The Effects of Water on DMSO and Effective Hydration Measurement

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DMSO has been used to prevent freezing injury to living cells for over half a century\(^1\). Lovelock\(^2\) concluded the principal protective action of a neutral solute (e.g. DMSO) was simply its ability to prevent excessive concentration of electrolytes and other substances that otherwise occur on freezing.

**Freezing Point Depression of DMSO**

However, DMSO solutions are prone to adverse effects, such as evaporation, precipitation, contamination and decomposition, to name a few\(^3\). As DMSO is highly hygroscopic, water absorption occurs quickly, which greatly affects the solubility of some compounds in DMSO in freezer storage and can significantly affect the value of the corporate compound collection. As seen in Figure 1, the freezing point of DMSO is greatly depressed by the presence of water, requiring much lower temperatures to freeze the sample.

**Figure 1: Freezing point of DMSO-water mixtures**

DMSO with water exhibits its maximum change at 33\% by weight water (figure 2), which corresponds to a molar ratio of 1 DMSO: 2 H\(_2\)O. The melting point decreases from 18°C to -73°C, the liquid phase becomes more structured and viscous, making it more difficult to form a cavity and therefore more difficult to dissolve a large lipophilic compound\(^4\).

**Figure 2: DMSO solubility dependence on H\(_2\)O**
Ostwald’s Rule of Stages – Entropy of Solubility

Ostwald’s Rule of Stages states that if a crystalline material can exist in several polymorphic forms, the one with the highest entropy will develop first:

1. Amorphous – highest level of entropy
2. Highest entropy crystalline polymorph
3. Lowest entropy crystalline polymorph

DMSO Solubility

Amorphous DMSO solubility is always higher and will be supersaturated relative to the thermodynamic DMSO solubility of a more stable crystalline form. Some compounds will remain in solution indefinitely, even though the solution is supersaturated. These are compounds whose concentration lies in a thermodynamically unstable but a kinetically stable “metastable” zone. This phenomenon only occurs when a supersaturated solution is created in the absence of crystalline solid. It does not occur if dissolution starts from the crystalline solid.

Other Effects of Hydrated DMSO

Water in DMSO increases super saturation and greatly decreases solubility as the now crystallised sample is in a lower energy state, has a higher melting point and is in a less DMSO soluble form. Therefore, freeze/thaw cycles increase the probability of crystallisation of samples from DMSO. The adverse effect of freeze thaw cycles on DMSO stock solutions stored in plate format, as a result of cherry picking operations, has led to the gradual replacement of plate-based storage with tube-based storage so as to minimise the number of freeze/thaw cycles.

Precipitation from DMSO

Compounds will precipitate from a DMSO stock solution if the kinetic conditions are appropriate; e.g. if enough time passes or enough freeze/thaw cycles are encountered as part of the compound in DMSO storage process. Amorphous compounds generally dissolve fairly easily at 30 or even at 60mM in DMSO, but noticeable precipitation can be detected after about a day. This occurs even at room temperature and even in the absence of any freeze/thaw cycles. If the compound falls out of solution, any analysis will be testing a DMSO/water solution alone and not the compound in question.

Advantages of amorphous compounds:

- Solubility in DMSO is always higher than crystalline compound
- Initially dissolve easily in DMSO
- Allow easy preparation of DMSO stocks

Disadvantages of amorphous compounds:

- Precipitation problems if DMSO becomes hydrated
- Precipitation problems if there are freeze/thaw cycles
- Precipitation problems if samples are cooled whilst liquid

Novel NIR Technique to Assess DMSO Water Absorption

Properties of a fluid are normally determined using invasive methods. These methods lead to possible contamination and/or consumption of the fluid sample. In the case of DMSO, it can also lead to hydration of the solvent from the atmosphere. In this instance, non-invasive measurement methods are preferred.

The NIR technique measures the combination overtone hydroxyl bands of water in the presence of DMSO in the NIR (near-infrared) region. It has been demonstrated that NIR can accurately and non-destructively assess water content in DMSO compound stock solutions.
**Tube Dowser – the Non-Invasive Method for Determining DMSO Hydration Levels**

The level of hydration of DMSO is rarely known and with such significant changes in melting point and solubility of compound Ziath has added the Tube Dowser (figure 3) from EDC Biosystems to their range of products.

![Tube Dowser from Ziath Ltd](image)

*Figure 3: Tube Dowser from Ziath Ltd*

**Figure 4: Schematic of a typical near-infrared (NIR) instrument**

Figure 4 shows the traditional NIR instrument set-up, but the technology within the Tube Dowser allows it to be much smaller, only 400g, and negates the need for all the separate instrumentation. This also means the Tube Dowser is much less expensive, and thus provides a cost effective method to manage compound collections.

**Tube Dowser – Method of Operation**

- Place the sealed tube into the Tube Dowser
- Read the measurement from the PC screen (<50msecs)

The Tube Dowser operation is quick, reliable, accurate to within 1% of hydration level of DMSO and is compatible with many storage tubes. DMSO concentration measurement range is from 50–100%. It’s ideal for integration into currently used robotic systems.
Tube Dowser Features

- Non-invasive measurement technology
- Accurate to within 1% of hydration level of DMSO
- Solvent concentration measurement range: 50-100%
- Operating temperature: 10°-45°C
- Measurement time: <50ms
- Wide range of tube diameters accepted
- No de-capping required
- Single-channel tube reading
- Easily adaptable to existing tube handling systems
- Powered by USB

See [http://www.ziath.com](http://www.ziath.com) for more information.

The Tube Dowser is the essential tool for any laboratory storing compounds in DMSO.

Tips for sample storage in DMSO

- Compound storage in a DMSO solution should be at -70°C or lower
- If multiple freeze/thaw cycles are anticipated, multiple replicate samples should be considered, rather than one master sample
- Keep samples well sealed to avoid water absorption
- Be alert to compound precipitation from DMSO
- Minimise the working time once DMSO stocks are diluted (1-2 days at RT)
- If hydration of the DMSO is suspected, use a non-invasive measurement technique to determine hydration level

5. Yan, B., *Analysis and purification methods in combinatorial chemistry*; Wiley Interscience 2004