

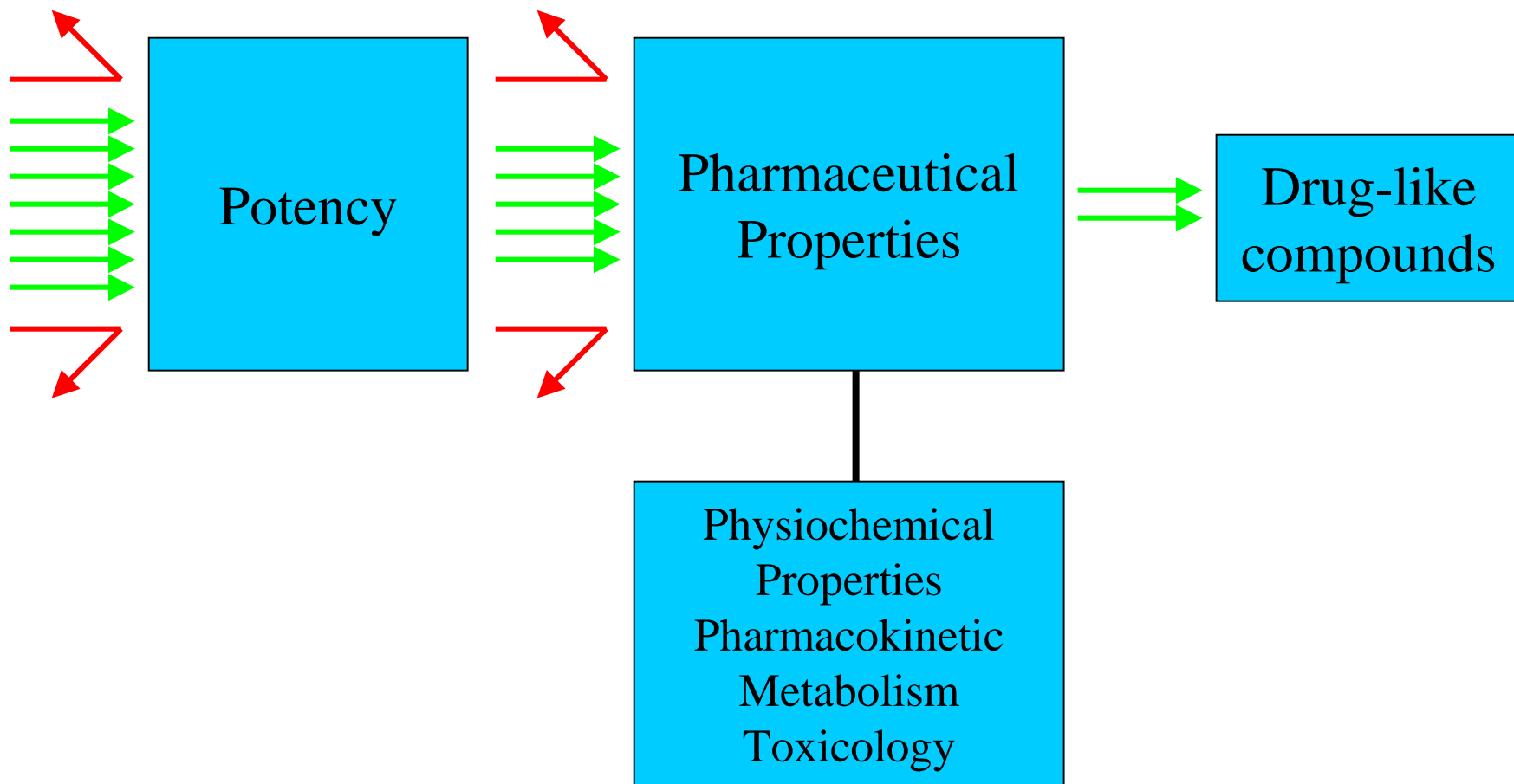
Importance and MTS Screening of Physiochemical Parameters In Drug Discovery

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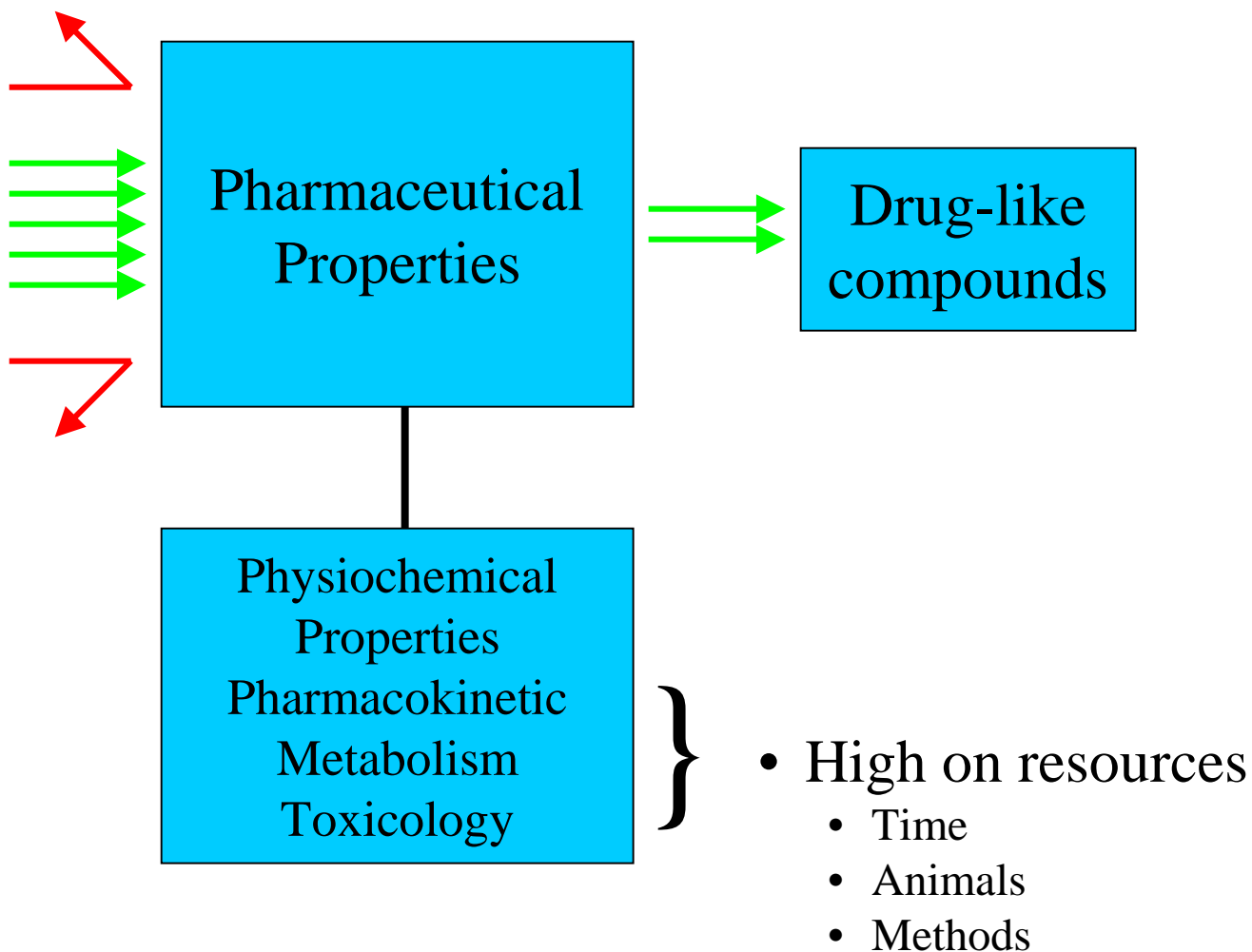
Outline

