Progressive Compound Management Strategies and Pitfalls

Rhett Affleck, PhD
LRIG
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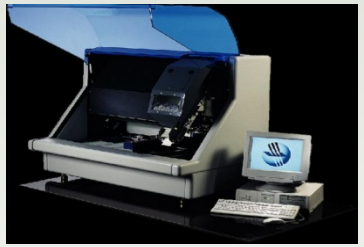
NEXUS Technology Platforms

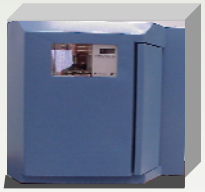
Combinatorial Chemistry Automation

IRORI™

RF MicroKan Synthesis Products

2D NanoKan & X-Kan Synthesis Products

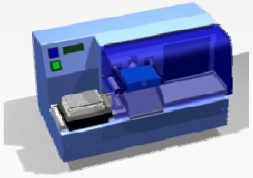




Protein Crystallography

Crystal Farm™

Automated Crystal Incubation & Imaging



Sample Management Solutions

Universal Store™

–20C, Nitrogen

Automated High-Speed Cherry Picking & Defrosting

Decapping and Desealing solutions

BioStore

-80°C

Automated picking and retrieval

NEXUS Biosystems
INNOVATION BY DESIGN

Best Storage Conditions

- **Ideal Storage for Stability**
  - Cold, inert, dry
  - Solid phase
    - Dry film, powder
- **Compound transfer is difficult**
  - Weighing
    - Slow
    - Doesn't work for dry films
    - Impractical for screening amounts
      - (1 – 1000 ng)
  - Volatile solvent transfer
    - Dissolve, transfer, evaporate
    - Slow
  - Apply to long term storage of compound reserve
  - Create a short term **Working Store**
    - **(compounds in solution)**

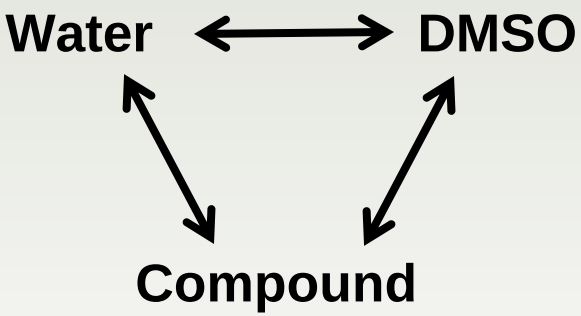
Working Store

Compound Storage and Transfer

- **Transfer solution-phase compound**
  - **Store dry:** Volatile solvent transfer
    - Store dry films
    - Dissolve – transfer - dry
  - **Store solutions:** Nonvolatile solvent transfer and storage
    - Flexible, fast, easy
- **Practical to store and transfer in DMSO**
  - DMSO solutions are a compromise
    - Some added reactivity
    - Complicated by its hygroscopic nature
      - Adds reactivity
      - Decreases compound solubility
  - For solution-phase storage is anything better than DMSO?
    - Universal solvent, plastic friendly, nonvolatile, decent biological compatibility
  - *Keep the water out!*

Compound Concentrations

Compound Storage and Transfer



DMSO/Water

- **Freezing point**
  - 0 ºC at 100% water
  - 18 ºC at 100% DMSO
  - -73 ºC at 33 mol % DMSO
    - 33% v/v water
  - -20 ºC at 55 mol % DMSO
    - 17% v/v water

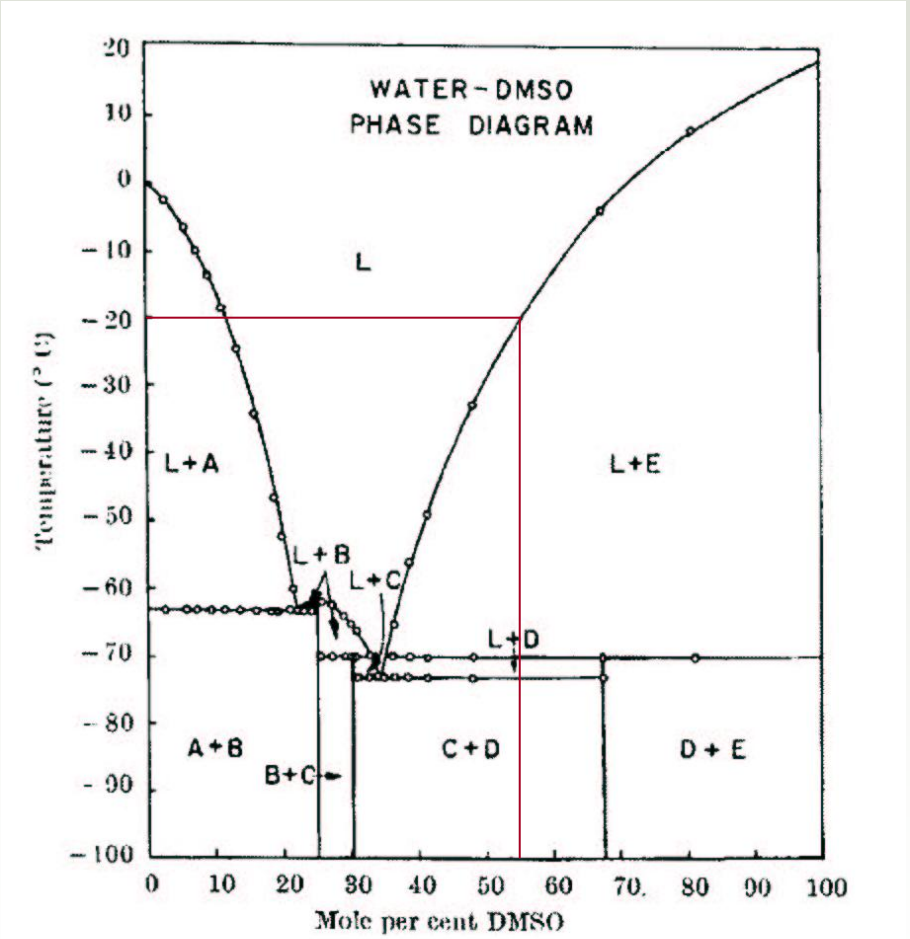


Fig. 2. Phase diagram for the water–DMSO system (plotted on a mole per cent basis). Symbols: L, liquid phase; A, H_2O (s); B, $DMSO.3H_2O$ (s); C, $DMSO.2\text{-}1/2\ H_2O$ (s)*; D, $DMSO.1/2\ H_2O$ (s)*; E, DMSO (s). *Tentative assignment.

