Determining Water Solubility of a Poorly Soluble Liquid; Beyond OECD 105

B. Retter, Scymaris Ltd, Freshwater Quarry, Brixham, Devon, TQ5 8BA, UK

Introduction

Water solubility of compounds is an essential parameter to understand how a chemical behaves in the environment and any hazards that may be associated with it, while also being a requirement in global chemical assessments and regulatory submissions.

The OECD 105 guideline currently uses two methods for water solubility determination; shake flask (solubility >10 mg/L), and column elution (solubility <10 mg/L). Each method comes with limitations, and while the column elution method is suitable for compounds of poor solubility, it is unsuitable for testing liquids.

Here we present a successful case of determination of water solubility using an alternative slow-stir method. This method using principles taken from the OECD 123[1] guideline and apply the criteria from the OECD 105 to determine the water solubility of a poorly soluble, liquid compound. Previous work conducted by D. Letinski *et al*.[2] demonstrated effectiveness of the methodology for medium chain saturated hydrocarbon compounds, while here we apply this methodology to a long-chain fatty acid methyl ester (FAME).

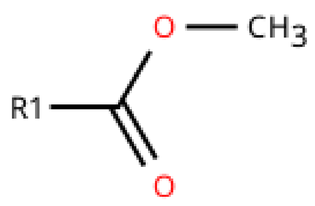

Figure 1 –Typical FAME structure (MW 354.5)

Apparatus and Materials

Analysis was performed on a TSQ Altis mass spectrometer, coupled to a Vanquish UHPLC (Thermo Scientific, USA). The HPLC methods used were performed on a C8 HPLC column, using a water/methanol mobile phase with formic acid modifier.
The test media used during the study was LC-MS grade water, to ensure a resistivity > 10 MΩ cm^{-1} was achieved. Glass test vessels with an approximate capacity of 1 L and a fitted tap at the base were used. A magnetic stirrer bar was added to the test vessel.

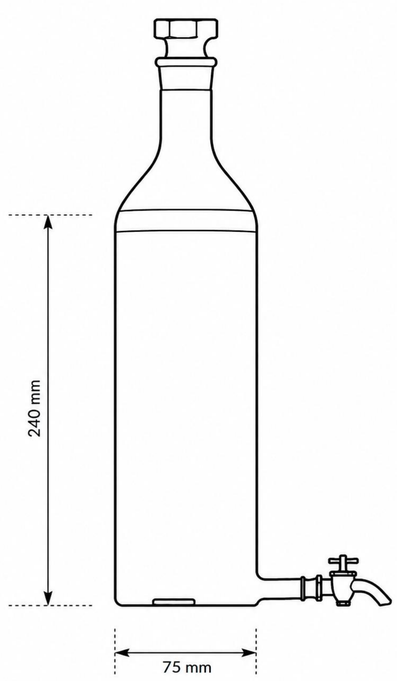

Figure 2 – Test Vessel Schematic

Method

The study was performed using duplicate test vessels. Each test vessel was filled with LC-MS grade water, and the test compound carefully dosed to the surface of the water using a glass syringe. The test vessels were placed onto magnetic stirrers and the stirring rate slowly increased until the vortex at the water/test substance interface, becomes visible but remains ≤ 0.5 cm.

The test vessels were equilibrated in the dark at 20°C for 7 days, prior to the initial sampling point (Day 0). Samples were then taken at Days 0, 1, 2, 3 and 7, following the equilibration period.

Day -7*	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
-	X	X	X	X	-	-	-	X

*Equilibration started
X = sampling point, - = no sampling required

Table 1 – Sampling Regime

On each sampling occasion the stirrers were stopped and the liquids allowed to settle. A visual assessment for the presence or absence of undissolved test substance at the surface of the test media was performed. Samples were taken from the tap at the base of the test vessels, collecting the initial discard from the tap for pH and Tyndall effect assessment. Remaining samples were taken, in duplicate, directly into glass vials containing an approximate equal volume of solvent, to minimise potential glass adsorption. The aliquot and vial weights were determined to allow calculation of exact dilution factors for increased accuracy.