- Discovery Paradigm Shift
- Definitions
- Relevance of Physiochemical Properties
- HTS and MTS
- What to do with the data
- Other Absorption Predictors and Relevance

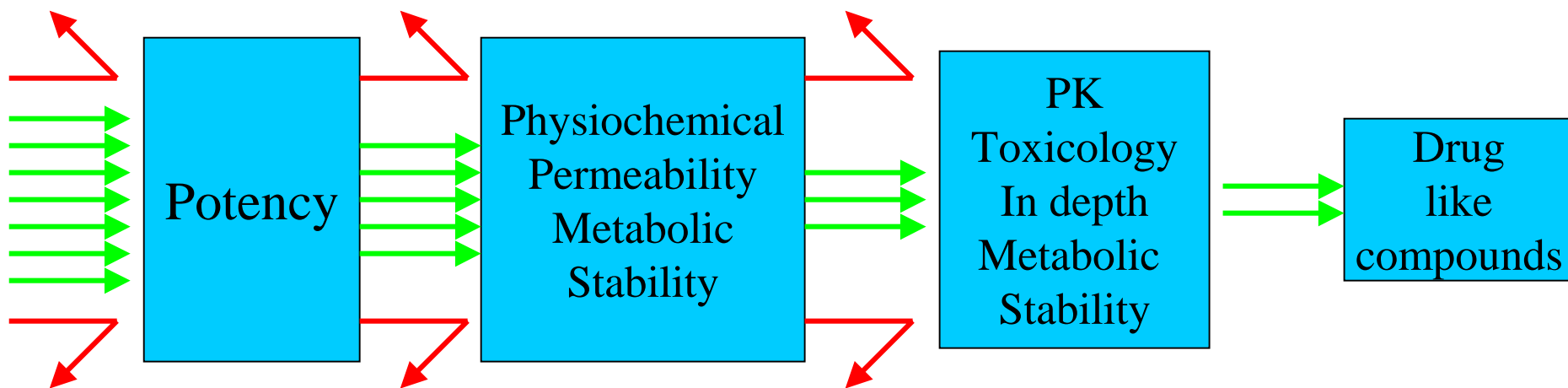
Discovery Paradigm Shift



Discovery Paradigm Shift



Discovery Paradigm Shift

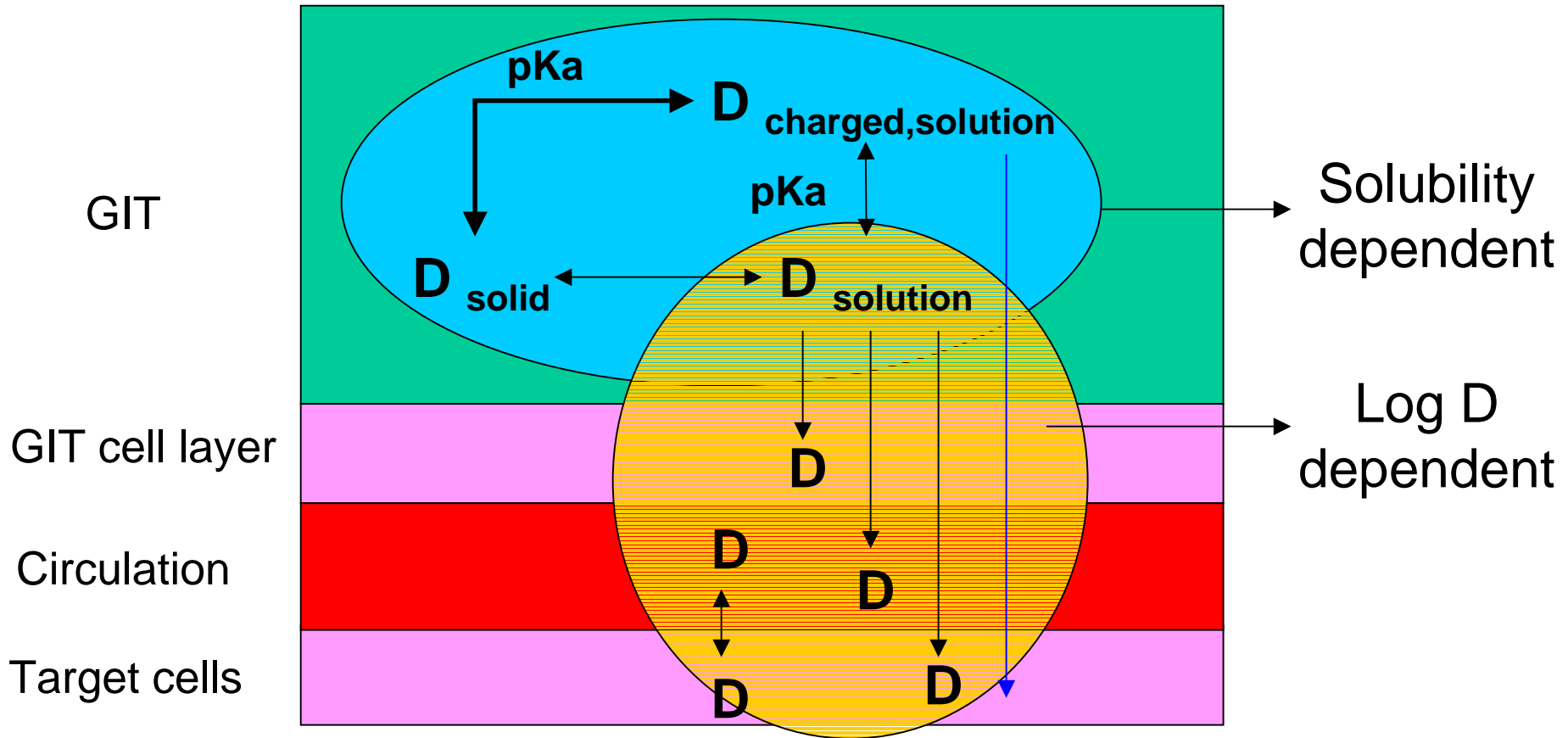


- Physiochemical
 - Solubility
 - pKa
 - partition coefficient
 - chemical stability

Definitions

- **Solubility**
 - Extent to which a solute dissolves in a solvent
 - Intrinsic solubility, pH solubility, excipient solubility
- **Partition Coefficient**
 - Measure of ability of molecule to transverse cell membranes measured as octanol water partitioning
 - Log P: partitioning ability of unionized form
 - Log D: partitioning ability at various pH (ionized + unionized).
- **High Throughput Screening**
 - Screening of >100 compounds/day/machine-operator
- **Medium Throughput Screening**
 - Screening of 10-50 compounds/day/machine-operator

pKa, solubility, and partition coefficients control absorption and diffusion across physiological layers



Solubility dependent

Log D dependent

Site	Stomach	SI	LI	Blood
pH	1 to 4	4 to 7	6 to 9	7.2 to 7.6
Avg	2	6.5	8	7.4

Physiochemical properties account for up to 33% of drug failures

Reason for Failure	Incidence %	
	1991 Survey	1997 Survey
Pharmacokinetics	40	28
Lack of Efficacy	30	11
Animal Toxicity	11	12
Human Toxicity	10	22
Commercial	5	5
Miscellaneous	4	5
Formulation	---	5
Strategic	---	12

High Throughput Screening (HTS)

- Requirements
 - Approximate value for ranking with reasonable accuracy
 - Rapid and immediate feedback to Chemistry on expected properties of various scaffolds
- Solubility
 - Turbidometry
 - Calculated value
- Log P
 - HPLC methods
 - Calculated value
 - Moriguchi Log P
 - cLog P (BioByte)
 - Kow (Syracuse)
 - Simulations Plus

Medium Throughput Screening (MTS)

- Requirements
 - Value for ranking with higher accuracy
 - Rapid and immediate feedback to Chemistry on expected properties of molecules within selected scaffolds

