



IMPROVED OECD 105 WATER SOLUBILITY TEST DESIGN

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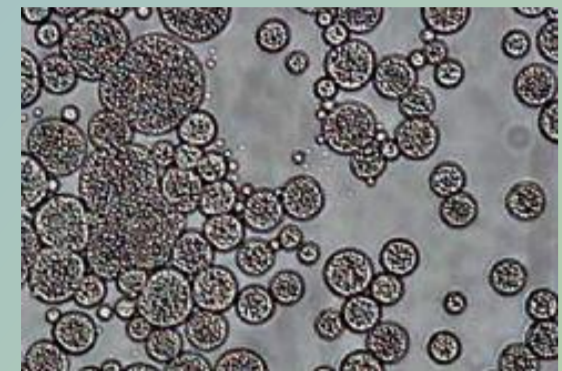
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Introduction

Water solubility is one of the critical parameters of water chemistry required for ecotoxicological risk assessment of chemical substances.

The OECD 105 Guideline¹ comprises two methods, the shake flask method for water soluble substances (liquids or solids) and the column elution method for poorly soluble solids (despite the fact that liquids are not formally excluded, the column elution is often not technically feasible, due to clogging for example).



Poorly soluble liquids therefore pose a problem as the shake flask method can lead to the formation of emulsions and potential overestimation of the solubility. Furthermore, the solubility of certain substances in pure water may differ, sometimes considerably, from their solubility in the more relevant environmental media potentially leading to misconceptions of the toxicity of the substance in certain cases.

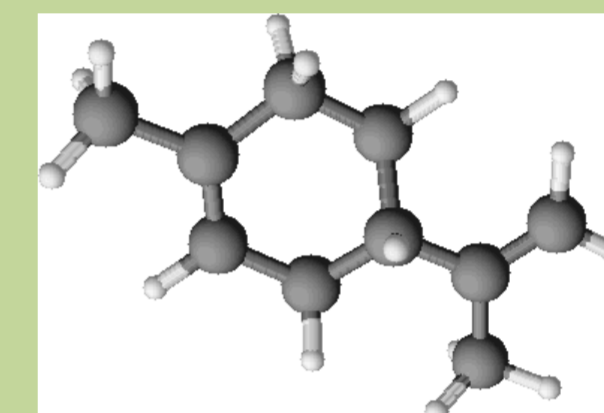
A method is proposed incorporating a simple modification that is imported from another OECD Guideline (OECD 123)² which can be used to improve solubility determinations for poorly soluble liquids and provide reliable indications of true solubility in aqueous media. A similar design was already proposed by Letinski *et al.* (2002)³ for measuring solubility aliphatic alcohols, phthalate and adipate diesters, as an alternative to the "WAF" (Water Accommodated Fraction) method. This method has the added advantage of also being useful for determination of solubility for slightly unstable substances and volatiles.

Moreover, in certain cases differences in solubility are found between pure water and media used in ecotoxicity tests due to the salt content of the latter. For this reason both media are tested.

Materials and Methods

Substance types tested:

A fragrance molecule – d-limonene, CAS # 5989-27-5, a high volatility (200 Pa at 25°C), low solubility (<10 mg/L) liquid.



Equipment

Standard glassware used for slow-stir octanol-water partition coefficient study (OECD 123)

Medium: Double distilled water and OECD 202 *Daphnia* medium.

Principle of the method:

2 replicates of 700 ml water per test medium.

At least 1 ml of test solution is added gently to the surface using a pipette and the system is stoppered to prevent volatile substances from leaving the system.