*Rasmussen et al, Nature vol.220.**1968**, 1315-1317

DMSO/Water

- **Non-ideal behavior**
- **1DMSO:2water complexes**
  - 33% v/v water

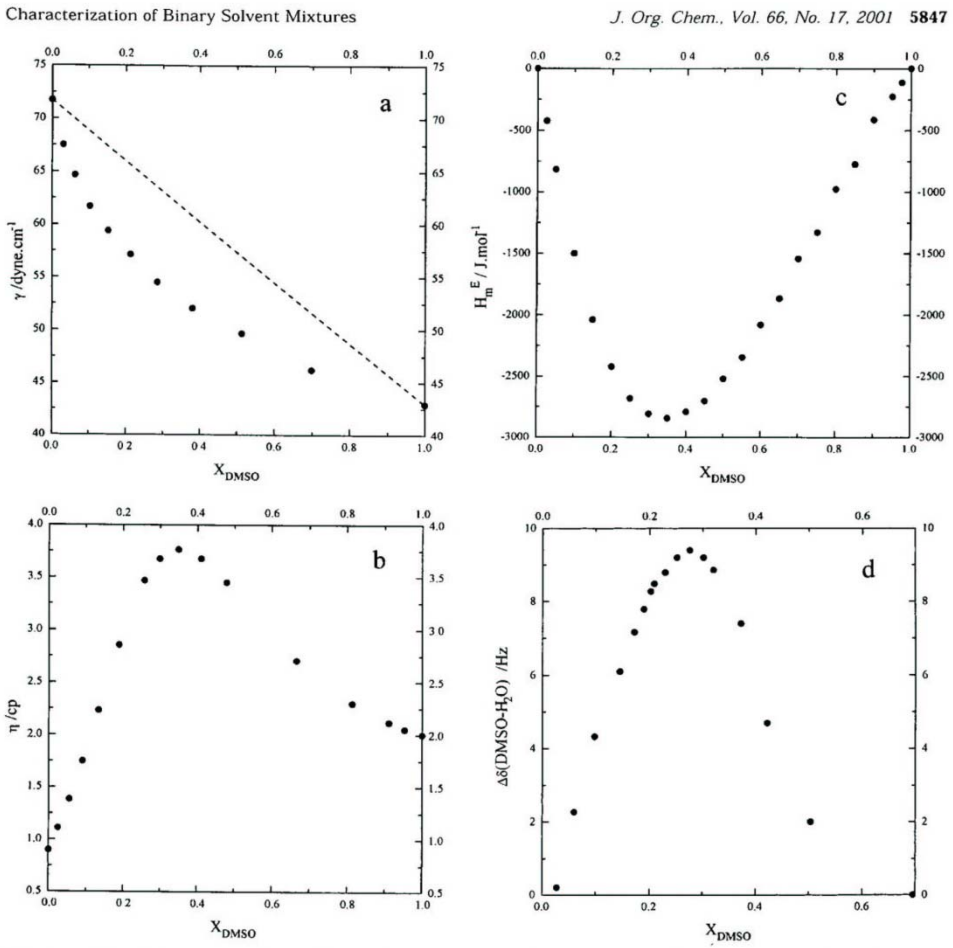
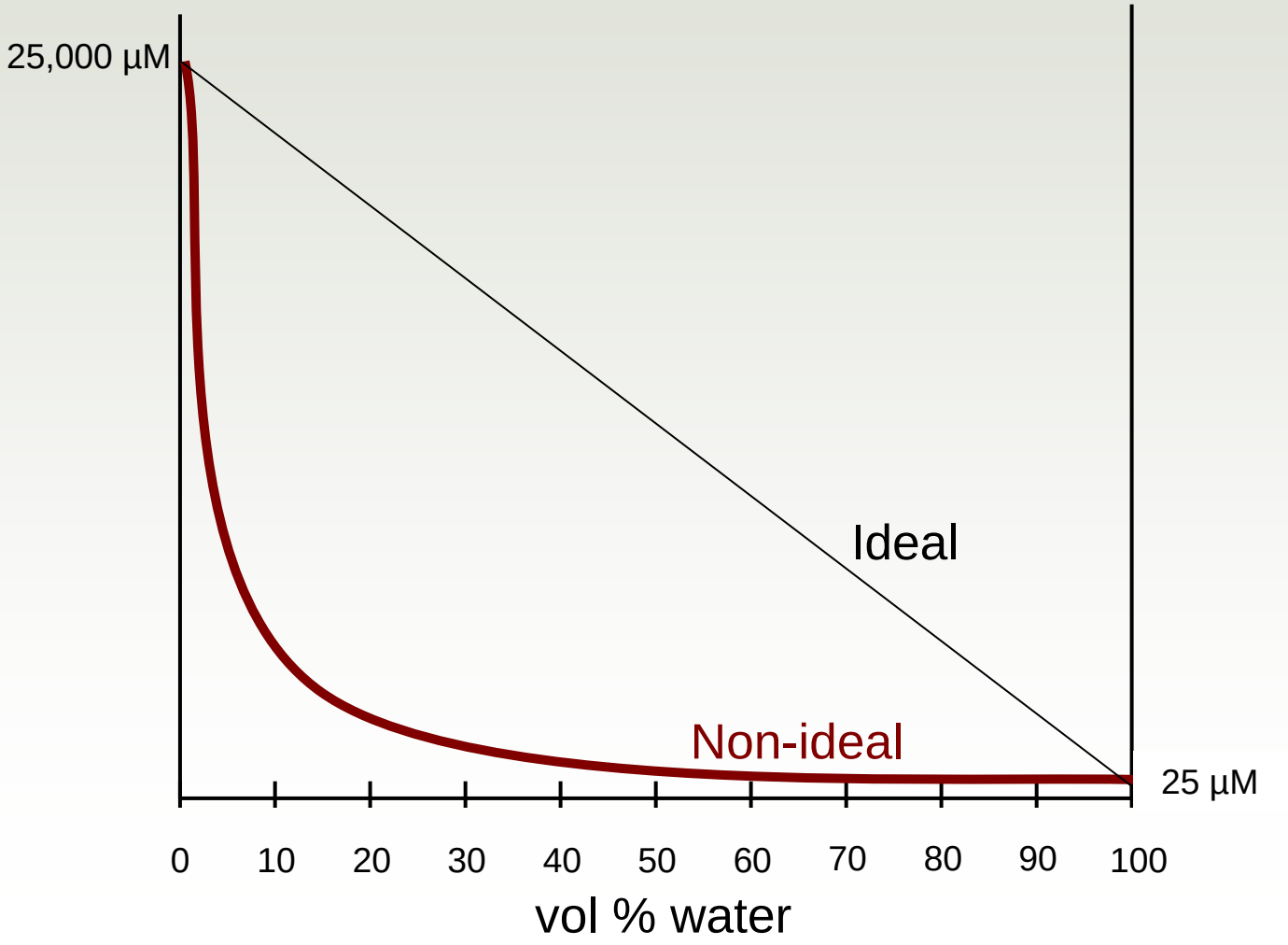


Figure 1. Plots of (a) surface tension, γ, (b) viscosity, η, (c) excess enthalpy of mixing, H_m^E, and (d) deviations from ideality for the chemical shifts of protons with respect to DMSO, $\Delta\delta$(DMSO − H_2O), vs the mole fraction, X, for the DMSO/water mixture.

*Catalan et al, J. Org. Chem.**2001**, *66*, 5846-5852

Compound Solubility
in DMSO/Water



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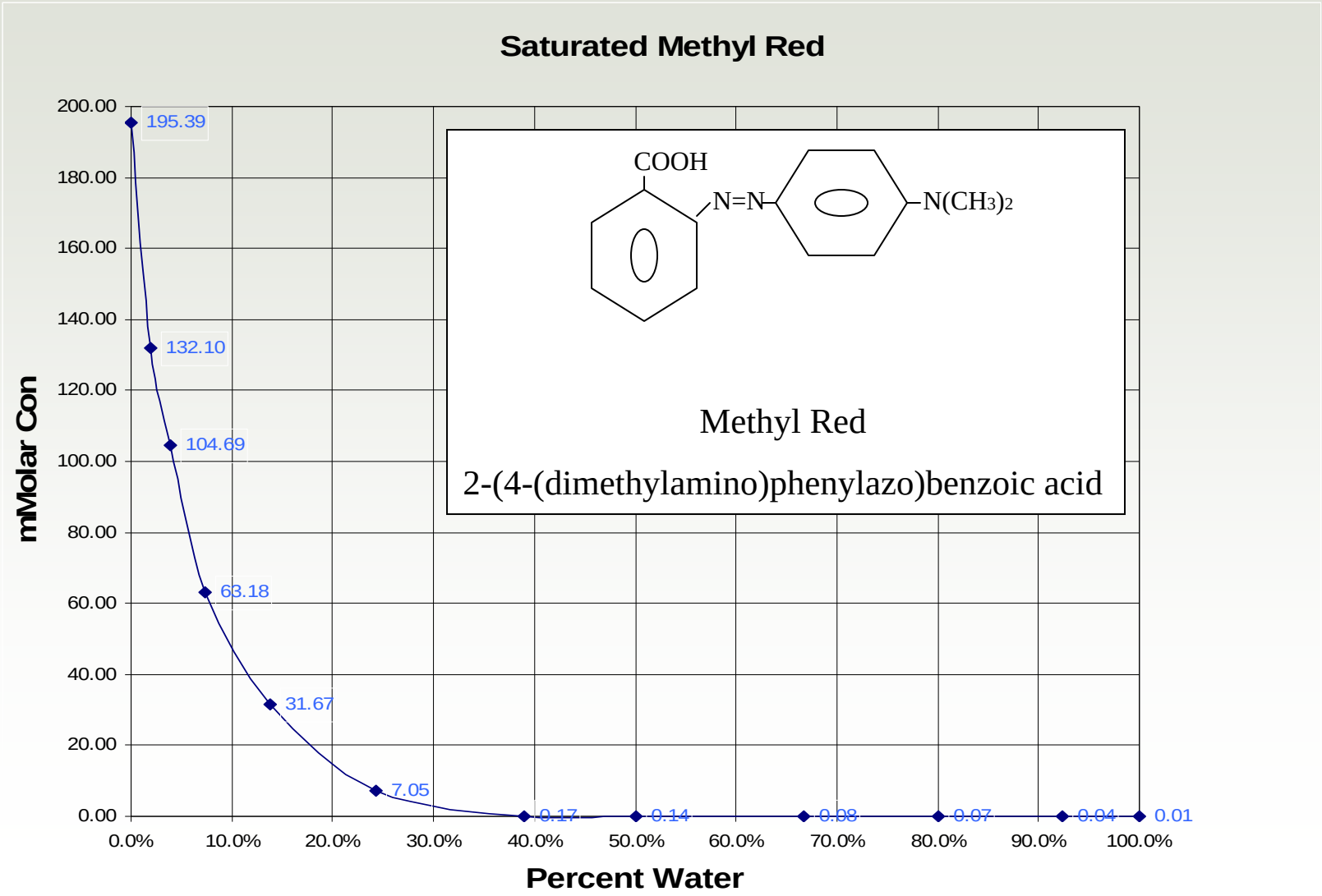