Solubility

- LC-UV-Vis
 - LC-MS
- Log P
 - Spectrophotometer
 - LC-MS

Different expectations from different throughput

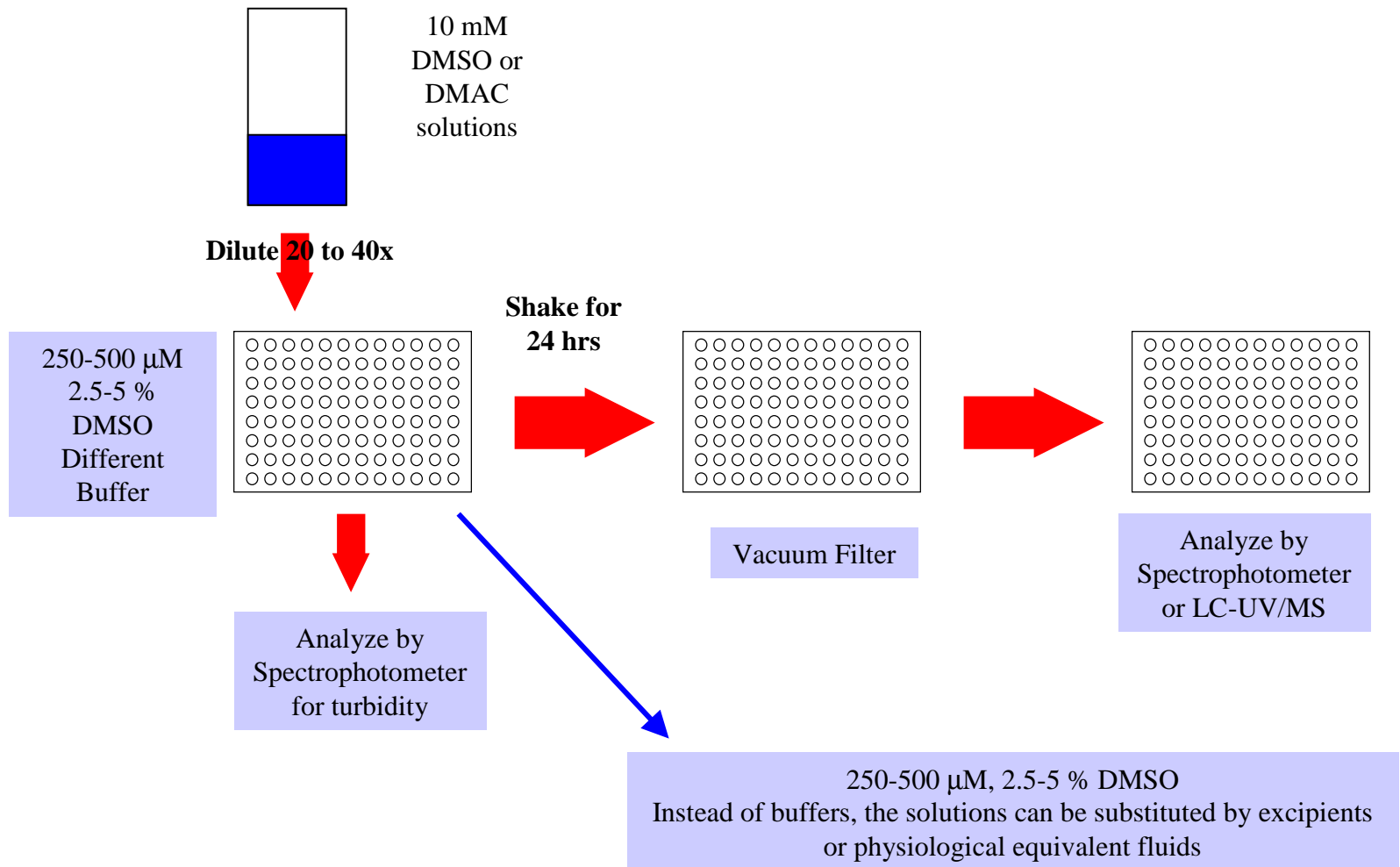
HTS

- >100 compounds/day
- 2-5 mg sample requirement
- 80-90% purity
- Provides binning data
- Aim is to categorize scaffolds
- Determinations are kinetic in nature
- Turbidometric/Nephelometric
- Target audience is mainly chemistry

MTS

- 50-100 compounds/day
- 10-20 mg sample requirement
- >90% purity, >95% preferable
- Provides numerical data
- Aim is to categorize compounds
- Determinations may be kinetic or thermodynamic in nature
- UV-Visible, mass spectrophotometric data
- Target audience includes chemistry, formulations, and ADME

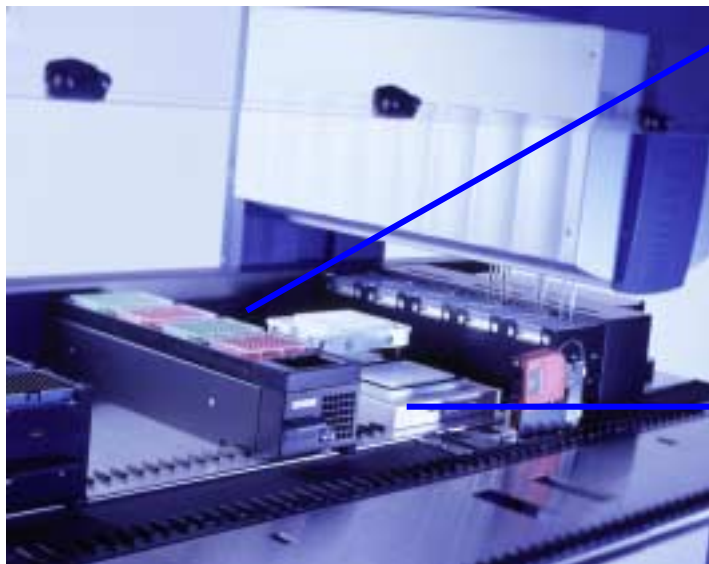
Experimental Setup - Solubility



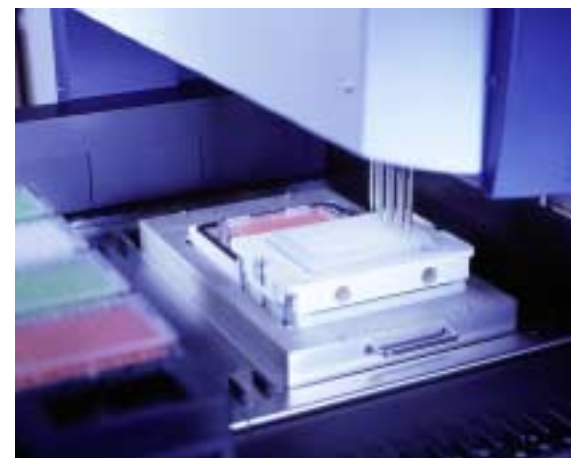
Automatic Liquid handling systems applications to determination of physiochemical properties have increased throughput