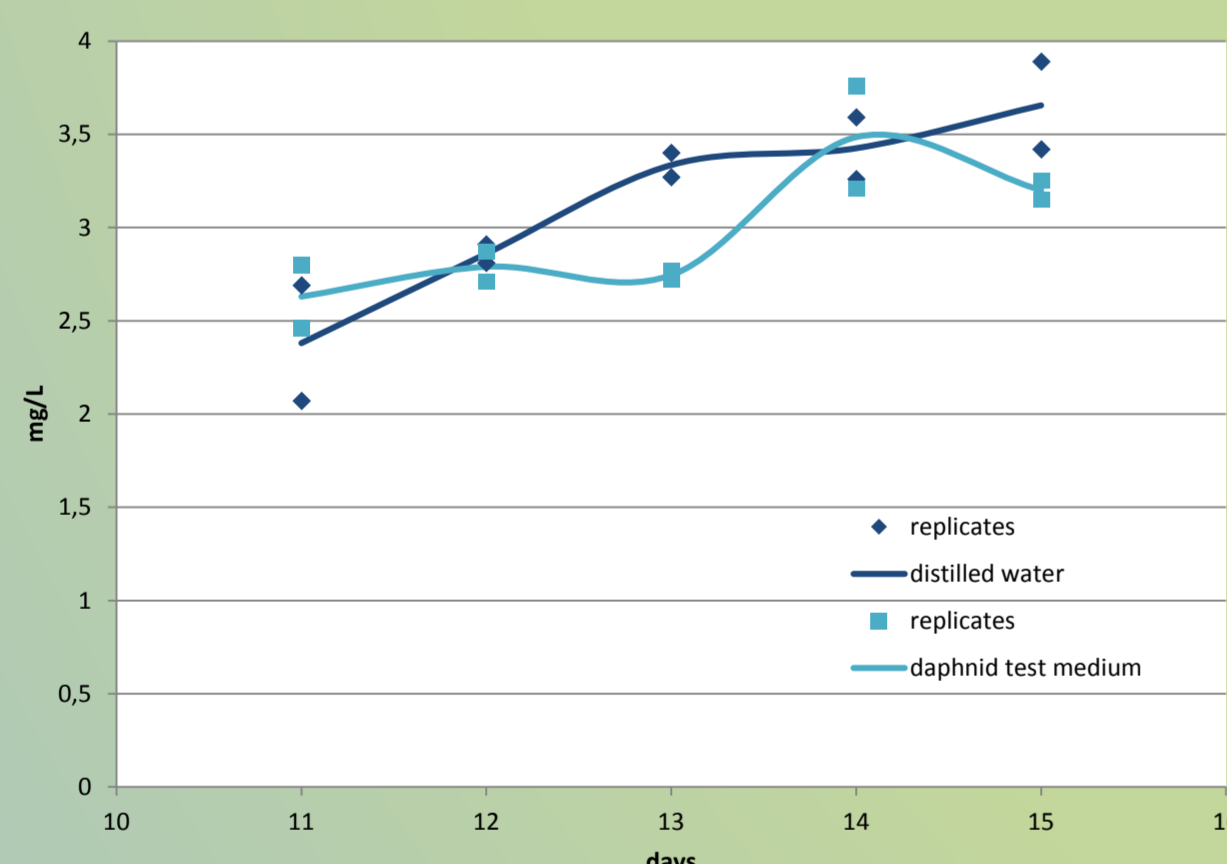
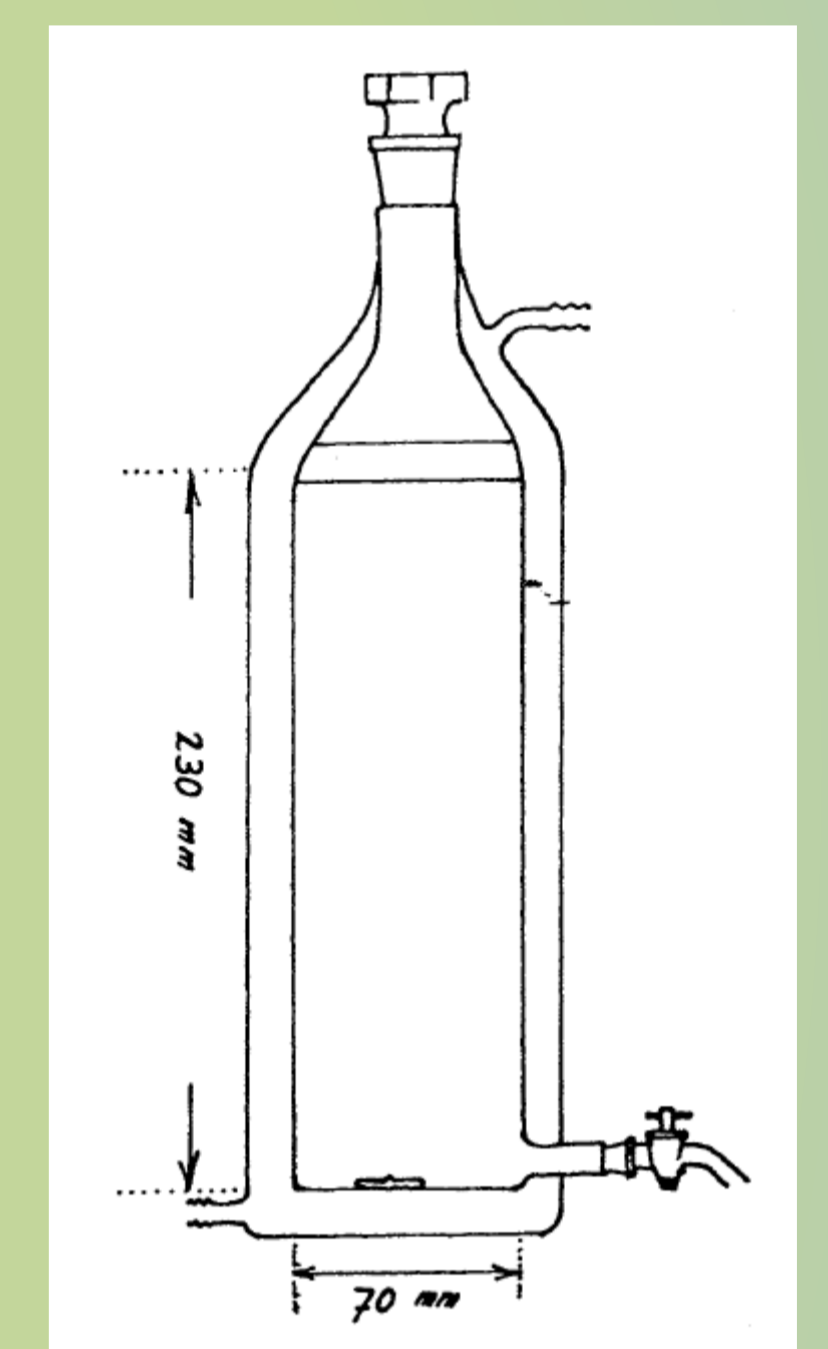
The system is slow stirred at 20 ± 0.5°C to avoid formation of droplets.