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INNOVATION BY DESIGN

Compound Solubility
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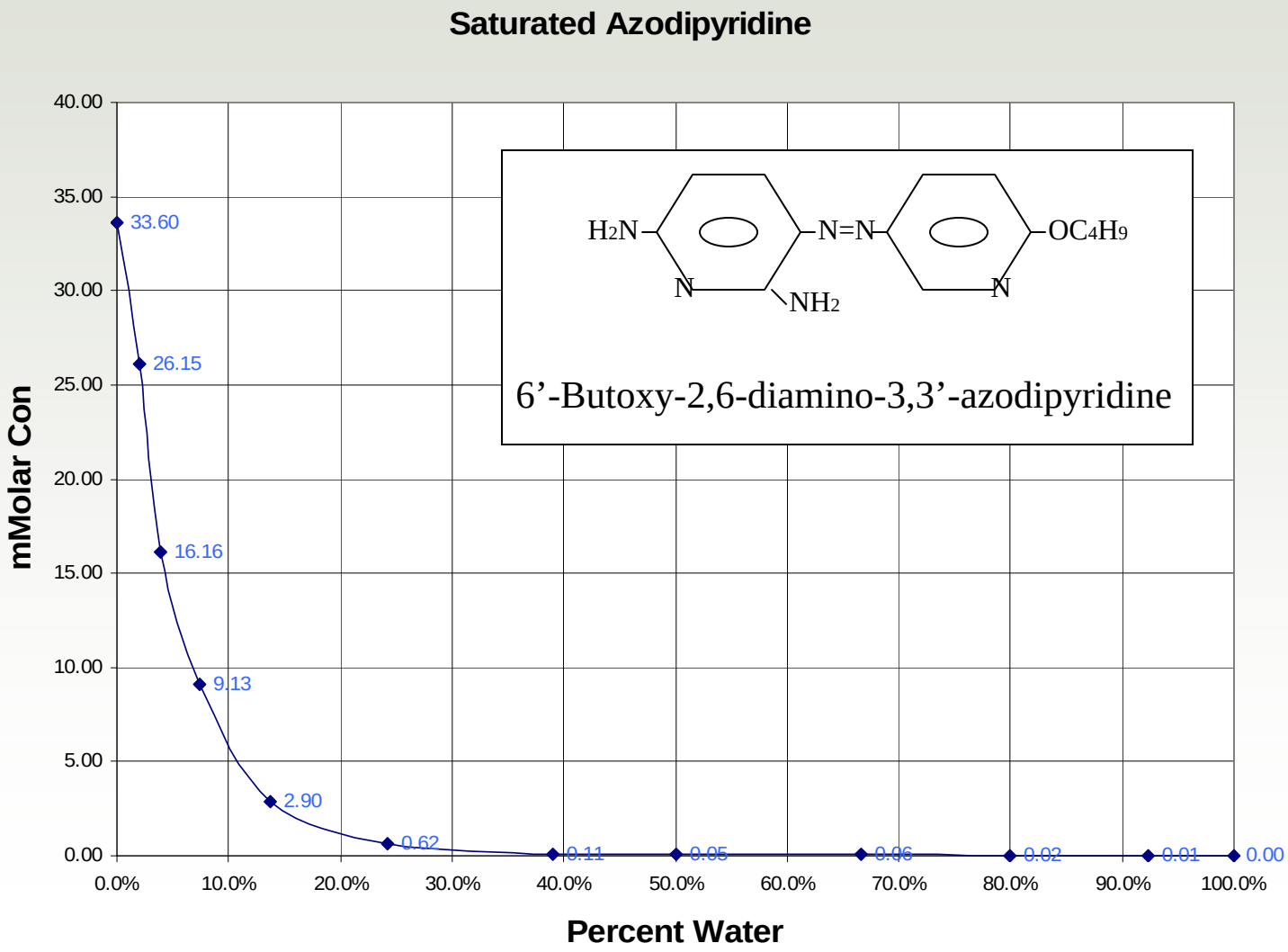

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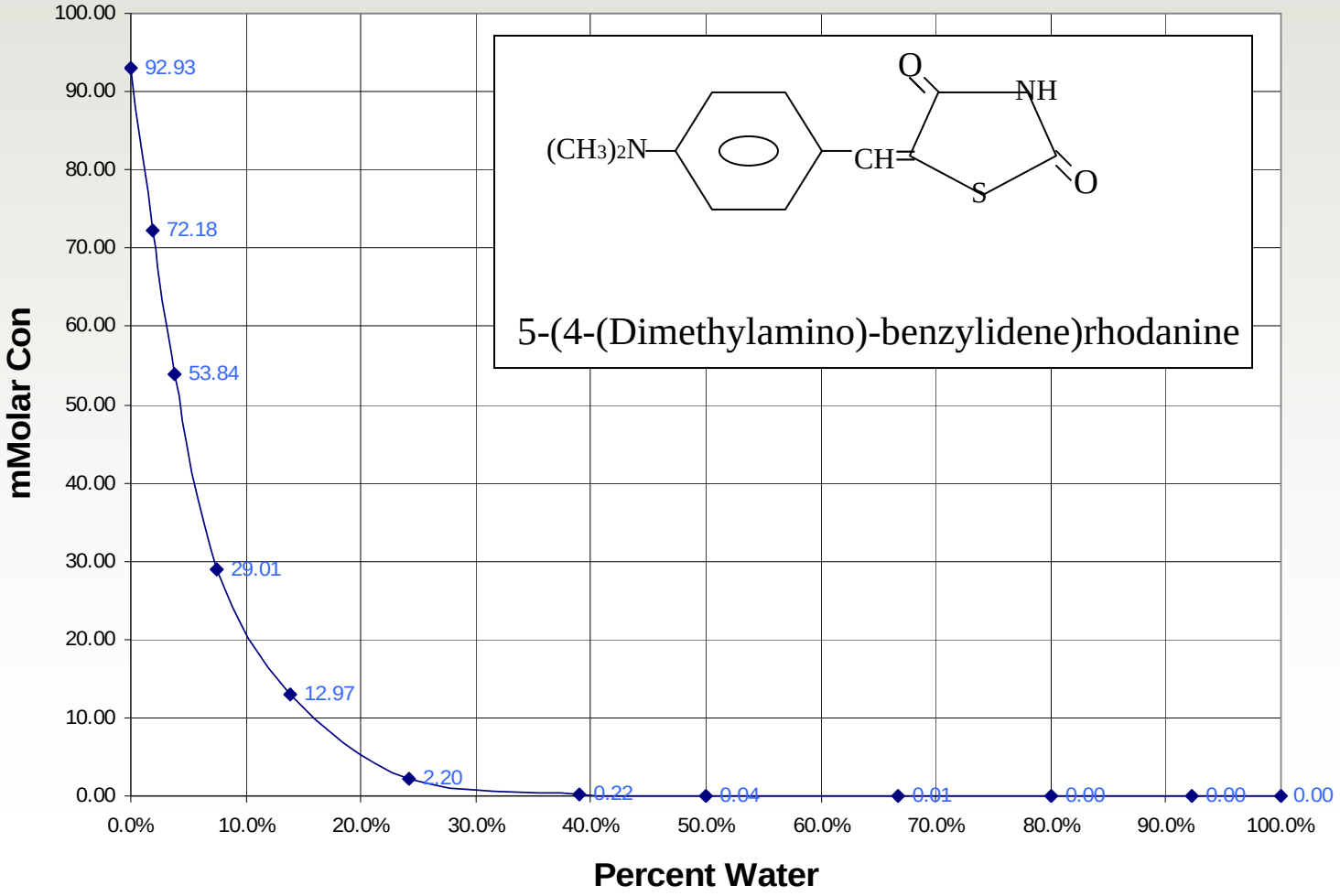
Compound Solubility in DMSO/Water