Temperature controller



Vacuum filtration

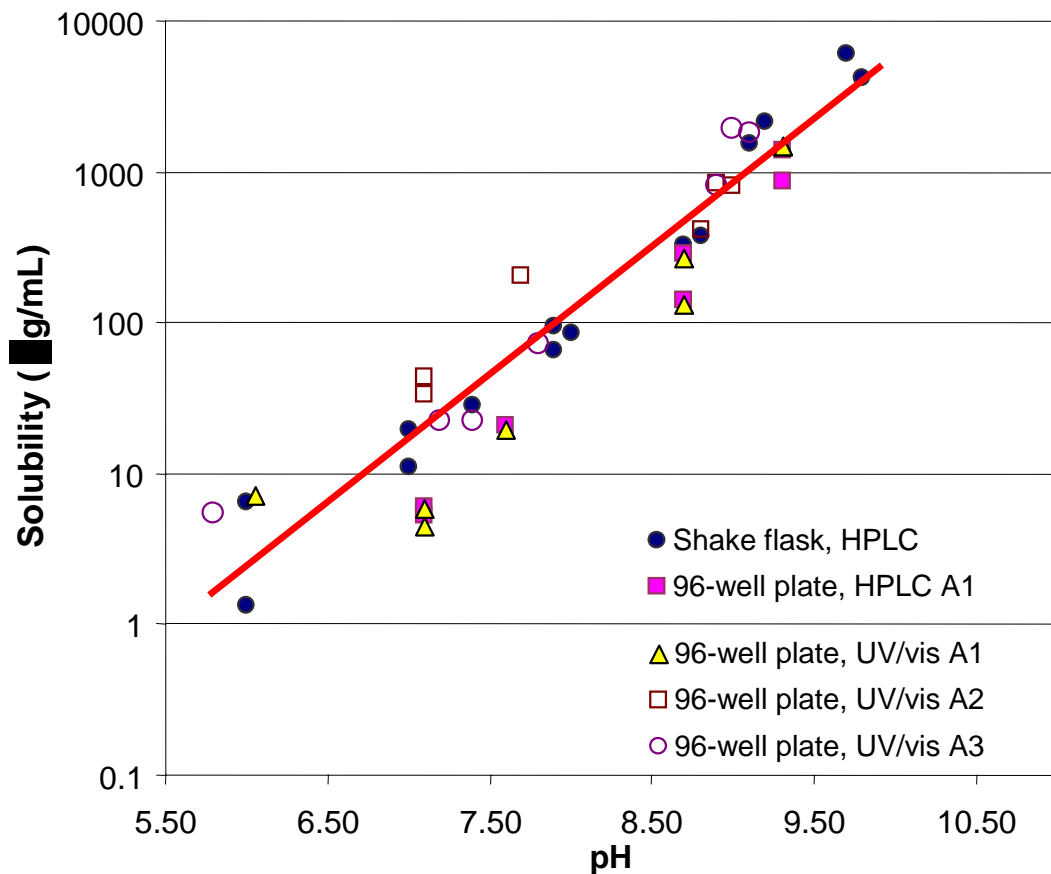


Hamilton MicrolabStar pictured



Shaker

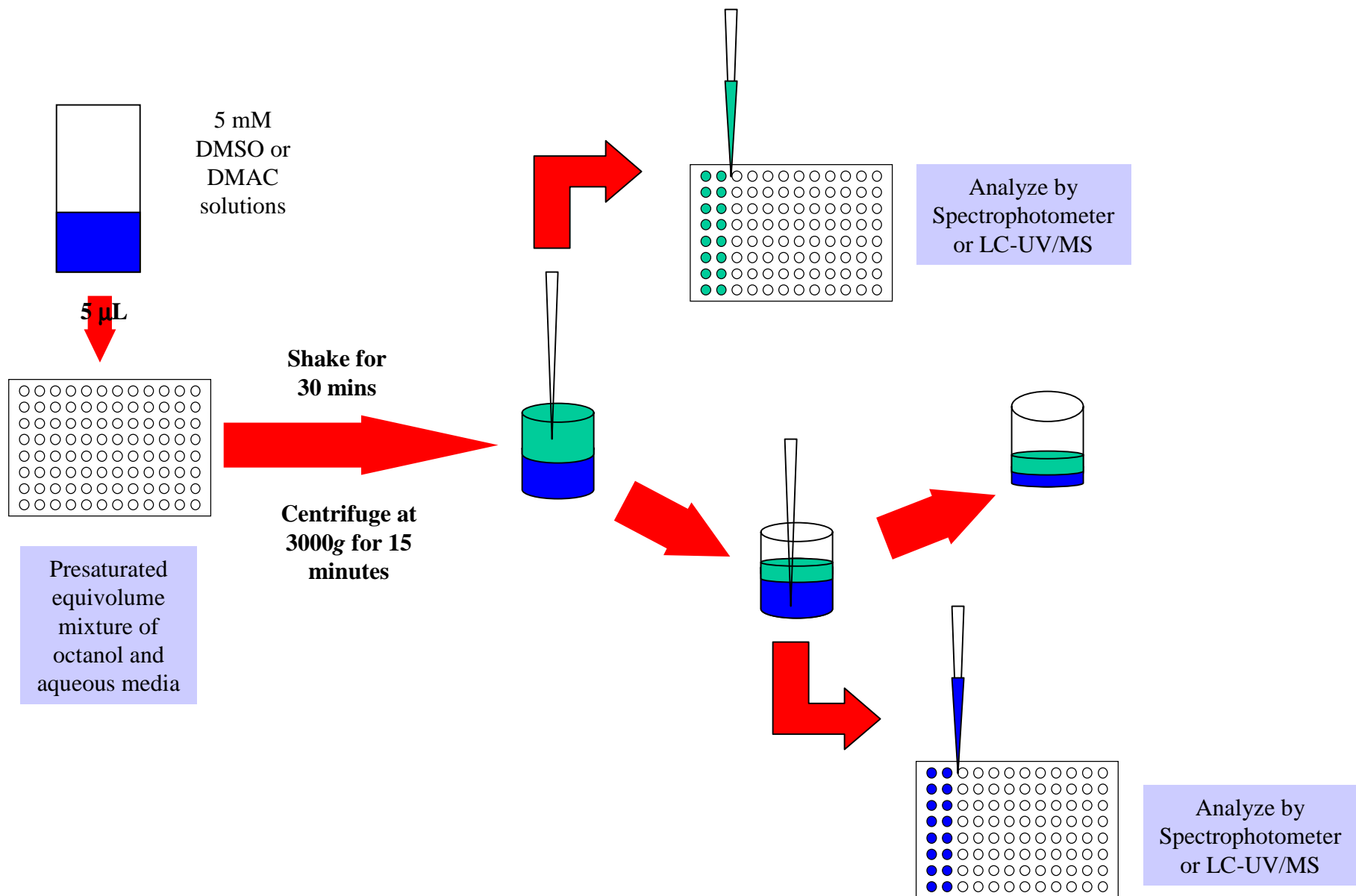
Good correlation seen between the shake flask method and the 96 well plate method



