Characterisation of water-soluble synthetic polymeric substances in wastewater using LC-HRMS/MS

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Figure S 5: Molecular weight dependent charge state distribution. The x-axis shows the molecular weight of PEG, namely 06-110 EO units, intensities are shown as normalized peak heights on the y-axis. Number of charges are color coded. For both ionization interfaces the most abundant ion specie, namely Na-adducts for APPI and NH₄-adducts for ESI are shown. a) Ionization performed using (+)-APPI: shows observed Na-adducts with charge states up to +3 for PEG 06-110 EO. b) Ionization performed using (+)-ESI: shows observed NH₄-adducts with charge states up to +8 for PEG 06-110 EO.

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Figure S7: Detected HS in the different wastewater treatment stages. Samples were pre-concentrated using vacuum-assisted evaporative concentration. Ionization was performed with APCI, APPI or ESI in negative mode. RU are depicted as m/z and indicated in the respective color. Hits from the suspect list are highlighted by a black asterisk.

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Sample preparation

In case of centrifugation, approximately 10 mL of sample were transferred to glass centrifuge tubes and centrifuged at 4160 x g, at 4 °C for 30 min (Megafuge 1.0R, Hereaus instruments). After adjusting an aliquot of the supernatant to the starting condition of the chromatographic separation the sample was stored at 4 °C until subjected to LC-HRMS/MS analysis. In case of vacuum-assisted evaporative concentration, the optimized conditions of Mechelke et al. (Mechelke et al., 2019) were employed (55 °C, 20 mbar and 200 to 300 orbital movements per minute using a vacuum-assisted evaporation system). Briefly, after evaporation of 10 mL until an approximate residual volume of 0.3 mL using the vacuum-assisted evaporation system (Syncore Analyst, BÜCHI Labortechnik AG, Switzerland), samples were centrifuged for 15 min at 2320 x g at 4 °C (5427 R, Eppendorf, Switzerland), transferred into a glass vial and visually adjusted to a final volume of 1 mL. This resulted to a pre-concentration factor of 10.

Table S2: Optimized parameters for the analysis of homologous series in wastewater using a C18 Xbridge column (2.1x50 mm, 3.5 μm), coupled to a ThermoScientific QExactive mass spectrometer. For the APPI source a Photomate Light Source from Synagen and the employed vacuum ultraviolet lamp is filled with krypton gas. a) Shows chromatographic separation parameters adjusted according to the different ionization sources employed. b) depicts optimized source conditions for ESI, APPI and APCI and c) lists MS and DD-MS2 parameters of the QExactive. Information on quality control can be found in e)

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<th>APPI</th>
<th>APCI</th>
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<td>H₂O, 2% MeOH, 5% IPA</td>
<td>H₂O, 2% MeOH</td>
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Table S3: Settings for enviMass v4.2beta (Loos 2018)

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<th>Parameters in enviMass</th>
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**Workflow section: Pre-processing**

- Mass recalibration: **Yes**
- RT alignment: **No**
- Median Intensity normalization: **No**
- Blank/ Blind detection: **detect: Yes, remove: No**
- Replicate Filter: **include: Yes**, use in profiling: **Yes**
- LOD interpolation: **No**

**Peak picking**

- RT filter range: **1 – 25min**
- Parameter estimation: **Yes**
- Maximum RT gap in an EIC: **118 s**
- Maximum m/z deviation of a centroid data point from its EIC mean: **8 ppm**
- Minimum number of centroid data points per peak: **5**
- Maximum RT window: **7 s**
- Maximum RT gap width to be interpolated: **6 s**
- Maximum RT width of a single peak, +/- from apex: **50 s**
- Minimum log10(intensity) threshold: **4**
- Minimum Signal/Intensity: **5**
- Minimum Signal/Base: **3**
- Maximum possible number of peaks within a single EIC: **5**
- Peak intensity: **intensoid(max int.)**
- Peak mass definition: **weighted mean**

**Mass recalibration**

- Reference compounds: target compounds; +/- m/z tolerance for peak matches: **8 mmu**, maximum permissible m/z correction: **4 mmu**
- RT tolerance: **30 s**

**Blind**

- No subtraction, use specified blind/blank samples per sample type
Homologous series componentization:

- File-grouping
- Isotopologue/Adduct componentization:
  - File-wise componentization: include:
    - isotopologue grouping:
      - M+3Na, M+2H, M+H+NH4, M+H+Na, M+H+K, M+2Na