Saturated Rhodanine



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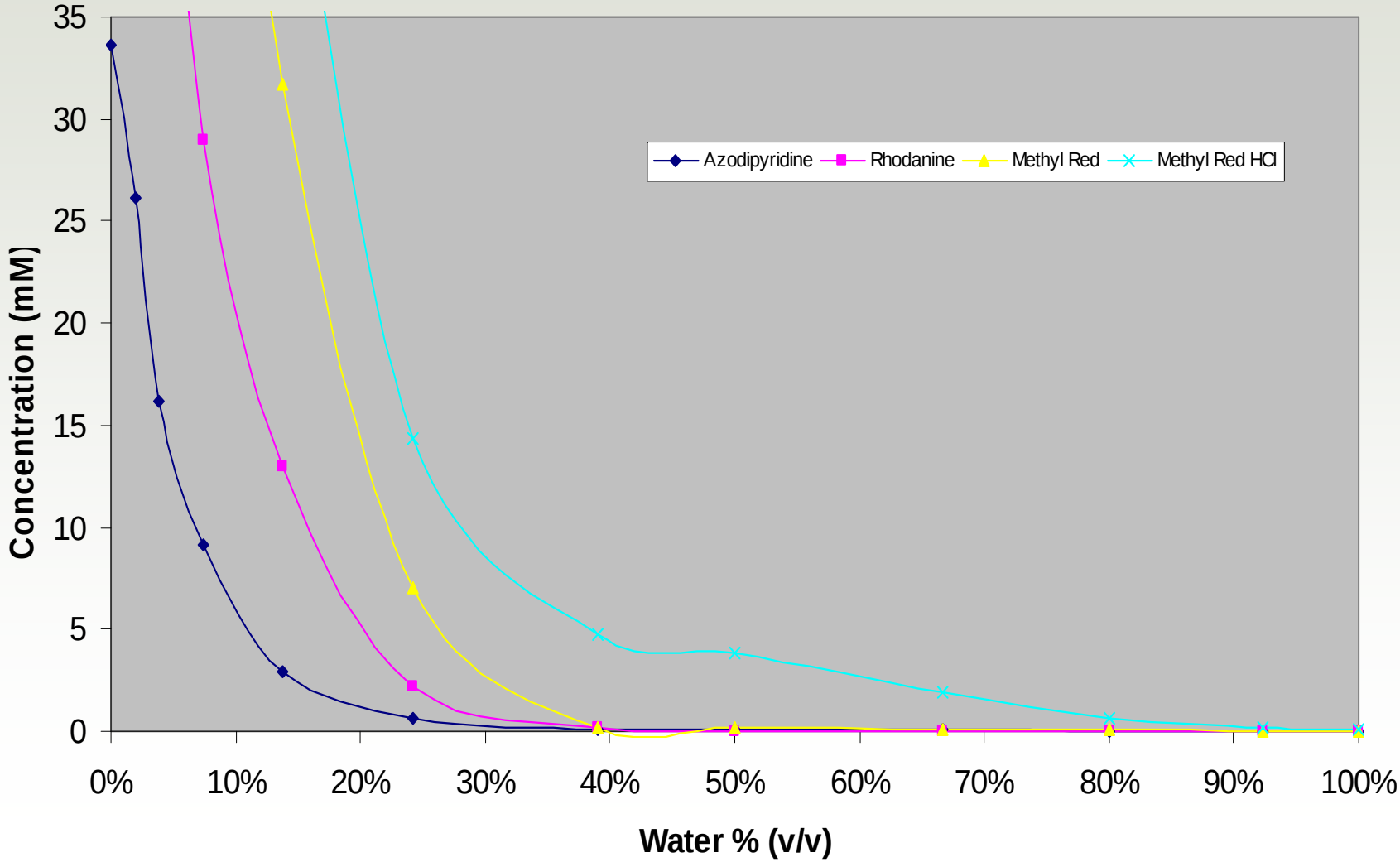
Compound Solubility in DMSO/Water



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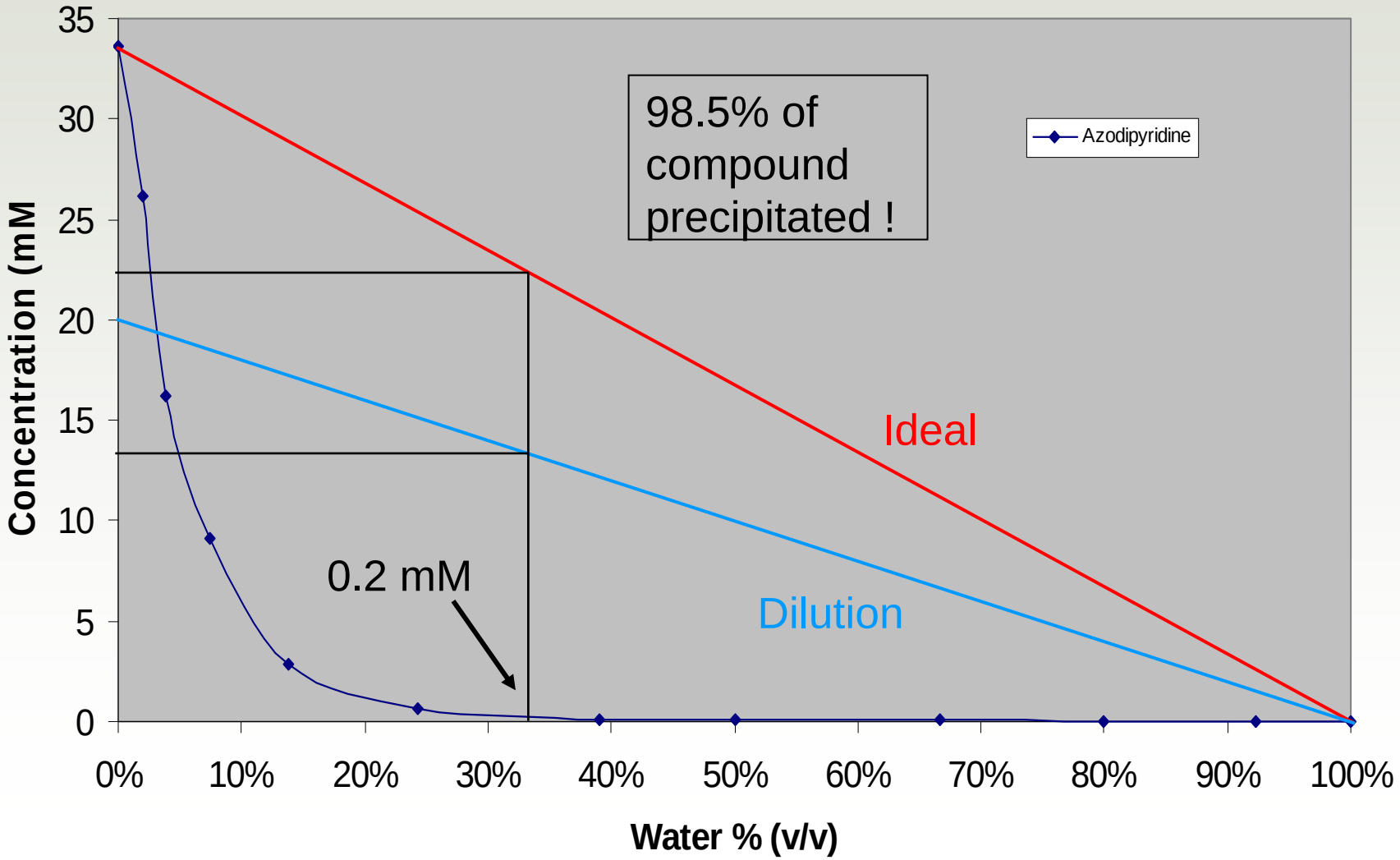

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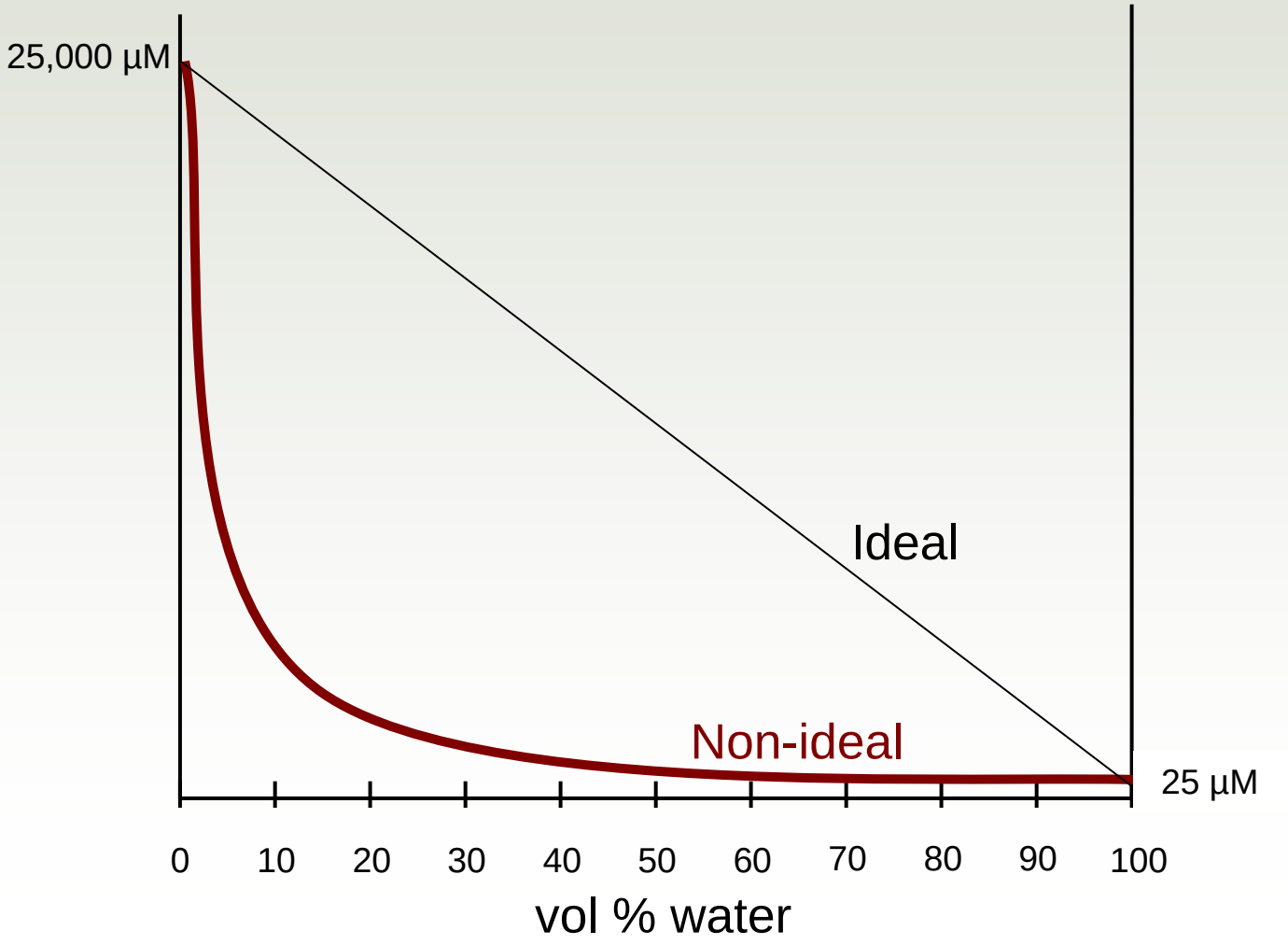