Results

Day	Vessel	% Difference within vessels	% Difference between replicates	Overall Mean Calculated Concentration (µg/L)	Water Solubility (µg/L)	RSD (%)
0	1	-2	38	129	31.4	8
	2	2				
1	1	-16	17	30.9		
	2	-14				
2	1	-17	13	33.1		
	2	-10				
3	1	-17	3	27.9		
	2	-16				
7	1	-13	2	33.5		
	2	4				

Table 2 – Overall Water Solubility Results

The results from the Day 0 analysis were much higher than the subsequent sampling points. This was suspected to be due to a build up of particles in the tap after the equilibration period and an insufficient initial waste volume. The results from this sampling point were therefore excluded from the final water solubility determination.

Vessel 1		Vessel 2			
Mean Conc. (µg/L)	Difference in Sampling Occasions (%)	Mean Conc. (µg/L)	Difference in Sampling Occasions (%)	Overall Mean Conc. (µg/L)	Difference Between Vessels (%)
32.7	-16	30	-9	31.4	9

Table 2 – Results Summary

The results show an RSD of 8% and the variability between the two test vessels is 9%, indicating a good level of precision between all sample concentrations and between the test vessels. This is further confirmed visually as shown graphically, and calculation of the Pearson correlation with α = 95% confidence showed the results were not statistically different from 0 (p stat = 0.618), confirming equilibration was reached.

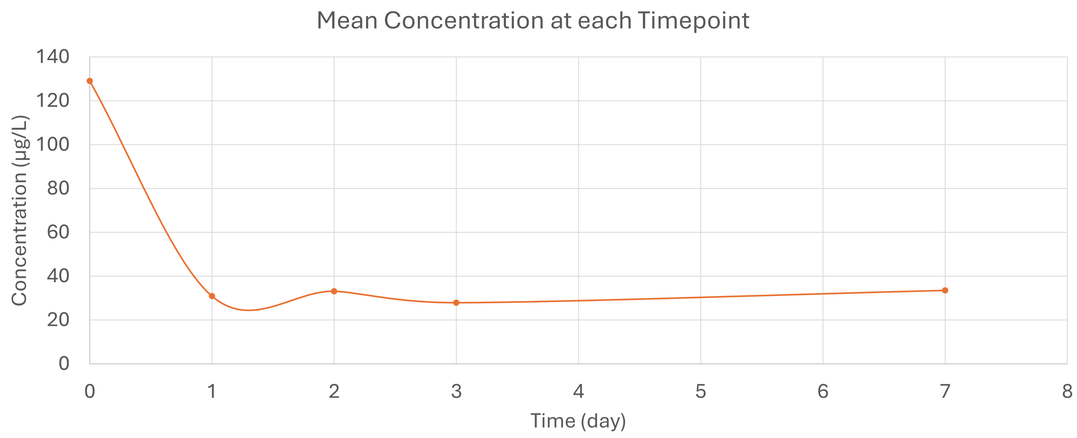

Figure 3 – Water Solubility Results

A calculation of the mean water solubility was taken from the results at equilibrium to determine an overall water solubility value of 31.4 µg/L.

Discussion

A preliminary test was performed in this study as per the OECD 105 guideline which returned a value at ppm level, in contrast to the ppb level determined in the definitive test. This shows an overestimation of the solubility, which is similarly reflected in QSAR predicted values. The use of the slow stir method allows us to accurately determine a more realistic value of water solubility for poorly soluble liquid compounds, allowing for better understanding of the chemical's behaviour within the environment.

The test so far has used liquids with a density < 1 g/mL, allowing for dosing on the surface of the water to maintain an interface. The test may similarly be applied to liquids with a density > 1 g/mL; dosing to the bottom of the vessel and sampling from the top.

Future work on this methodology would look at applying the test to a wide range of poorly soluble liquids, of varying densities, to understand the applicability and robustness of the method and to become a widely recognised and adopted method for water solubility determination.

Conclusions

Overall, the slow-stir water solubility test successfully determined the water solubility value of a poorly soluble liquid test compound, with a density of < 1 g/mL. The mean solubility values within and between each test vessel did not exceed ±20% indicating consistent results, and that an equilibrium was reached within the test period.

References

[1]OECD 123, OECD Guideline for the testing of chemicals – partition coefficient (1-octanol/water): slow-stirring method. 2006 :1-16. https://doi.org/10.1787/9789264015845-en

[2]D. Letinski, A. Redman, H. Birch, P. Mayer, Inter-laboratory comparison of water solubility methods applied to difficult-to-test substances, *BMC Chemistry*, 2021, 15:52, DOI: 10.1186/s13065-021-00778-7