Samples in DMSO: What an end user needs to know

Christopher A. Lipinski Adjunct Senior Research Fellow Pfizer Global R&D, Groton New London Labs Christopher_a_lipinski@groton.pfizer.com

Who is an end user

- Stores HTS plates in lab for a few weeks to 3 months
- Room temperature chemical degradation
 - 5% at 3 months
 - 10% at 6 months
- By analogy to p = 0.05 store samples for up to 3 months then dispose
- CAVEAT
 - trifluoroaceticacid (TFA) contamination

Why is DMSO hygroscopic?

- Liquid A in equilibrium with vapor of liquid B
- Ideal behavior case
 - liquids have no interactions
- Sealed container molar composition depends on:
 - starting molar concentrations of A and B
 - relative vapor pressures of A and B
 - 20°C vapor pressure ratio is 41.66 H2O to 1 DMSO
- DMSO and water have pronounced interactions
- Non ideal behavior
- Driver for DMSO hygroscopic behavior

DMSO and water, non-ideal behavior

- DMSO plus water exhibits very non ideal behavior
 - change maximum at 33% by weight water
 - corresponds to molar ratio 1 DMSO : 2 H2O
 - liquid phase is very structured
 - ice-1 like lattice structure
 - more H-bonding, more order than in pure water
- Melting point declines from 18 deg C to -73 deg C
- Viscosity increases as water is added to DMSO
- Solution gets colder as water is added to DMSO
- DMSO is very hygroscopic
 - (J Org Chem2001, 66, 5846-5852)



<u>Compound solubility in DMSO and H₂O</u>

- As H₂O is added solvent is more structured
- More difficult to form a cavity
- More difficult to dissolve a large lipophilic compound
- Solubility non ideality is expected to be worst at 33% by weight $\rm H_2O$



DMSO – water phase diagram



DMSO containing 9% water is unfrozen in the typical lab refrigerator

<u>Very bad practice</u>. Typical biology procedure of storing samples in DMSO in the non-freezer part of a lab refrigerator

Nature (1969), 220, 1315-1317

Storage Pod to keep DMSO plates / tubes dry





http://www.4ti.co.uk

Compound storage and compound concentration

- Nobody knows compound concentration
- 2 4 x concentration error is insignificant in HTS
- 10 –100 x concentration error is significant
- IC-50 orders get scrambled
- Selectivity panels are inaccurate
- Compounds precipitate from DMSO
- Compounds precipitate from buffer
- LLQ issue in buffer quantitation

Streamlined System for Purifying and Quantifying a Diverse Library of Compounds and the Effect of Compound Concentration Measurements on the Accurate Interpretation of Biological Assay Results. Anal. Chem. (2004), 76(24), 7278-7287.

Improving quality by analytical

- Compound identity
- Compound purity
- Compound concentration
 - Adding analytical quantitation to HTS is the single greatest HTS quality advance possible
 - Technically requires 100-1000 x improvement in technology efficiency
 - CLND detection
 - ELSD detection
 - reduced solvent suppression MS

New Matrical / Pfizer freeze thaw data

High Throughput Sonication: Evaluation for Compound Solubilization

Kevin Oldenburg¹, Douglas Pooler¹, Kurt Scudder¹, Christopher Lipinski2 and Michele Kelly²*.

¹ MatriCal, Inc., Spokane, WA; ² Pfizer Global Research and Development, Groton, CT. Accepted for publication Combinatorial Chemistry and High Throughput Screening 2005, 8, 499-512

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 minimize the number of freeze thaw cycles. Compound solubility in DMSO is markedly decreased by uptake of small quantities of water Finally, we demonstrate that **precipitation of compound from DMSO stock solutions is synergistically enhanced by water uptake into DMSO compound stock solutions as well as by increasing the number of freeze thaw cycles.**

Sonication puts 50% of solids into solution



Disposable 384-sonication lid



Sonic horn transfers energy directly to samples via lid

Microplate Gasketed lid design ensures airtight seal during sonication

Floating pins

Sonic Horn

Gasket

http://www.matrical.com/SonicMan.htm

Sonic / Acoustic effects on re-dissolution

- Expected effects with contact sonication
 - excellent mixing
 - efficient cell lysis
 - speed up equilibration time
 - minor effect -may induce precipitation
- Unexpected beneficial effects
 - 50% dissolving of precipitated samples
 - light scattering data
 - rescue of expired HTS plates
 - similar dissolving with contact-less acoustics
- Theory of re dissolving is speculative

Sonication, why does it work?

- Redissolution from both contact and external sonication
- Redissolved material is thermodynamically more stable, less soluble so dissolution is counter to thermodynamics
 - modest bulk temperature increase, Temp < 60°C
 - no chemical degradation by HPLC
- Explanation?
 - cavitation-local temperature, pressure gradients?