Working Store
Compound Concentration (in DMSO)

- **In practice: 1 – 30 mM**
- **Lower is better (for compound management)**
  - Solubility
    - 16% not soluble at 10 mM
    - 65,500 member drug-like compound set*
  - Liquid handling easier (µL instead of nL)
- **Higher is better (for biology)**
  - Minimize DMSO at screening concentrations (1 µM – 10 µM)
- **Compromise at ~ 1 mM**
  - 1 mM → 10 µM, 1% DMSO, or
  - → 1 µM, 0.1% DMSO, or
  - → 50 µM, 5% DMSO

→ If needed: evaporate DMSO

*Balakin et al, J. Biol. Screen. 9(1); 2004

Working Store

Freeze/Thaw Cycles

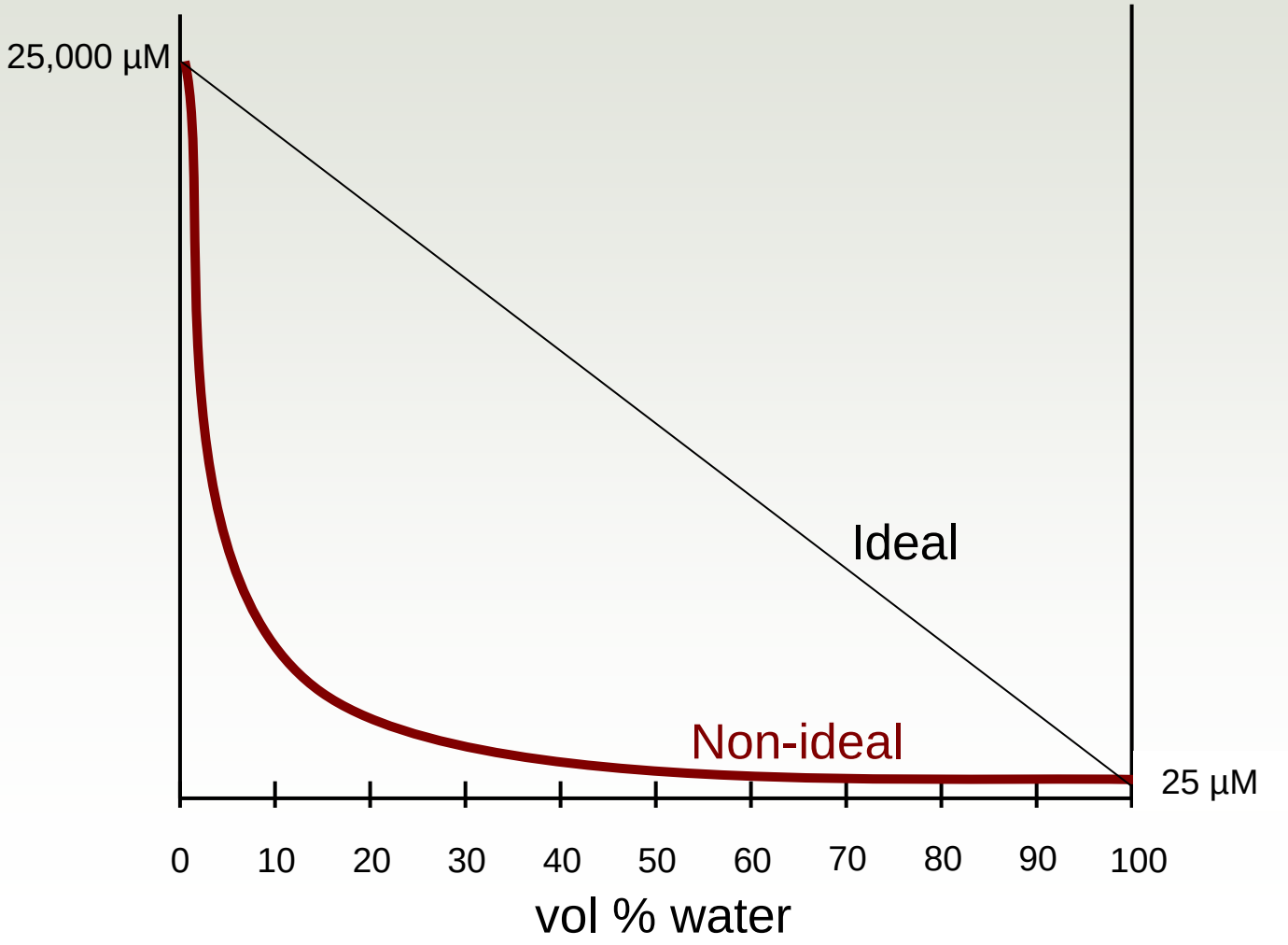
- **Freezing and thawing does not degrade compounds**
- **Freezing DMSO solutions can cause some compounds to come out of solution**
  - Equilibrium solubility at 25 °C does not change
  - Dissolution kinetics can change
    - More stable crystals can form
- **Compounds will re-dissolve!**
  - Make sure that thawed solutions are mixed well

Intermediate Transfer Plates

- **Storage at remote sites**
  - Are remote sites equipped with storage, liquid handling, and personnel to take multiple aliquots?
    - Example: seal and unseal samples at point of liquid handling

- **Intermediate dilution required**
  - 1 µL @ 20 mM in DMSO delivered
  - 10 µM in buffer desired
  - Need to dilute 1:2000
  - For example:
    - Dilute 1:10 with 10 µL buffer,
    - then transfer 1 µL to assay plate and dilute to 200 µL

Compound Solubility
in DMSO/Water



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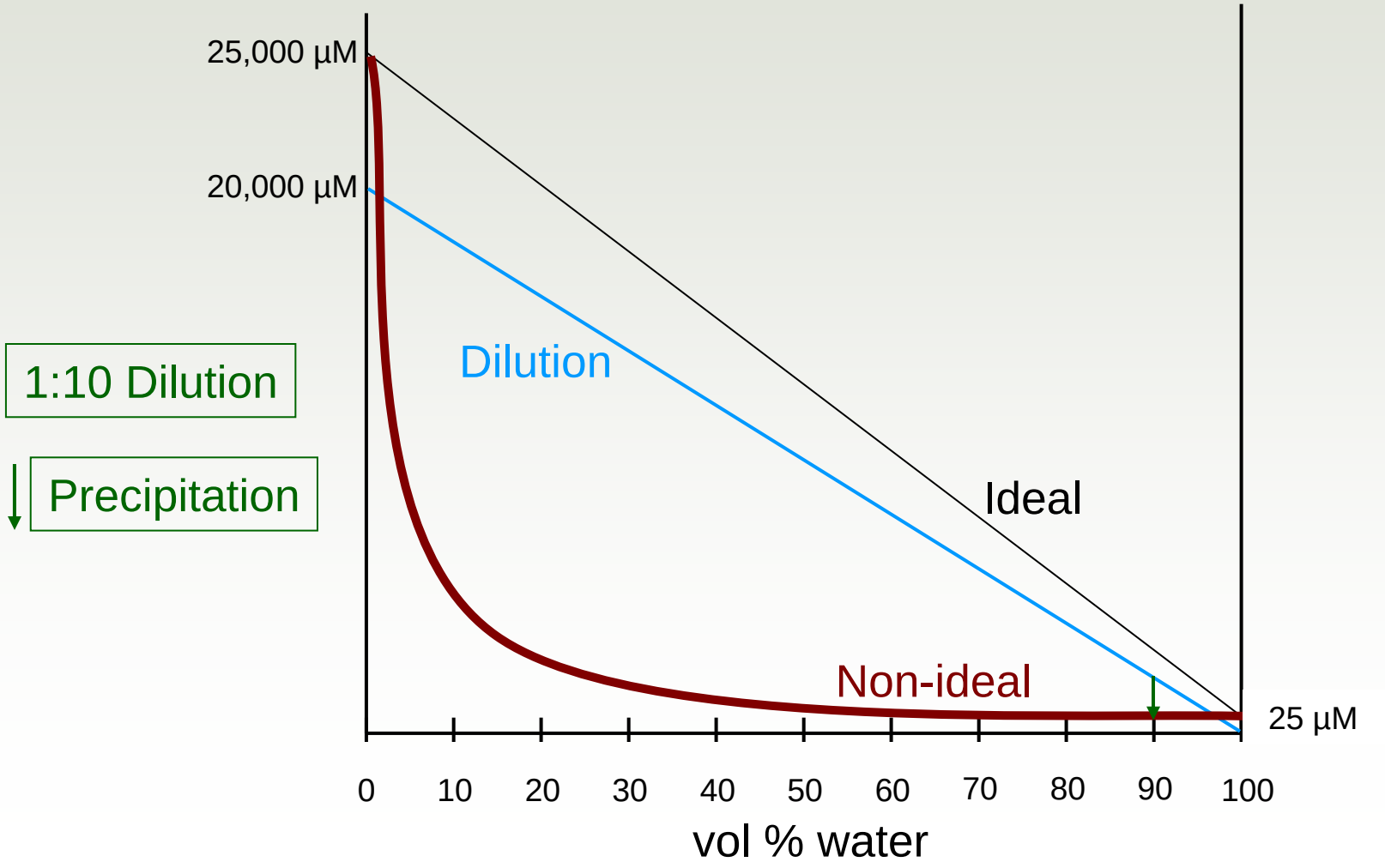

