Assessment of methods for measuring the membrane-water partition ratio (K_{MW}) for surfactants

Steven Droge[1*], Nina Jansen[1], Elin Barrett[2], Adriana Bejarano[3], Jens Bietz[4], Kristin Connors[5], James Dawick[6], Marc Geurts[7], Geoff Hodges[2], Eoin Kearney[8], Mark A. Miller[8], Diederik Schowanek[5], Sabrina Wilhelm[9]

[1]Environmental Risk Assessment, Wageningen Environmental Research; Netherlands; [2]Safety and Environmental Assurance Centre, Unilever, UK; [3] Shell Global Solutions, US; [4]Clariant Produkte (Deutschland) GmbH; [5]Procter & Gamble Brussels Innovation Centre; [6]Innospec Limited; [7]Nouryon; [8]Department of Chemistry, Durham University, UK; [9]BASF Personal Care & Nutrition Gmbh [*steven.droge@wur.nl]

Background & Objective

The Environment and Health – Risk Assessment & Management (ERASM) is a joint research platform of the European Detergents and Surfactants Industries. The ERASM 'Membrane Water Partitioning of Surfactants' project aims to evaluate the alignment between 3 experimental and 3 computational methods to derive the **phospholipid membrane-water partition ratio (K_{MW}),** covering 4 surfactant types. K_{MW} previously has been shown to provide a New Approach Methodology (NAMs - 3R principle) to assess the bioconcentration factor (BCF) when applied with *in vitro* biotransformation data as part of a tiered approach (Droge et al. 2021). Here we discuss results from 3 experimental methods: liposome vesicles in dialysis systems, Solid Supported Lipid Membranes (SSLM: bilayers covering silica), and immobilized artificial membrane chromatography (IAM-HPLC: monolayer coated silica). For computational methods, see Poster Corner TUE 3.12 Kearney et al.

Results

- Even for non-surfactant chemicals, K_{MW} range ~1 log units for the 3 methods (**Figure 1**).
- There is reasonable consistency between the K_{MW} by SSLM and dialysis. The high density (HD) and low density (LD) SSLM sorbent dilution series deviated up to a factor 3 in K_{MW}. Care is needed in selecting the right density **(Figure 1**).
- K_{MW} by IAM-HPLC are >10x higher than SSLM for zwitterions, nonionics and C12-E4SO4, but lower for some cations (**Figure 1**). The ethoxylate chains (coloured units in molecules) cause strong overestimation for IAM-HPLC, no explanation yet for zwitterions.
- K_{OW} is predictive of K_{MW} for nonionic surfactants, but K_{OW} may strongly underestimate the favourable interactions with phospholipids for ionic surfactants. Biotransformation data maybe required for some surfactants to determine relevant BCF values. For most ionic surfactants, K_{MW} >> K_{OW} (**Figure 2**).
- Experimental feasibility for the experimental methods is limited to logK_{MW} <6. In a proposed Tier 1 baseline BCF assessment (no biotransformation, 1% phospholipid fraction as key sorptive phase, (Droge et al. 2021)) logK_{MW} 5 ≡ 1000 L/kg for ionic surfactants. Validated computational approaches to predict K_{MW} are thus needed to extend the chemical domain for challenging (technical) surfactants.

Figure 1: Membrane-water partition ratios from 3 different methods, LD = low density; HD = high density