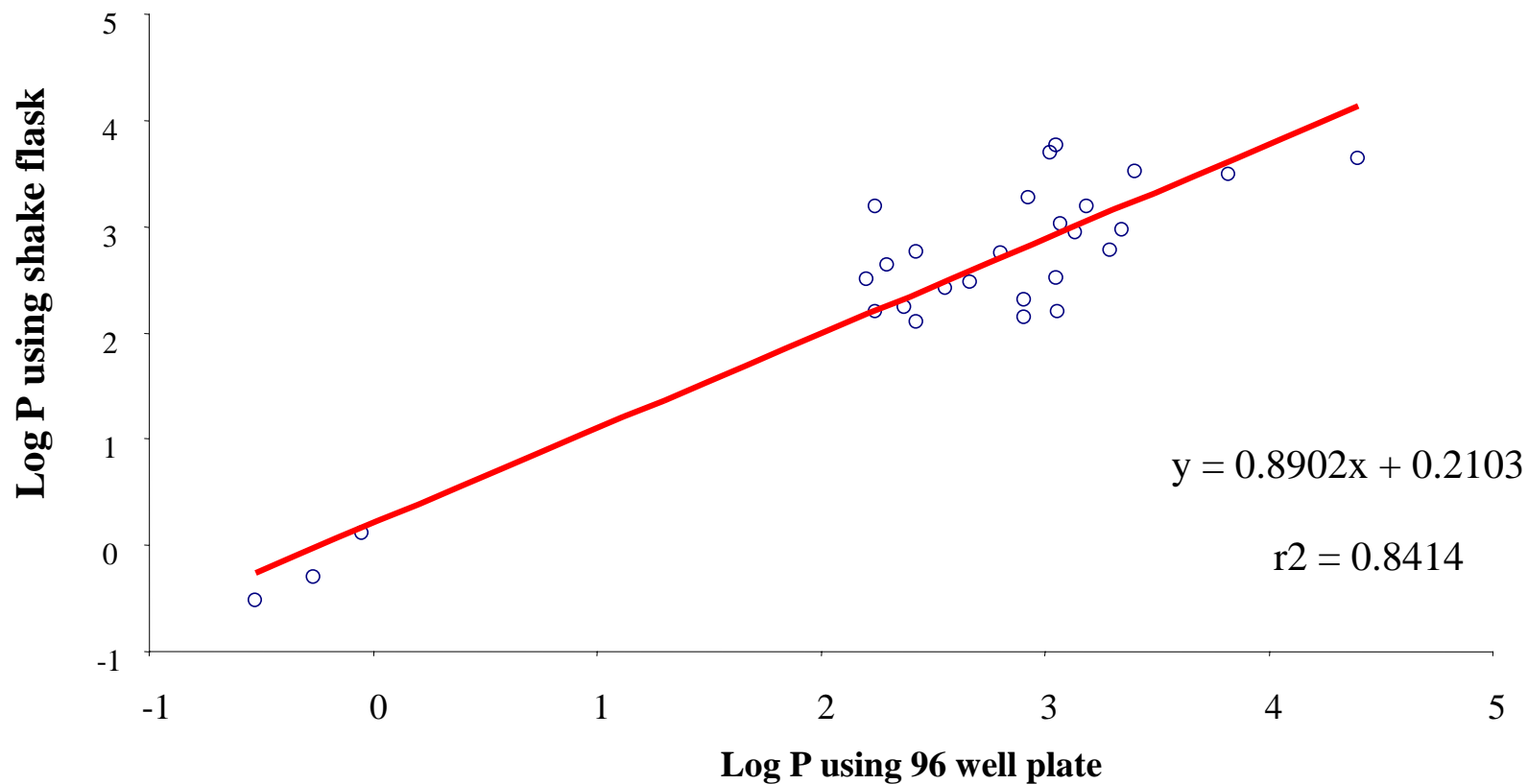
- $R^2 = 0.89$
- Difference between the theoretical curve and experimental points for the shake-flask method was *ca.* ± 0.1 on the log scale of the solubility expressed in mg/mL.
- For solubility values <10 mg/mL, the deviation becomes much larger (± 0.4 on the log scale).

Reference: A. Blasko, AAPS Annual Meeting 2002

Experimental Setup – Log P



Good correlation seen between the 96 well plate and the shake flask method



Pros of the MTS Assay

- Gives a numerical value for solubility
- Throughput of >100+ compounds/week
- Generic Fast Gradient HPLC with UV-Vis-MS
- Spreadsheet data handling makes data analysis quicker and more powerful
- Multiple wavelength detection allows the best spectrum to be used for quantification
- MS detection may avoid separation problems

Cons of the MTS Assay

- Compound must be UV-Vis/MS active to be detected
- Dissolved compound may adhere to the filter, giving a low value for solubility
- Limited to a maximum of 192 compounds per assay per HPLC unit due to the layout of the autosampler
- Assuming reasonable purity of the compound in question, impurities do not interfere

