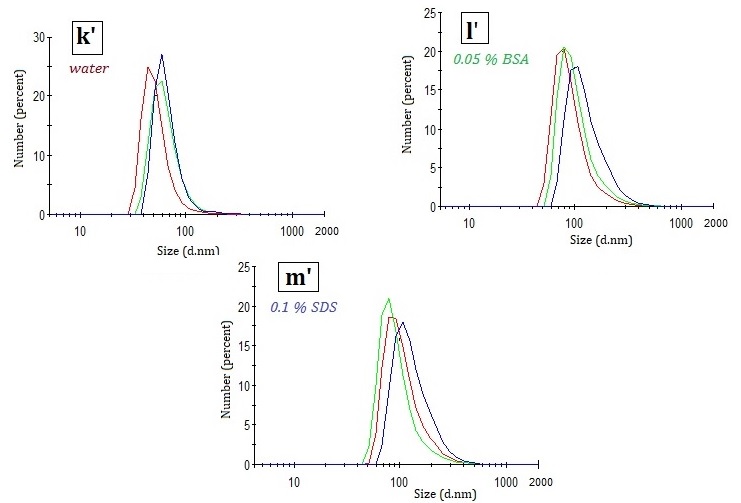
**Figure S7.** Size distributions (by **intensity percentage**) of **NPs washed** suspended in different dispersants: **water, BSA (0.05%) and SDS (0.1 %)** are presented. Size distributions are shown for NPs obtained from combinated fractions: **NP-40, NP-50 and NP-60**.

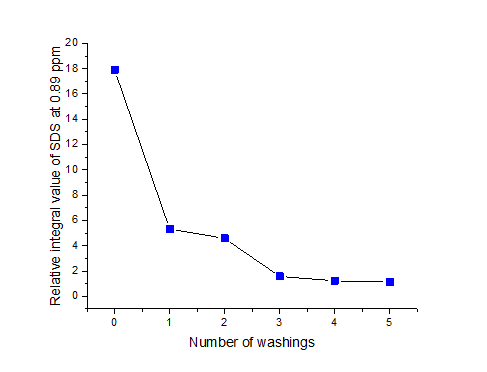


**Figure S8.** Size distributions (by **number percentage**) of **NPs washed** suspended in different dispersants: **water, BSA (0.05%) and SDS (0.1 %)** are presented. Distributions are shown for NPs obtained from combinated fractions: **NP-40, NP-50 and NP-60**. Additionally multiple consecutive measurements (lines with different colors) are presented.