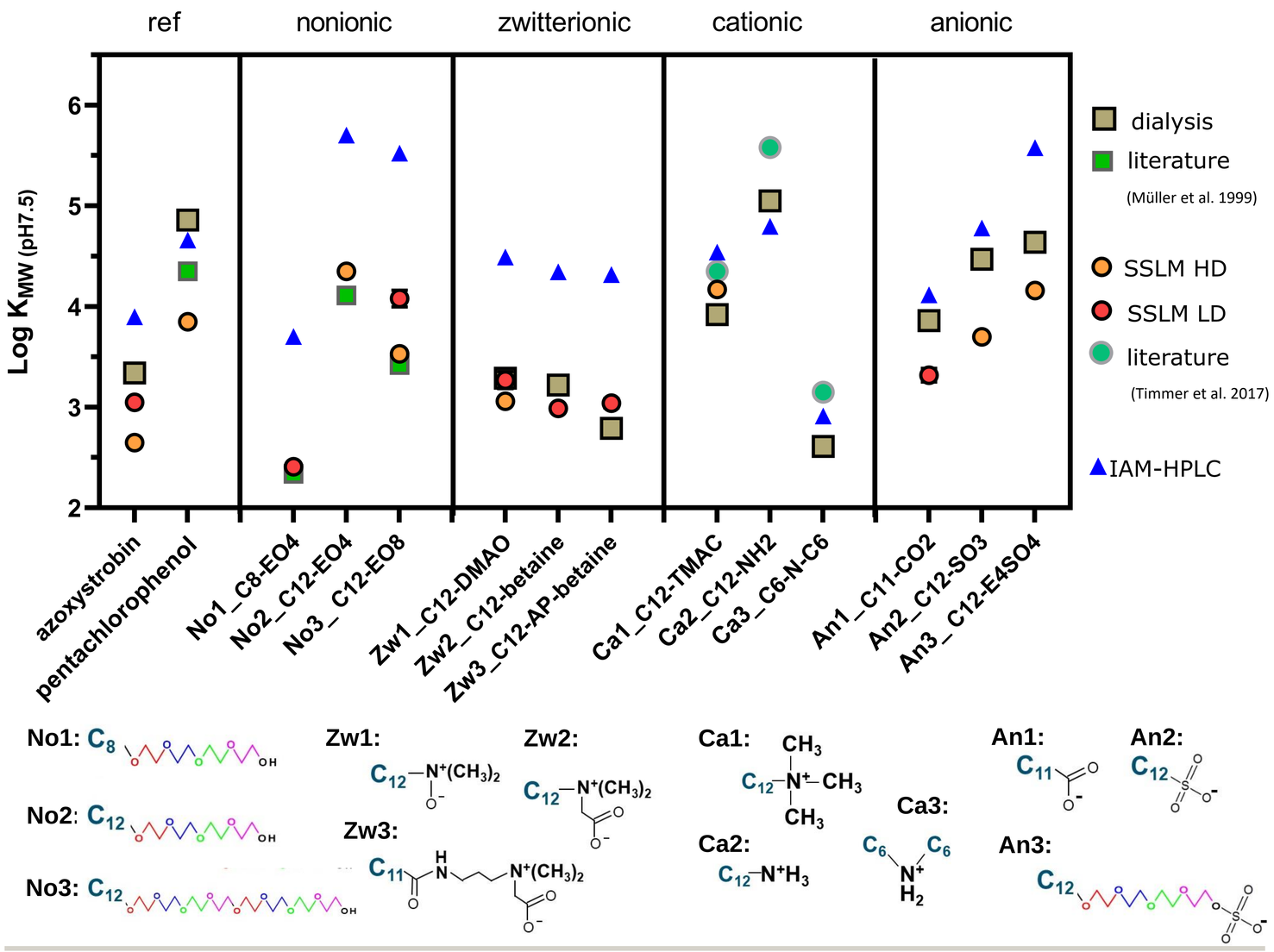
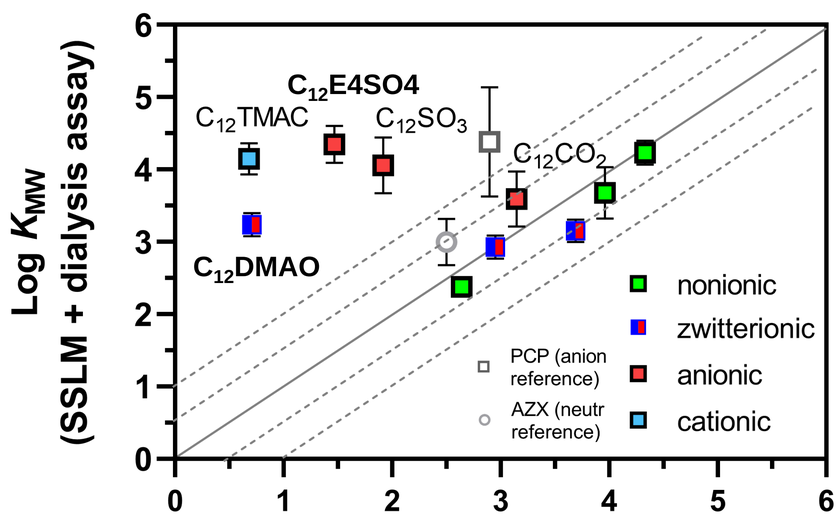