Adducts:
- RT: IS, Targets& suspects: RT tolerance of peaks relative to their expected same analyte across replicate sample:
- Peak Mass deviation within profiles: +/- m/z tolerance: 3 ppm; peak deviation within profiles: RT tolerance: 30 s
- Rt tolerance window of peak caused by the M+FA
- M+3H, M+2H+Na, M+H+2Na, M+3Na, M+2H, M+H+NH4, M+H+Na, M+H+K, M+2Na
- (+): M+H, M+NH4, M+Na, M+K, M+, M+3H, M+2H+Na, M+H+2Na, M+3Na, M+2H, M+H+NH4, M+H+Na, M+H+K, M+2Na

File-wise componentization: Isotopologue/ Adduct grouping:
- +/- m/z tolerance: 3 ppm; RT tolerance of peaks within an isotope pattern: 1.5 s
- Adducts:
  - (+): M+H, M+NH4, M+Na, M+K, M+, M+3H, M+2H+Na, M+H+2Na, M+3Na, M+2H, M+H+NH4, M+H+Na, M+H+K, M+2Na

Workflow section: Targets
- Screening IS, Targets& suspects: RT tolerance of peaks relative to their expected RT: 30 s; RT tolerance of peaks within an isotope pattern: 1.5 s; mass tolerance: 3 ppm
- Adducts:
  - (+): M+H, M+NH4, M+Na, M+K, M+, M+3H, M+2H+Na, M+H+2Na, M+3Na, M+2H, M+H+NH4, M+H+Na, M+H+K, M+2Na

Profiles
- Peak Mass deviation within profiles: +/- m/z tolerance: 3 ppm; peak deviation within profiles: RT tolerance: 30 s

Workflow section: Nontargets
-Peakshape correlation: Yes; File-wise componentization: include: isotopologue grouping: Yes; adduct grouping: Yes; homologous series detection: Yes; Profile componentization: Yes; Blacklist screening: No

Replicates
- +/- m/z tolerance: 3 ppm; RT tolerance window of peak caused by the same analyte across replicate sample: 3 s
- m/z tolerance: +/– 3 ppm
C15H28N2O2, C17H36Si, C18H36O, C16H18N2O2, C16H23O4, C18H28Si,
C16H18O4, C18H29NO, C13H8O5S, C18H12O3, C15H19NO4, C6F10O, C16H30N2O2,
C13H17NO4S, C18H203, C16H28O4, C19H12O3, C12H16O8, C17H24N2O2,
C19H28O2, C17H26N2O2, C19H18O3, C20H28Si, C20H40O, C21H32O,
C18H26N2O2, C18H24O4, C15H8Cl2O3, C20H12N4, C22H44, C20H22O3,
C18H34N2O2, C20H42Si, C21H16O3, C16H12C2O3, C19H36N2O2, C20H24O4,
C22H37NO, C25H32, C24H18N2, C22H24O3, C24H48, C22H26O3, C20H38N2O2,
C20H36O4, C21H20O5, C24H36O2, C23H18O4, C24H38O2, C16H8F6O3,
C22H10N2O4, C22H22O5, C24H36OSi, C26H24O3, C26H18O3, C22H10N2O5,
C29H40, C16H10Cl4O3, C26H16O4, C23H10N2O5, C24H16O4S, C26H16NO,
Charges z: (+)-ESI: 1-6; (+)-APPI, (+)-APCI, (-)-ESI, (-)-APPI, (±)-APCI: 1-3; minimum change in RT from one homologue to next: 2 s; maximum change in RT from one homologue to next: 240 s; tolerance by which the RT differences between two adjacent homologue pairs are allowed to change: 5 s; +/- m/z tolerance: 3 ppm; minimum number of homologues in a series: 4; filter peaks by sample-vs-blind intensity ratio: yes; sample-vs-blind intensity ratio: 3; Check series smoothness with: spline up to series of length 7; maximum number of homologues in a series: 50; Filter homologous series: reduce redundant combinations, merge gapped series, only keep series with a majority of monoisotopic peaks, reduce co-eluting series to most intense one

File-wise componentization: Peak shape correlation
RT tolerance window for candidate peak pairs: 1.5 s; min number of MS1 scans over which peak pairs co-elute to check for their peak shape correlation: 8; min. spearman correlation coefficient: 0.95

Workflow section: Concentration
Calibration: No; Quantification: No; Recovery: No

Workflow section: Profiling
Profile extraction: Yes; Profile filtering: Yes; subtract internal standard peaks: No, Substract target compound peaks: No; subtract peaks, which have also been detected in blind/blank samples: No, peaks from spiked files: No; Profile blind detection: Yes, Trend detection: Yes, Comparison: No
Non-target HS detection: Advances on the embedded non-targeted HS extraction tool, envihomolog:

A first advance was to detect and merge sets of systematically gapped series that overlap in their peak composition. These are series which have multiples of RU mass differences of each other, and which share subsets of peaks in alternating series positions as set by their RU mass difference. Such gapping arises from decreasing peak intensities, e.g., at the end of series, with non-detected peak signals leading to overlapping but differently mass-spaced series. The series with the smallest RU mass difference was retained for each such merging. Second, another advance was made to reduce partly overlapping and redundant peak series combinations over isobaric and close-eluting peaks and based on all possible peak series combinations formed by the original envihomolog algorithm. For this purpose, series were selected by their decreasing intensity summed of their contained peaks, and in a way that the next selected series had no peaks in common with any of the previously selected series. Unselected HS peaks remaining after this procedure were represented by the most intense (again by intensity peak sum) series they were contained in, and even if such a series overlapped in its peak composition with previously selected series. Third, series can form over the different isotopologues of the same ion species of a homologous analyte, and are then redundant in representing this analyte. Therefore, the nontarget components of HS peaks were checked for non-monoisotopic series. The HS with the largest fraction of monoisotopic peaks was retained, and other series in the individual components discarded. Fourth, and despite the named reduction to monoisotopic series, HS redundancy can still arise over the different ion species of a homologous monoisotopic analyte. The simultaneous co-elution over several peaks between HS as a criterion for grouping and further reduction is an advantage over non-HS componentization, and can even be applied to differently charged and possibly yet unidentified HS ion species. Here, monoisotopic series which coeluted with at least four peaks were reduced to the most intense series to finally represent a homologous analyte.
Optimization: S-Lens of Orbitrap Q-Exactive HRMS

tested parameters: S-Lens (50-100 arbitrary unit): 70

...depends on m/z and stability of analytes of interest:

่อ S-lens RF level: δ fragmentation of fragile ions
          ↑ low m/z transmission
          AND: δ high m/z transmission

Figure S 1: Optimization of S-Lens of QExactive HRMS instrument using two model homologous series, that is polyethylene glycol (PEG) and polypropylene glycol (PPG). For the optimization a sample taken from the primary clarifier, diluted 1:5 with nanopure water was used. To account for repeatability, a rather over-estimated value of 15% relative standard deviation was applied.
Figure S 2: Optimization of electrospray ionization interface using two model homologous series, that is polyethylene glycol (PEG) and polypropylene glycol (PPG). For the optimization a sample taken from the primary clarifier, diluted 1:5 with nanopure water was used. To account for repeatability, a rather over-estimated value of 15% relative standard deviation was applied. Tested parameters were the capillary temperature and the auxiliary temperature.

Mobile phase A: H₂O, 1 mM NH₄FA, 2% MeOH, 0.01% FA (pH 3.5)
Mobile phase B: MeOH, 1 mM NH₄FA, 2% H₂O, 0.01% FA
200 µL min⁻¹ flow
Optimization: **atmospheric pressure chemical ionization**

tested parameters: **Vaporizer T** (300- 450 °C): **400 °C**

**Capillary T** (280- 320 °C): **300 °C**

Mobile phase A: H₂O,2% MeOH

Mobile phase B: MeOH, 2% H₂O

200 μL min⁻¹ flow

Figure S 3: Compared to ESI, in case of APCI, solvent is evaporated by heating after the sample solution is sprayed into a fine mist of droplets. By applying a high voltage to a needle close to the exit end of the tube and creating a corona discharge, reagent ions are formed through a series of chemical reactions, involving the sheath gas nitrogen and solvent molecules. Sample molecules are ionized by reacting with the formed reagent ions. Polarity, thermal stability and molecular weight of the analytes of interest are the key parameters responsible for ion formation (Gruendling et al., 2010).

Optimization of atmospheric pressure chemical ionization interface using two model homologous series, that is polyethylene glycol (PEG) and polypropylene glycol (PPG). For the optimization a sample taken from the primary clarifier, diluted 1:5 with nanopure water was used. To account for repeatability, a rather over-estimated value of 15% relative standard deviation was applied. Tested parameters were the capillary temperature and the vaporizer temperature.
Optimization: atmospheric pressure photoionization

tested parameters: Vaporizer T (300-500 °C): 350 °C
Capillary T (280-320 °C): 300 °C
addition of dopant (post column 5-10% of flow rate; toluene, acetone, none): none

In the final analytical method 5% isopropanol was added to both eluents. Isopropanol is also considered as dopant.

Figure S 4: Ion formation in APPI can be distinguished classified into either direct or indirect/dopant assisted photoionization. A direct analyte photoionization occurs if the ionization potential of the analyte molecule is lower than the energy of the photon emitted by the light source. In the latter approach dopant-assisted photoionization, primary reagent ions are generated by using either dopant or appropriate solvents. This dopant or solvent is amenable to direct photoionization and produces thereby reagent ions which can subsequently ionize the analyte (Andrea and Alessandro, 2003), (Terrier et al., 2011).