What to do with the data ?

- Ranking
 - Desired ranges
- Attempts to predict
 - Maximum Absorbable Dose
 - Absorption Potential

Lipinski's "rule of five"

Poor absorption/permeation can be expected

- More than 5 H-bond donors
- Molecular weight is over 500
- $\text{Log } P > 5$ (Moriguchi $\text{Log } P > 4.15$)
- Sum of N's and O's is over 10

- Substrates for transporters and natural products are exceptions.

What pKa or Log D values do we need?

– pKa

- IV - Acidic compound (<4.4) and Basic compound (>10.4)
- Oral - Acidic compound (<4.5) and Basic compound (>8.5)

– Log D (IV at pH 7.4 and Oral at pH 6.5)

- IV – Introduced into the circulation and need partitioning into cells. Higher Log D values may be beneficial (1.5 to 3.5).
- Oral – Need to cross multiple layers: GIT cell → Systemic circulation → Target cells

Log P/Log D values	Ranking	Why???
<0 or >3.5	Low	In water or in lipids
0-1.5 and 2.5-3.5	Intermediate	Some difficulty in partitioning through different layers
1.5 to 2.5	High	Ability to partition through various barriers easily

What solubility values do we need?

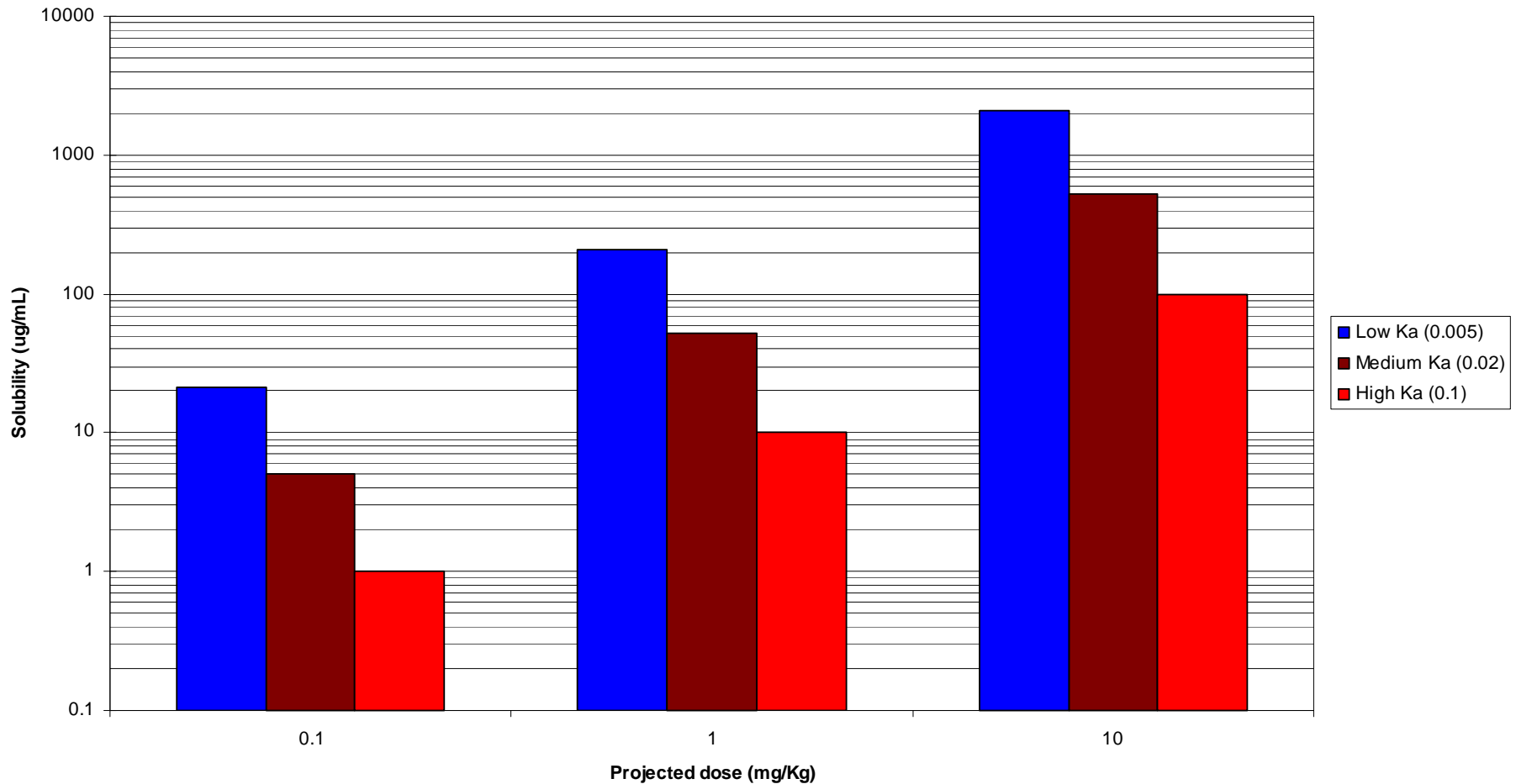
– Solubility (IV at pH 7.4, Oral at pH 2 to 6.5)