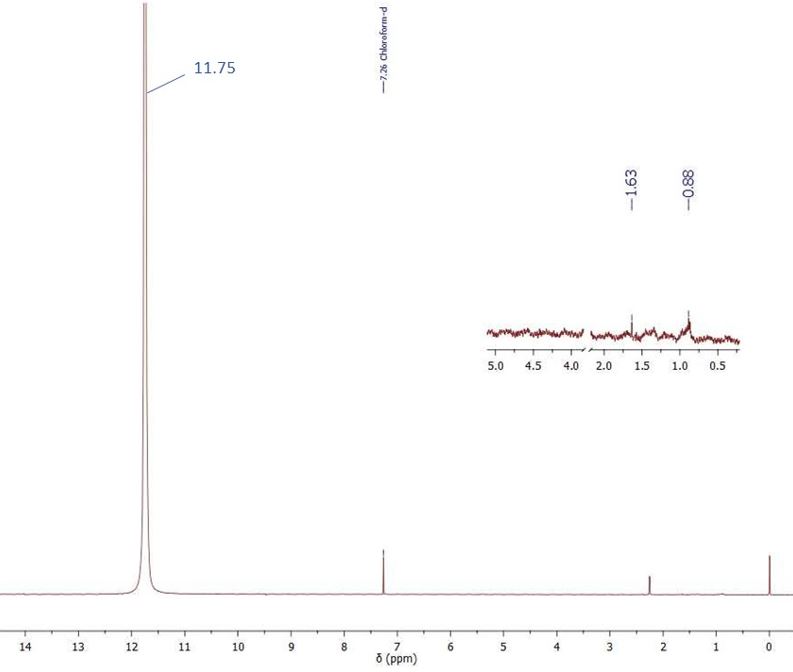


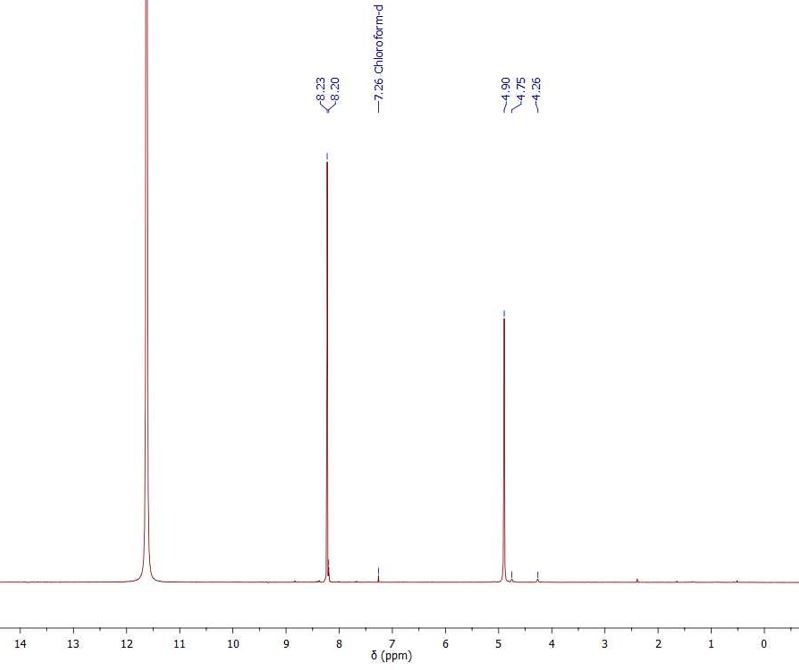
**Figure S9.** Size distributions (by **intensity percentage**) of **NPs washed** suspended in different dispersants: **water, BSA (0.05%) and SDS (0.1 %)** are presented. Size distributions are shown for NPs obtained from combinated fractions: **NP-70, NP-80 and NP-90**.

**Figure S10.** Size distributions (by **number percentage**) of **NPs washed** suspended in different dispersants: **water, BSA (0.05%) and SDS (0.1 %)** are presented. Distributions are shown for NPs obtained from combinated fractions: **NP-70, NP-80 and NP-90**. Additionally multiple consecutive measurements (lines with different colors) are presented.