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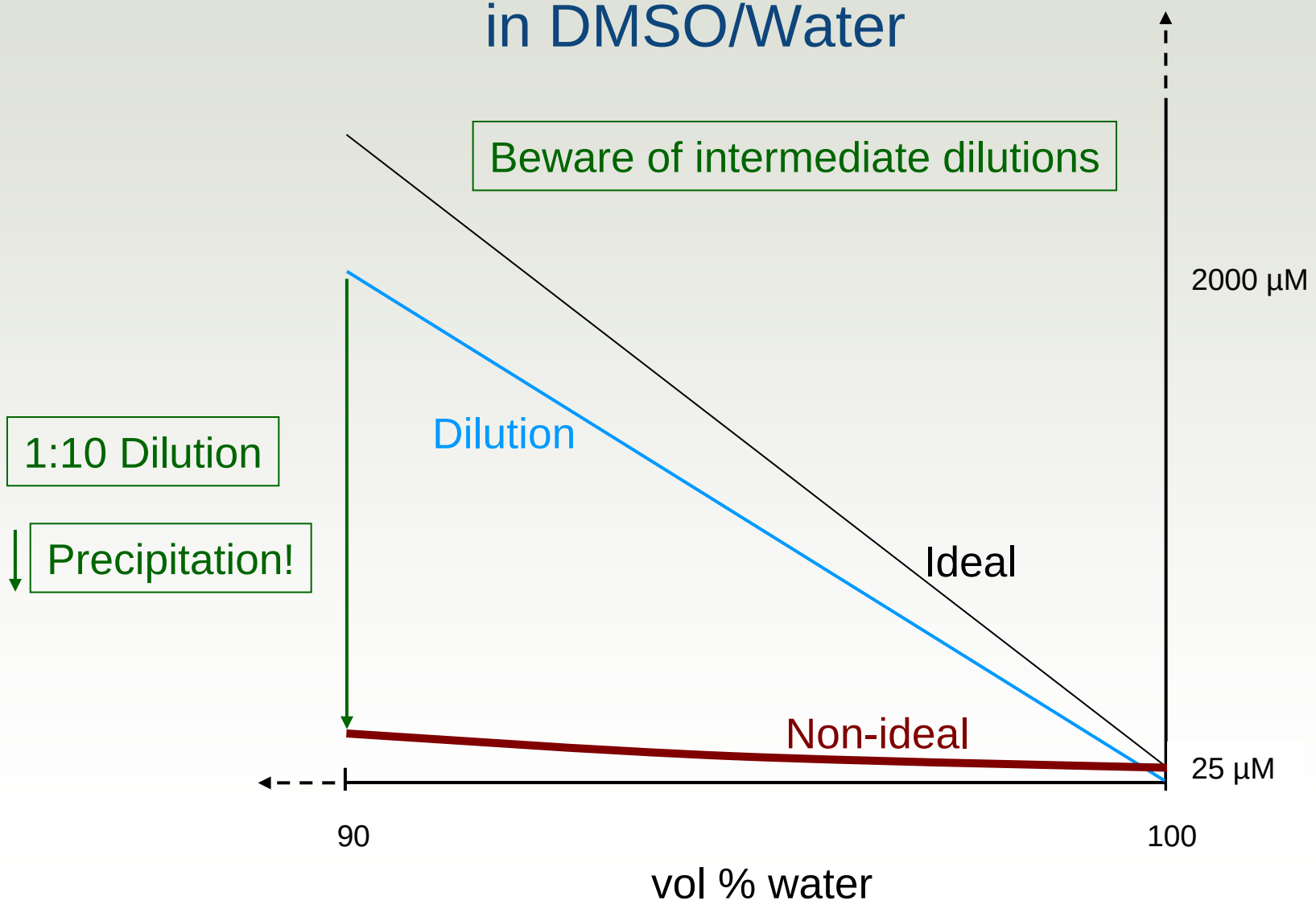

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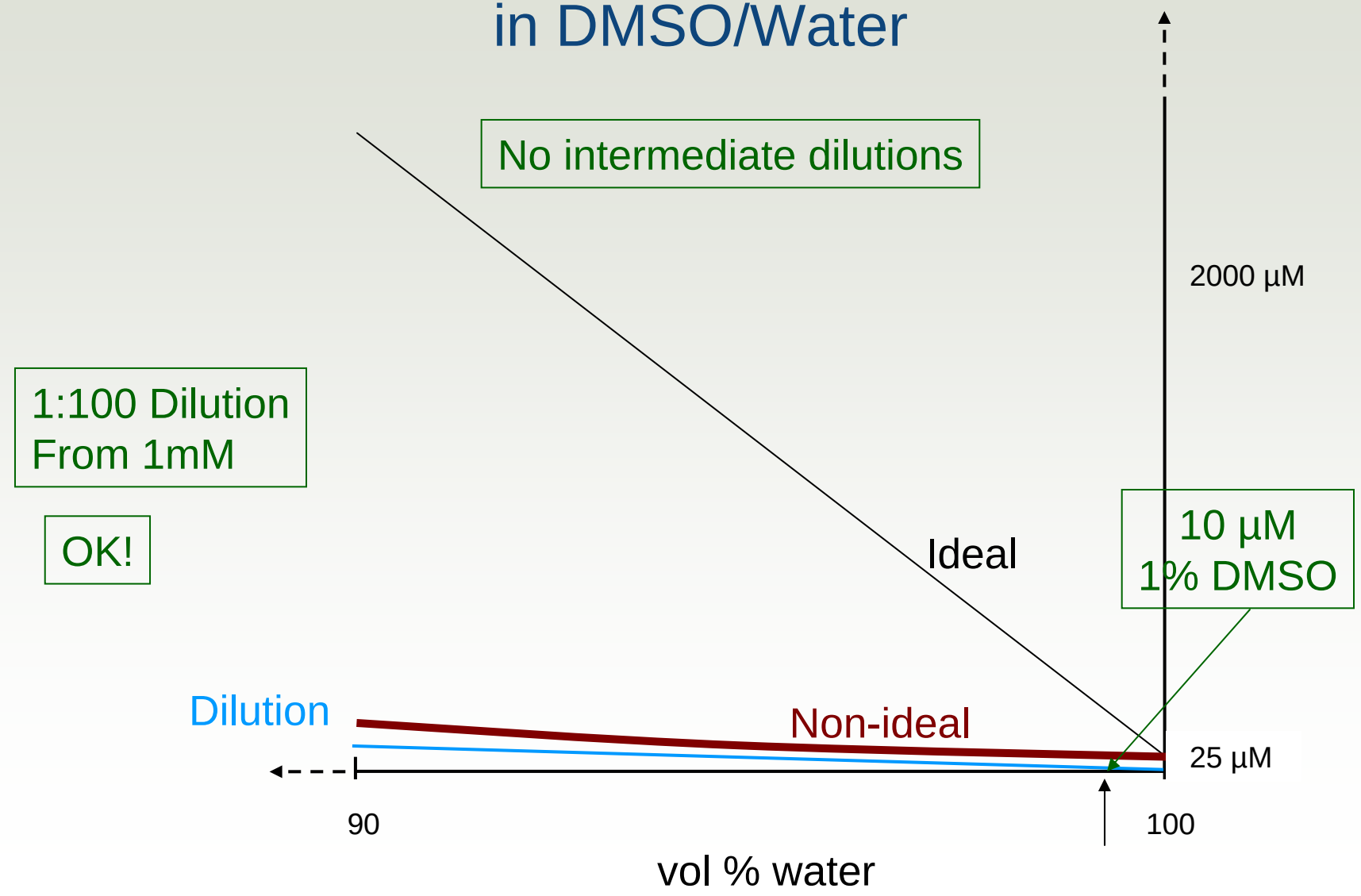