Solubility	IV Dose (mg) (200 mL)	Oral Dose (mg) (500 mL)
• 0-50 ug/ml	0 - 10	0 - 25
• 50-250 ug/ml	10 - 50	25- 125
• > 250 ug/ml	>50	>125

– Solubility numbers required depend on

- Dose required
- Permeability - Oral

What solubility values do we need – Oral focus?



Reproduced from C.A. Lipinski. Journal of Pharmacological and Toxicological Methods, 44 (2000), 235-249.

Concept of Maximum Absorbable Dose - Oral

$$MAD = S \times K_a \times SIWV \times SITT$$

Where

MAD = Maximum Absorbable Dose

S = Solubility (thermodynamic or equilibrium) in $\text{mg}\cdot\text{mL}^{-1}$ at pH 6.5

K_a = transintestinal absorption rate constant (min^{-1})

SIWV = Small Intestine Water Volume (~ 250 mL)

SITT = Small Intestine Transit Time (~ 270 minutes)

So typically for a compound with medium permeability and a dose of 1 and 10 mg/Kg, solubility of 52 and 520 $\mu\text{g}/\text{mL}$ is required at pH 6.5

Reference: W. Curatolo, Pharmaceutical Science & Technology Today, 1;9 (1998), 387-393.

MAD is a good conceptual tool in indication of problem compounds

Notes

- Projected value: If dose is greater than this, incomplete absorption may be expected.
- Conceptual tool for rank ordering compounds and setting targets

Challenges

- Determination of solubility
 - Media – buffer solutions or simulated fluids (Accurate estimation of ionic/surfactant levels)
 - Thermodynamic vs. Equilibrium solubility?
 - Solubilization in stomach → Precipitation rate in intestine?
- Determination of transintestinal absorption rate constant
 - Typically estimated using rat intestinal perfusion model or Caco2 cells