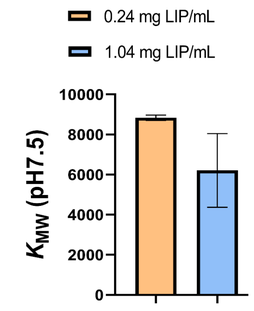
Figure 2: Average membrane-water partition ratios (SSLM and dialysis) plotted against the most reliable K_{OW} values using the slow stirring method (Hodges et al. 2019).



Examples shown for anionic C_{11}-CO_2

Dialysis method

Average K_{MW} obtained at 50% and 80% sorbed fraction with triplicate samples.



Lipid: Bilayer liposomes vesicles by extrusion cycles over 100 nm

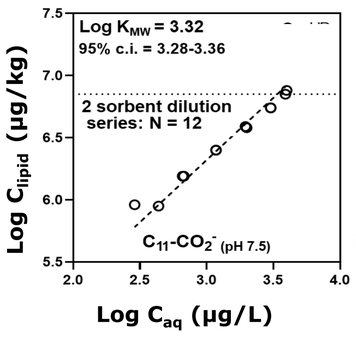
Pros: Benchmark method, adjustable lipid material, various commercial suppliers

Cons: Equilibrate time 2-4 d, Limited shelf life lipids, variable batch quality, adsorptive losses at high K_{MW} lipid dose needs good K_{MW} est.

SSLM method

Duplicate sorbent dilution series.

K_{MW} obtained by fitting a linear isotherm slope of 1.



Lipid: Solid supported lipid membrane bilayers

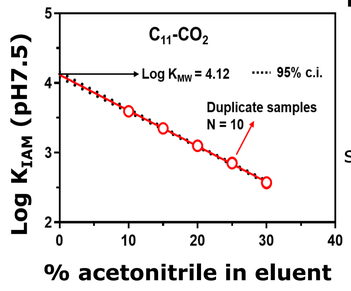
Pros: Standardized pre-dosed lipid material, rapid (<1h) equilibration, easy centrifugation.

Cons: Transfer into glass vials required for surfactants.

IAM-HPLC method

Chromatographic peak elution series of solvent/water, extrapolated to 100% water.

Retention capacity factor x phase ratio = partition ratio K_{IAM}



Lipid: Monolayer coating phospholipid on silica

Pros: Cost-effective, direct analysis, eluent 100% water or solvent series, easily transferable inter-lab

Cons: Sensitive to eluent pH and ionic strength, influence of silica, poor prediction Non&Zwitterions

Conclusions & Outlook

- We completed a systematic K_{MW} data matrix (**Figure 1**) to inform risk managers which type of membrane-water assay has a high confidence level, for which chemical applicability domain, and for further work towards regulatory acceptance.
- Commission Regulation (EU) Amendment 2021/979 states that: "an experimental bioaccumulation study cannot be waived on the basis of low K_{OW} alone, if the substance is surface active or ionisable at environmental pH (4–9)." This is confirmed for several ionic surfactants (**Figure 2**).
- The K_{MW} can be most effectively measured for all surfactant types using the SSLM method. K_{MW} determined by the SSLM method demonstrates good consistency with K_{MW} determined by the benchmark liposome dialysis method (< factor 3). IAM-HPLC can roughly indicate K_{MW} for anionics and cationics.
- A ring test K_{MW} study is being considered to support possible development of OECD test guideline for K_{MW} analysis.
- Study results support earlier assessments (Hodges et al. 2019, Droge et al. 2021) that the K_{MW} is a biologically relevant & methodologically alternative to K_{OW} for ionizable surface-active chemicals.

DOI for cited references: Muller 1999 - 10.1002/etc.5620181011; Timmer 2017 - 10.1021/acs.est.6b05662; Hodges 2019 - 10.1186/s12302-018-0176-7; Droge 2021 - 10.1039/d1em00327e.