Aqueous samples are removed using the side arm.

Frequency of sample removal depends on expected solubility kinetics of the test substance and its stability and may vary from hours (for hydrolysing substances) to days (for stable very poorly soluble substances).

Samples should be removed regularly until a plateau is observed.

At least five successive samples are taken such that equilibrium can be demonstrated (i.e. concentrations do not differ by more than ± 30% in the last 3 samples).



Results

d-Limonene was measured by GC-FID from day 8 of stirring. Solubility values ranged between 2.07 and 3.89 mg/L in double distilled water, and between 2.71 and 3.76 mg/L in reconstituted *Daphnia* medium. Replicates varied by approximately 0.3 mg/L on average for both solutions.

Solubility was calculated to be 3.5 mg/L in double distilled water and 3.3 mg/L in *Daphnia* medium when equilibrium was considered reached after ca. 14 days of stirring.

Discussion

According to the literature⁴ the solubility of d-limonene is 12.3 mg/L using the shake flask method. This was considered to be a reliable study. Other shake flask studies varied between 6.13 to 20.4 mg/L. This overestimate was due to the capacity of d-limonene to form stable emulsions in the shake flask method.

The plateau appeared to be reached after 14 days and this suggests that maintaining a 15 day test duration may be justified in cases where the plateau is not visibly attained earlier.

The system can also help to obtain a good approximation of solubility for hydrolysing substances with a half-life of 12h or more due to the excess of test substance at the surface. However, hydrolysis of the test compound may produce surfactant-like degradation products which could increase apparent solubility of the substance.

The present study supports the suitability of the slow-stir method for volatile compounds. Further studies are ongoing to verify its accuracy and precision for unstable substances.

Conclusions

This test system has been found to be superior in design than the existing OECD 105 methods for testing low solubility (<10 mg/L) organic liquids with a relative density <1. The system also functions for volatile substances. As the test substance is in excess, the occasional opening of the system to avoid vacuum during sampling is unlikely to alter the final results.

The limonene study will be repeated to verify inter-laboratory reproducibility of the method.

References

1. OECD guideline 105 (1995) Water solubility.
2. OECD guideline 123 (2006) Partition coefficient (1-octanol/water): slow-stirring method.
3. Letinski, D.J., Connolly, M.J., Peterson, D.R. and Parkerton, T.F. (2002) Slow-stir water solubility measurements of selected alcohols and diesters, *Chemosphere*, 48, 257 – 265
4. Tamura K and Li H (2005) Mutual solubilities of terpene in methanol and water and their multicomponent liquid-liquid equilibria, *J. Chem. Eng. Data* 50(6): 2013–2018.