Absorption Potential as an alternative

$$AP = \log \left(P \cdot F_{non} \cdot \frac{S \cdot V_L}{X_0} \right)$$

Where,

P- Partition Coefficient

F_{non} - Fraction unionized at pH 6.5

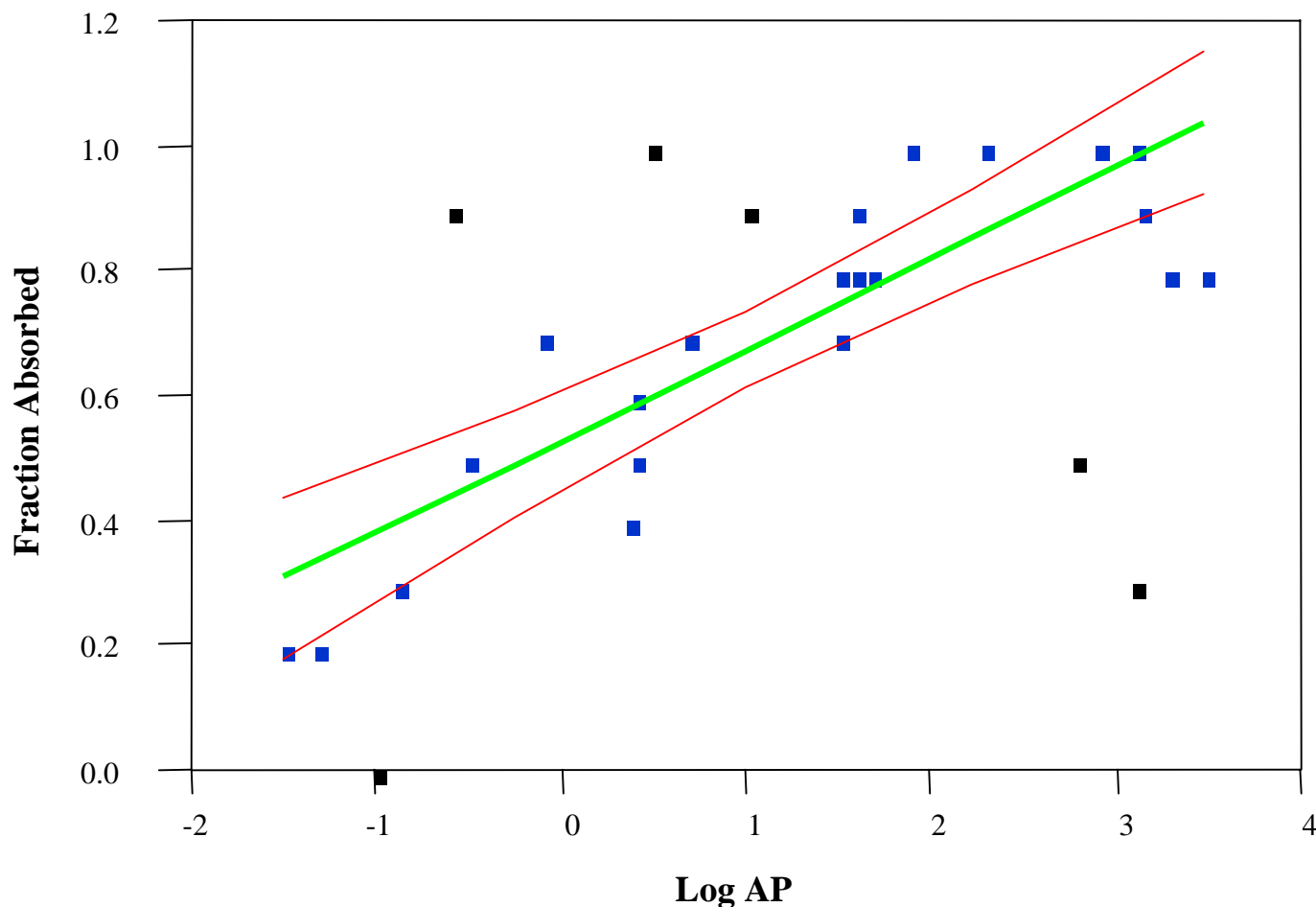
S- Solubility at pH 6.5

V_L - Lumenal Volume (~ 250 mL)

X_0 - Dose Administered

Sanghvi et al., Pharm. Res. 18;12, (2001), 1794-1796.

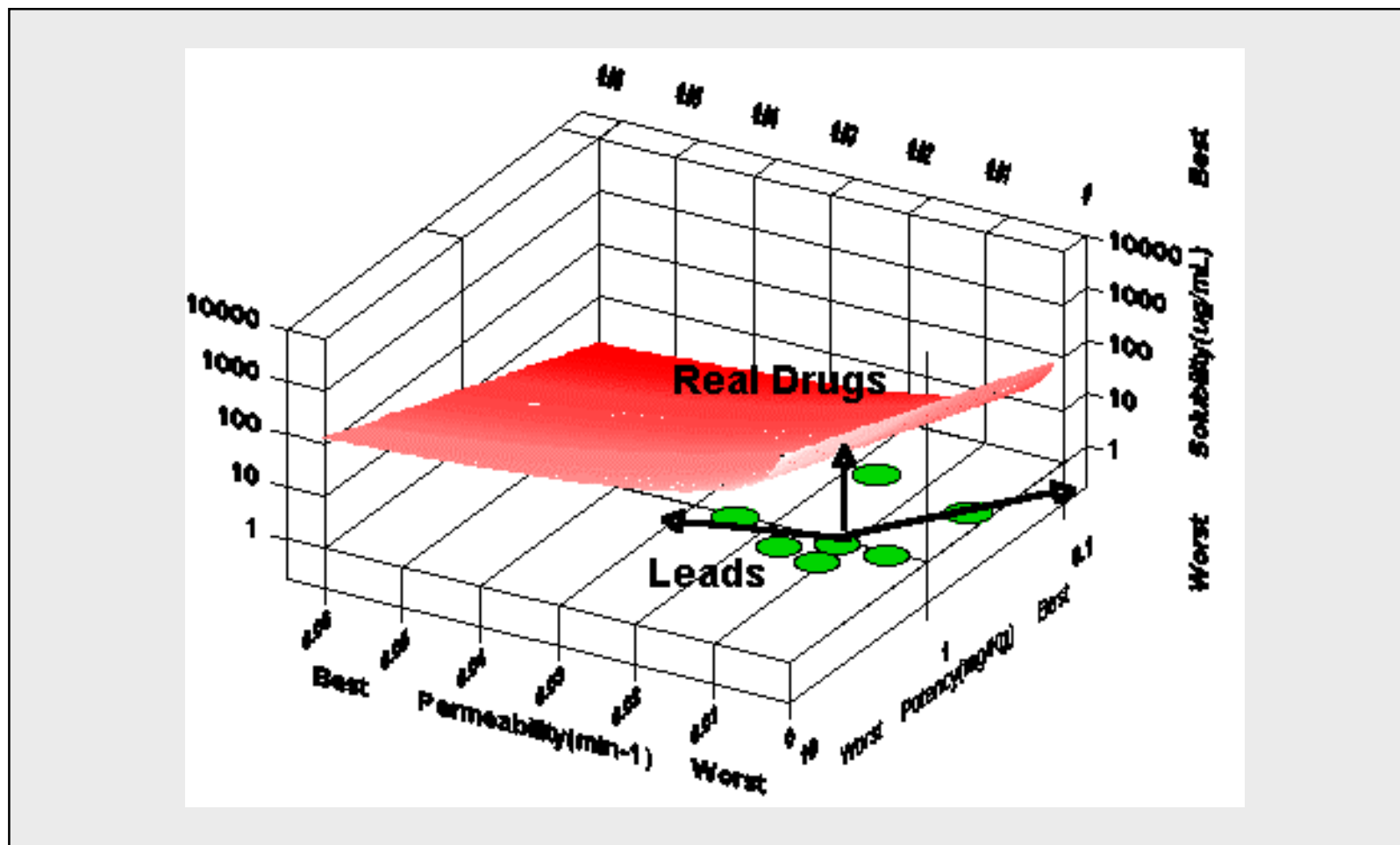
Correlation between Fraction Absorbed of marketed compounds and Absorption Potential



Reproduced with modification
from Sanghvi et al., Pharm.
Res. 18;12, (2001), 1794-
1796.

- 28 compounds evaluated. 6 complete outliers
- $r^2 = \sim 0.73$

Feedback to chemistry on solubility and permeability is critical to avoid oral absorption problems



Reference: C.A. Lipinski, Tripos, March 21, 2003

Permeability is another parameter used to attempt predict absorption

- Determined via
 - Caco2 cells
 - Parallel Artificial Membrane Permeability Assay
- Relevance
 - Determination at relevant concentration
 - Effect of solubility on parameter estimation

References

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Acknowledgments

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