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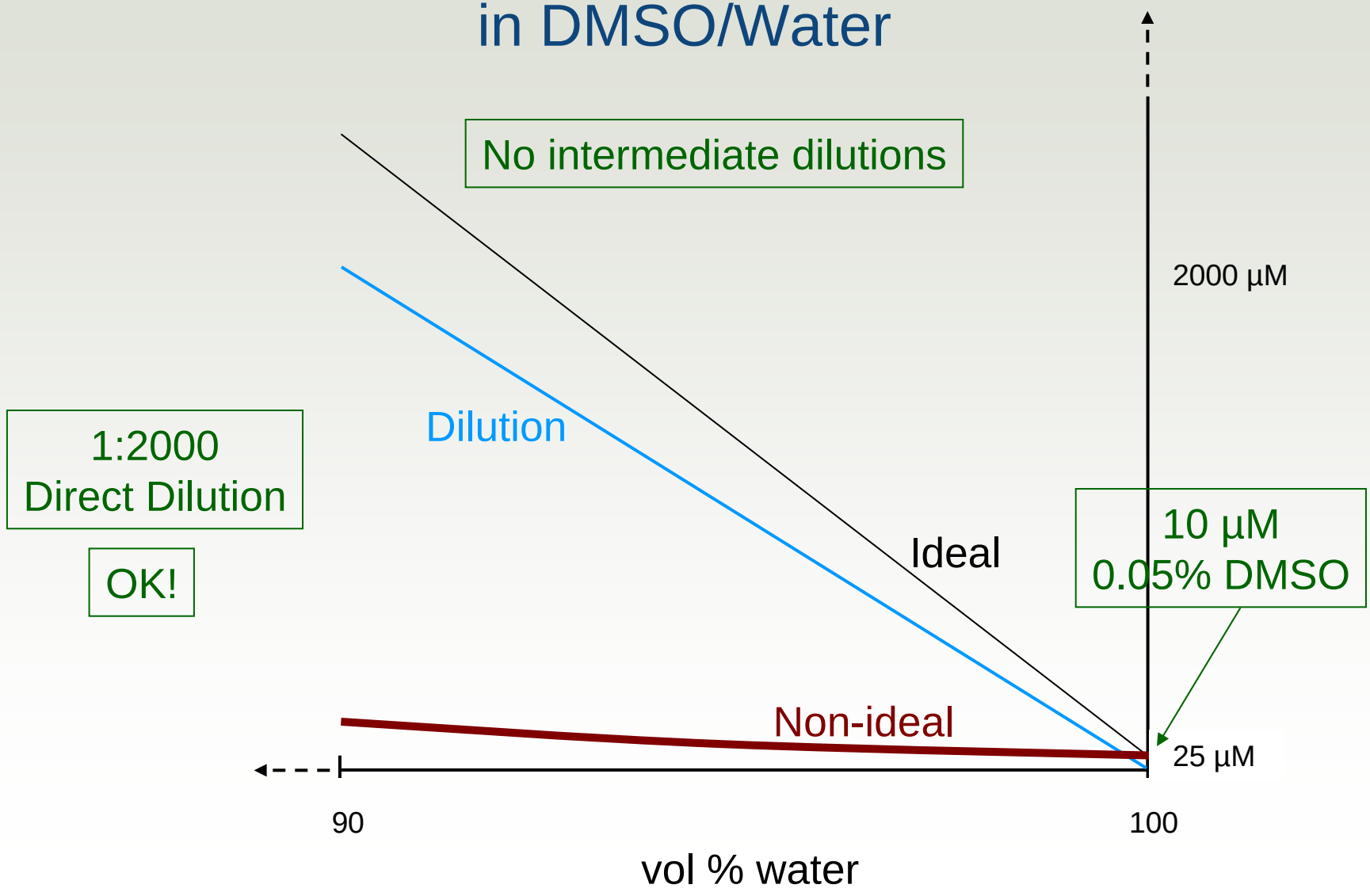


Intermediate Transfer Plates

- ~~**Storage at remote sites**~~
- **➔ Compound handling only at compound management sites**

- ~~**Intermediate dilution required**~~
- **➔ Make Assay-Ready Plates**

Screening Concentration

- **In practice: 1 – 30 µM**

- **Solubility**
  - Amphora Discovery*
    - ~85% of compounds below expected 50 µM
    - ~55% of compounds below 10 µM
    - Buffer with 5% DMSO
    - 2,000 member diversity library
  - Pfizer**
    - ~95% of compounds below expected 200 µM
    - ~45% of compounds 25 µM – 100 µM
    - ~50% of compounds below 25 µM
    - Water with 5% DMSO
    - 1,000 member diverse set

*Popa-Burke et al, Anal. Chem. 76(24); 2004

**Nakayama, San Diego HTS Discussion Group, Dec. 7, 2005

Screening Concentration Too High?

- **Many compounds not soluble at 10 µM!**
  - Best would be to measure concentration
    - Not practical for HTS
  - Anticipate insoluble compound

- **Insoluble and soluble, high-concentration dependent aggregates act as promiscuous inhibitors***
  - Study used 30 µM and 5 µM solutions diluted from 10mM DMSO stock

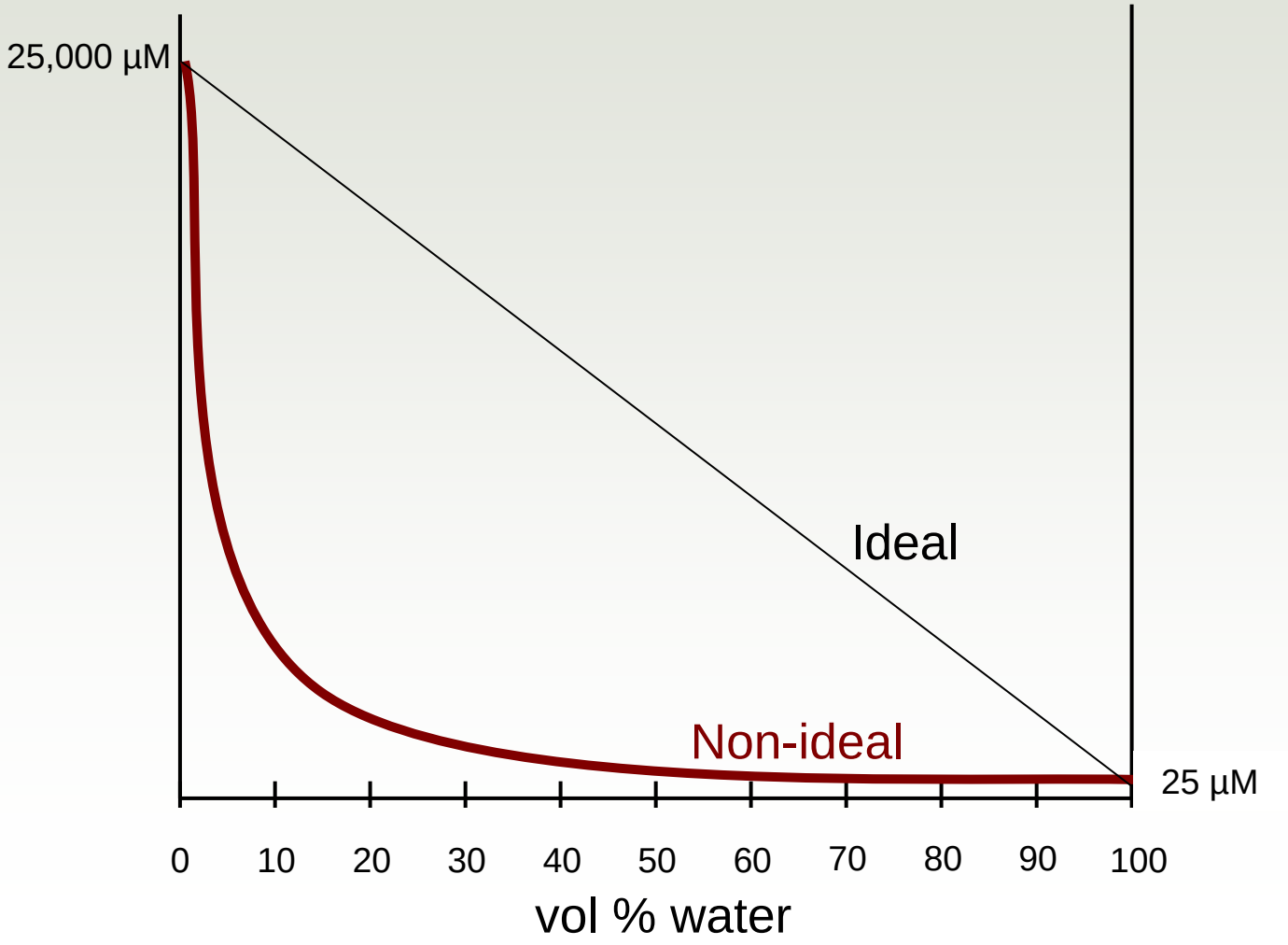
*B.Y. Feng et al, Nature ChemBio. 1(3), 2005

Screening Concentration Too High?

Why does this matter to compound management?

- **Screening “demands” push DMSO stock concentration**
  - Lower screening concentrations could lower DMSO stock concentrations

Compound Solubility
in DMSO/Water



Remove DMSO before Screen?

