Novel passive sampling for steroid hormones in water using Diffusive Gradients in Thin films and the ERE-CALUX bioassay

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Framework

- Water Framework Directive consisting of 33 priority (hazardous) pollutants and 8 other chemicals under Annex II (2008/105/EC) in water quality assessment
- Stringent detection limits for AA-ESQ of watchlist chemicals (2013/39/EU)
  - Hormones at 0.05 ng/L and 0.4 ng/L for, respectively, EE2 and E2
- Classic spot sampling and sample pretreatment requires high volumes with current chemical detection methods (GC/MS-MS-M) in the range of 0.1-1ng/L.

Objectives

- Development of a time-integrated passive sampler for in-situ determination of estrogens in water using diffusive gradients in thin films (DGT) and having E2 as a model contaminant
- Independence of flow properties of the sampling environment requiring no post-calibration for uptake and binding characteristics
- Combined DGT approach with in vitro effect directed analysis for assessment of mixture activity otherwise missed by conventional methods during field work

Materials and Methods

Diffusive Gradients in Thin Films

- The total mass of analyte (M) accumulated on a resin over time (t) after passing through a well-defined area (A) with a known gradient (Dg), can be modeled according to Fick’s first law to determine the bulk water (Cw) concentration:

  \[ D_g \frac{M}{A} = C_w \frac{M}{A} \]

  Whereby Dg is the effective diffusion coefficient of organics in the diffusive gel which can be calculated from lab experiments and corrected for temperature variations in the field

- DGT sampler:
  - Teflon base (2.5cm)
  - HVLP Durapore filter membrane (PVDF 0.45µm; 0.017cm thick)
  - Agarose diffusive gel (0.025-0.125cm thick)
  - XAD18 Resin gel (0.05cm thick)

  - Resin layers are fabricated in pre-heated casts and stored in 0.03M NaCl until use
  - After sampling, resins are collected and extracted using an ASE 200 (Dionex)
  - Spiked water samples are extracted using Oasis HLB (6cc; 200mg) cartridges

Bioanalytical estrogen activity measurements using CALUX

- Chemically Activated Luciferase gene Expression for ER binding
- VM7Luc4E2 (variant breast cancer MCF7) recombinant luciferase reporter assay
- Measure total endocrine activity as opposed to individual compound conc.

- Determine biological equivalence to E2 reference compound by calculating a BEQ or EEQ expressed as ng E2-equ./L (ng EEQ/L)
- Experiments carried out according to optimized XDS LUMI-CELL® and OECD TC455 protocols and guidelines

Results & Discussion

Uptake capacity of XAD18 resin and experimental Dg

- Adsorption to DGT components is minimal (less than 5%)
- The XAD18 resin accumulates linearly and with an efficiency close to 100%

- A theoretical diffusion coefficients Dg,29°C for E2 (5.17 x 10^-6 cm^2 s^-1) in diffusive gel is in close agreement to an experimental value for Dg,20°C of (4.65 ± 0.37 x 10^-6 cm^2 s^-1) and is comparable to the literature value in water Dg,0 of 4.88 x 10^-6 cm^2 s^-1

Field application and DBL (6) measurement

- Effluents of three sewage plants in Beijing, China were sampled by DGT and grab sampling (sampling time: 6hrs)

- DBL (6) measurements in the lab (6: 0.021cm) and field (6: 0.022cm) justify the use of a combined Dg of 5.16 x 10^-6 cm^2 s^-1 for estrogens
- Spot sampling (mix of samples t5 and t6) is not different from DGT sampling for Gaobedian (GBD), Qinghe (QG), and Liangxiang (LX) stations

Conclusion

- Development of a novel passive sampling DGT device capable of measuring low levels of estrogens (MDL of 0.026 ng E2-equ./L) and independent of river flows
- Effective diffusion coefficient Dg,29°C of (4.65 ± 0.37 x 10^-6 cm^2 s^-1) for E2 in agarose and δ determination of 0.022cm (field) that is non-negligible compared to Dg,0 (0.092cm)
- Future applications with multiple DGTs in combination with hyphenated MS techniques for EU WFD compliance monitoring

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