Synergy between water uptake and freeze thaw

- Super saturation in DMSO is high
 - possible to see freeze thaw effect
- Super saturation in DMSO is low to moderate
 - difficult to see freeze thaw effect
- Water in DMSO increases super saturation
- Water in DMSO greatly decreases solubility
- <u>A little water in DMSO decreases compound solubility</u> <u>a whole lot</u>

<u>Fraction of Pfizer Groton compounds having melting</u> <u>point field information</u>



<u>Compounds differ in aqueous and DMSO</u> <u>solubility based on crystalline form</u>

- Ostwalds "rule of stages"
- Sequence of compound batch isolation proceeds towards thermodynamically most stable form
 - 1. amorphous highest energy solid form
 - 2. highest energy crystalline polymorph
 - 3. lowest energy crystalline polymorph
- Amorphous is the highest energy form
 - most soluble in water and DMSO

Dimension of the solubility problem

- 40% of compounds have poor aqueous solubility
- Half the problem due to size / lipophilicity
- Half the problem due to crystal packing
- DMSO no better than water for compounds insoluble due to crystal packing
- <u>Suggests an upper limit of 20% compounds insoluble in</u> <u>dry DMSO at 10uM</u>
 - supported by NCI Frederick, MD studies
- Will this limit ever be achieved?
- Depends on whether a nucleation event occurs
- Depends on number of freeze thaw cycles

Taking advantage of amorphous states

- Amorphous state enhances dry DMSO solubility
 - negligible problems at 60 mM
- Keep the DMSO dry
- Avoid cooling in less than 3 months storage
- No freeze thaw cycles

Solubility and compound purity

- Pressure on chemistry to increase output
 - crystallization has disappeared
- Combinatorial compounds are now being purified by automated procedures
 - 85-95% pure by ELSD or UV
- Compounds "appear" more soluble
 - amorphous state
 - impurities enhance solubility
 - crystal growth retardation
- Compressed samples (multiplexing)
 - multiple compounds in a well
 - solubility is enhanced

Pro's and con's of amorphous compounds

- Pro:
- Amorphous DMSO solubility is always higher than when compound is crystalline
- Amorphous compounds from combichem or medchem initially easily dissolve in DMSO
 - allows easy preparation of DMSO stocks
- Con:
- Sets stage for later precipitation problems
 - if the DMSO gets wet
 - if there are freeze thaw cycles
 - if samples are cooled while liquid

Timing factor in compound DMSO solubility

- Once a compound crystallizes from DMSO it will not easily re-dissolve
 - crystallized compound is in a lower energy, higher melting point, less DMSO soluble form
- Narrow working window (time window) for keeping most compounds dissolved in DMSO
 - 1 to 2 days at room temperature
 - explains why compounds are active when freshly made but not when stored
- Freeze thaw cycles increase the probability of crystallization

Alternatives to DMSO?

- Organic solvent –evaporate store as film
 - chemical stability issues
 - poor control of plasticity crystallization
 - wet-ability issues glycerol in DMSO
- Additive in DMSO to retard crystallization from H₂O
 - pluronic copolymer
 - block ethylene /propylene oxide copolymer
 - very low CMC
 - two solubilization mechanisms are possible
 - micellar solubilization
 - kinetic retardation of crystal growth

https://worldaccount.basf.com/wa/NAFTA/Catalog/ChemicalsNAFTA/pi/B ASF/Brand/pluronic

Functionality most sensitive to precipitation

- Carboxylic acids
 - 4 x more likely to precipitate from wet DMSO
 - DMSO poor solvation for anions
- Zwitterionic compounds

Beware of trifluoroaceticacid (TFA)

Trifluoroacetate, a contaminant in purified proteins, inhibits proliferation of osteoblasts and chondrocytes J. Cornish,¹ K.E. Callon,¹ C. Q.-X. Lin,¹ C. L. Xiao,¹ T. B. Mulvey,² G. J. S. Cooper, ^{1,2} and I. R. Reid¹ ¹Department of Medicine and ²School of Biological Sciences, University of Auckland, Auckland 1001, New Zealand Am. J. Physiol. 277 (Endocrinol.Metab. 40): E779–E783, 1999.

10 nMTFA is cytotoxicin a 24 hour cell culture

Dry DMSO maximises stability with TFA present

Studies of the Relative Stability of TFA Adducts vs Non-TFA Analogues for Combinatorial Chemistry Library Members in DMSO in a Repository Compound Collection Jill Hochlowski,* Xueheng Cheng, Daryl Sauer and Stevan Djuric

Abbot Laboratories, 100 Abbot Park Road, Abbot Park, Illinois, 60064 J Combinatorial Chemistry, Vol 5, No. 4, July/August 2003

Heterocycle sensitive to TFA acid hydrolysis



2,5-substituted-1,3,4-oxadiazole

Acknowledgements

The generous support of Pfizer Global R&D, Groton Labs in my post retirement activities is gratefully acknowledged