- **Deliver compounds in DMSO or dry?**
  - In DMSO
    - Advantage:
      - Rapid mixing
    - Disadvantage
      - Crashes out of solution unpredictably
      - Forms promiscuous aggregate activity?
  - Dry
    - Disadvantage
      - Slow dissolution, so good mixing needed
    - Advantages:
      - Assay can dictate % DMSO (if any) ←
      - Insoluble compound stays on well surface ← **Better for biology**
      - Easy to transport (dry) ←
      - Working Store concentration can be low! ← **Better for compound management**

My Experience with Dry Films

- **Pharmacopeia**
  - Single bead libraries
    - Extracting compounds from beads
    - Assays run from dry films
  - 1536-well reformatting
    - 1 µL assays

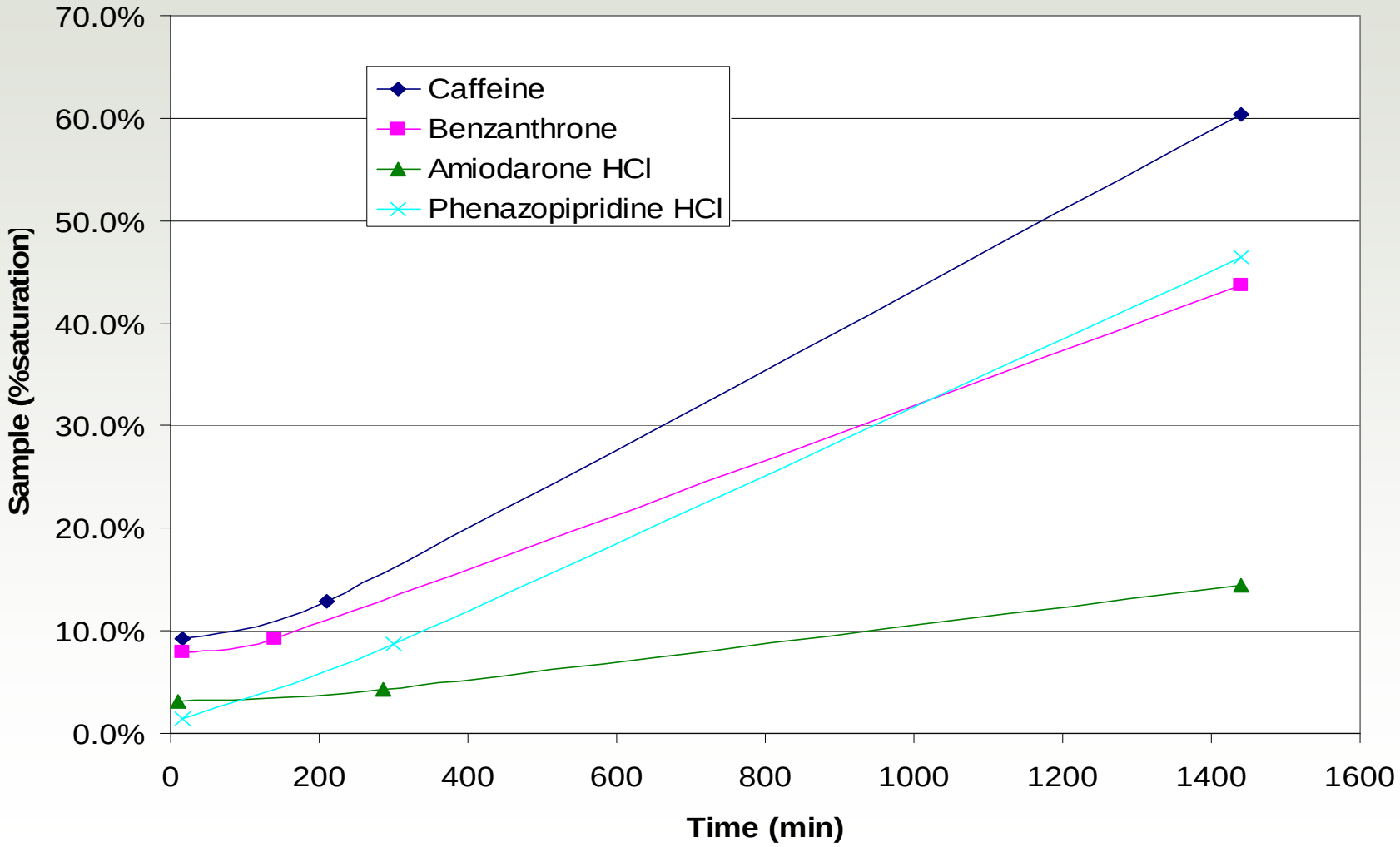
- **Discovery Partners**
  - µARCS
    - Reformat compounds onto 10,000 spot cards
    - Assays run as compounds dissolve from dry film into agarose gels

Microplate Mixing

- **Sonication**
  - Variable effectiveness
- **SonicMan, Matrical**
  - Pros: high throughput
  - Cons: exposed samples, wash probes, heat
- **Advalytix**
  - 100MHz, 20 µm,
  - Pros: Whole plate, no water bath
  - Cons: low energy surface acoustic waves, mixing near surface, needs hard plate
- **AFA, Covaris**
  - Pros: sealed samples, isothermal, non-contact, high energy
  - Cons: water bath

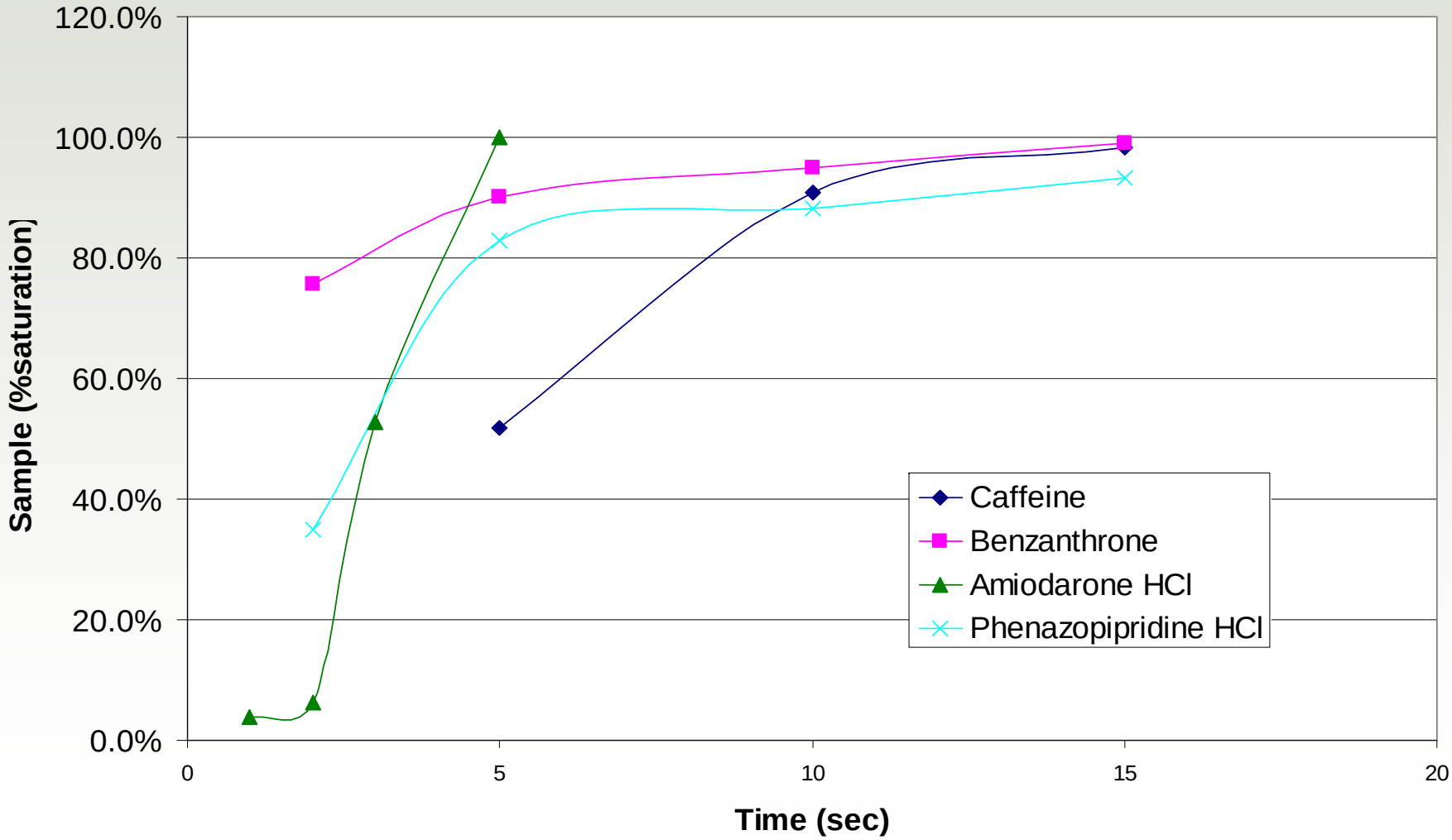
Orbital Table Mixing

150 cycles/min



NEXUS Biosystems
INNOVATION BY DESIGN

Covaris AFA Treatment



Summary

- **Keep water out of your DMSO samples**
  - Working store
  - Intermediate plates
    - Intentional or not
- **Store and manipulate compounds only at expert facilities**
  - Centralize
  - Properly equip and train remote sites
- **Consider:**
  - JIT, individual-use, assay-ready plates
  - Lowering concentrations of DMSO stock
  - Delivering compounds dry