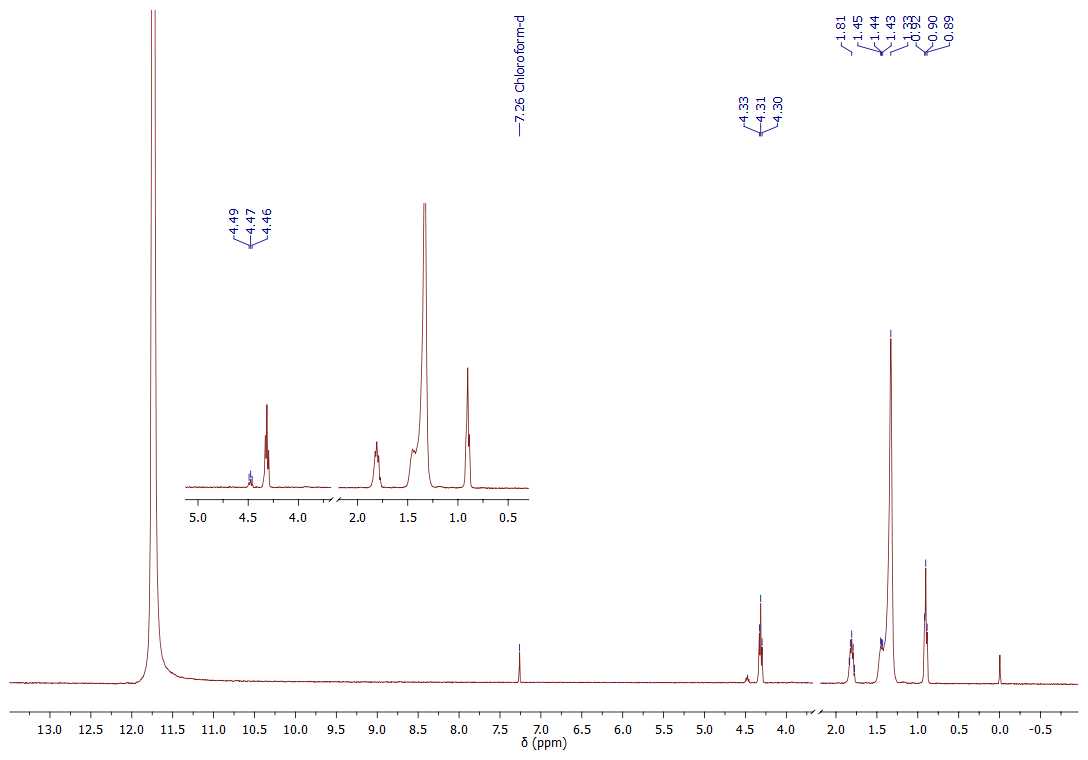


**Figure S11.** Relative quantification of SDS contamination in relation to PET signal in NMR.

**Figure S12.** 1H NMR spectrum (400 MHz) of trifluoroacetic acid (TFA) recording with addition of deuterated chloroform (CDCl3) in the ratio 4:1 (*v,v, respectively*). Region importance for process monitoring of washing process of NPs (0.5- 2.0 ppm and 4.0- 5 .0 ppm) is zoomed. Signal at 11.75 ppm originates from TFA; signals with chemical shifts values 0.88 and 1.63 ppm indicate the presence of impurities in very low concentrations.



**Figure S13**. 1H NMR spectrum (400 MHz) of PET pellet (starting material) recorded in the mixture of TFA and CDCl3 in the ratio 4:1 (*v/v*). The use of this mixture containing the fluorinated solvent is advantageous, since it allows room temperature spectra [[](https://www.zotero.org/google-docs/?0WzQfx)1[]](https://www.zotero.org/google-docs/?hCGZdV). Characteristic signals at 8.20- 8.23 (aromatic protons), 4.90 (internal methylene groups), and low intensity signals at 4.75 and 4.26 ppm (the methylene protons adjacent to the hydroxyl end functions) are consistent with reported literature data [[](https://www.zotero.org/google-docs/?0WzQfx)2,3[]](https://www.zotero.org/google-docs/?hCGZdV).



**Figure S14**. 1H NMR spectrum (400 MHz) of sodium dodecyl sulfate (SDS) recorded in the mixture of TFA and CDCl3 in the ratio 4:1 (*v/v*). The peaks at regions 0.89-0.92, 1.33-1.45, 1.81 and 4.30-4.33 ppm originate from SDS. In the magnified part of the spectrum a low intensity signal with chemical shift of 4.46-4.49 ppm is visible. The intensity of this impurity decreases during the washing process of NPs from SDS; already after third washing, this peak is not visible in the NMR spectrum (Figure 1B).

**References:**

1. Fox, B.; Moad, G.; Van Diepen, G.; Willing, I.; Cook, W. D. Characterization of poly(ethylene terephthalate) and poly(ethylene terephthalate) blends. *Polymer,* ***1997****, 38(12), 3035-3043,* doi:10.1016/s0032-3861(96)00872-5.
2. El Mejjatti, A.; Harit, T.; Riahi, A.; Khiari, R.; Bouabdallah, I.; Malek, F. Chemical recycling of poly(ethylene terephthalate). Application to the synthesis of multiblock copolyesters. *EXPRESS Polym. Lett.* ***2014***, 8, 544553, doi: doi:10.3144/expresspolymlett.2014.58.
3. Saint-Loup, R.; Jeanmaire, T.; Robin, J.J.; Boutevin, B. Synthesis of (polyethylene terephthalate/polyϵ-caprolactone) copolyesters. *Polymer,* ***2003****, 44, 3437-3449*, doi: 10.1016/S0032-3861(03)00257-X.