Optimization of atmospheric pressure photoionization interface using two model homologous series, that is polyethylene glycol (PEG) and polypropylene glycol (PPG). For the optimization a sample taken from the primary clarifier, diluted 1:5 with nanopure water was used. To account for repeatability, a rather over-estimated value of 15% relative standard deviation was applied. Tested parameters were the capillary temperature, the vaporizer temperature and the post-column addition of the dopants toluene or acetone.
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<table>
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<tr>
<th>Abbreviation of HS family</th>
<th>Name of HS family</th>
<th>n, m</th>
<th>x</th>
<th>Molecular structure</th>
<th>Level of confidence</th>
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<th>APCI (-)</th>
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<th>APPI (-)</th>
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<td><strong>Cn - AS</strong>&lt;br&gt;n= 8- 16</td>
<td>alkyl sulfate</td>
<td>CH₂</td>
<td></td>
<td>D (2)</td>
<td></td>
<td>Cent.: P, B&lt;br&gt;VEC: P</td>
<td></td>
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<tr>
<td><strong>Cn- SAS</strong>&lt;br&gt;n, m = 10- 21</td>
<td>secondary alkane sulfonate</td>
<td>CH₂</td>
<td></td>
<td>D (2)</td>
<td></td>
<td>Cent.: P&lt;br&gt;VEC: P</td>
<td></td>
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<tr>
<td><strong>Cn- AExS</strong>&lt;br&gt;n= 12- 18; x= 1-12</td>
<td>alkyl ethoxy sulfates</td>
<td>CH₂</td>
<td>C₂H₄O</td>
<td>D (2)</td>
<td>Cent.: P&lt;br&gt;VEC: P</td>
<td>Cent.: -&lt;br&gt;VEC: P</td>
<td>Cent.: P&lt;br&gt;VEC: P</td>
<td>Cent.: P&lt;br&gt;VEC: P</td>
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<tr>
<td><strong>Cn - DATS</strong>&lt;br&gt;n, m= 4- 19</td>
<td>dialkyl tetralinsulfonates</td>
<td>CH₂</td>
<td></td>
<td>D (2)</td>
<td></td>
<td>Cent.: P&lt;br&gt;VEC: P</td>
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<tr>
<td><strong>Cn- LAS</strong>&lt;br&gt;n, m = 8- 14</td>
<td>linear alkylbenzene sulfonate</td>
<td>CH₂</td>
<td></td>
<td>D (2)</td>
<td></td>
<td>Cent.: P&lt;br&gt;VEC: P</td>
<td>Cent.: P, B&lt;br&gt;VEC: P, B</td>
<td></td>
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<tr>
<td><strong>SPA-nDC</strong>&lt;br&gt;n, m = 1- 15</td>
<td>sulfophenyl alkyl dicarboxylated</td>
<td>CH₂</td>
<td></td>
<td>D (2)</td>
<td></td>
<td>Cent.: -&lt;br&gt;VEC: P,S,O</td>
<td></td>
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<tr>
<td><strong>STA-nC</strong>&lt;br&gt;n, m = 1- 12</td>
<td>sulfotetralin alkyl carboxylated</td>
<td>CH₂</td>
<td></td>
<td>D (2)</td>
<td></td>
<td>Cent.: S,O&lt;br&gt;VEC: S,O</td>
<td>Cent.: O&lt;br&gt;VEC: P,O</td>
<td></td>
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</table>
Figure S 5: Molecular weight dependent charge state distribution. The x-axis shows the molecular weight of PEG, namely 06-110 EO units, intensities are shown as normalized peak heights on the y-axis. Number of charges are color coded. For both ionization interfaces the most abundant ion specie, namely Na-adducts for APPI and NH₄-adducts for ESI are shown. a) Ionization performed using (+)-APPI: shows observed Na-adducts with charge states up to +3 for PEG 06-110 EO. b) Ionization performed using (+)-ESI: shows observed NH₄-adducts with charge states up to +8 for PEG 06-110 EO.
Figure S 6: Prevalent Ion species, depending on the employed ionization interface and molecular weight.

a1) EIC of most abundant ion species for PEG-21EO, using (+) APPI as ionization source. a2) EIC of most abundant ion species for PEG-42EO, using (+) APPI as ionization source. b1) EIC of most abundant ion species for PEG-21EO, using (+) ESI as ionization source. b2) EIC of most abundant ion species for PEG-42EO, using (+) ESI as ionization source. c1) EIC of most abundant ion species for PEG-21EO, using (+) APCI as ionization source. c2) EIC of most abundant ion species for PEG-42EO, using (+) APCI as ionization source.
Figure S7: Detected HS in the different wastewater treatment stages. Samples were pre-concentrated using vacuum-assisted evaporative concentration. Ionization was performed with APCI, APPI or ESI in negative mode. RU are depicted as m/z and indicated in the respective color. Hits from the suspect list are highlighted by a black asterisk.