

XRPD and Chemometrics Approach in Phase Separation Analysis (of lyophilized systems)

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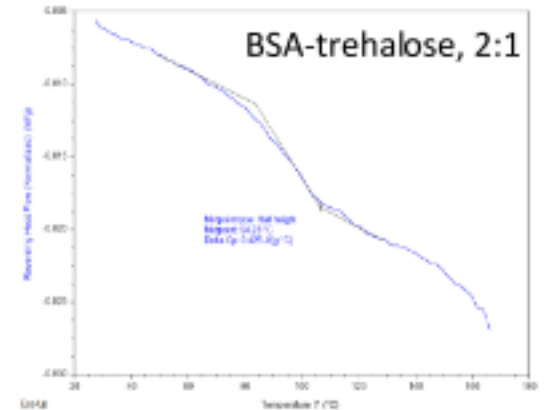
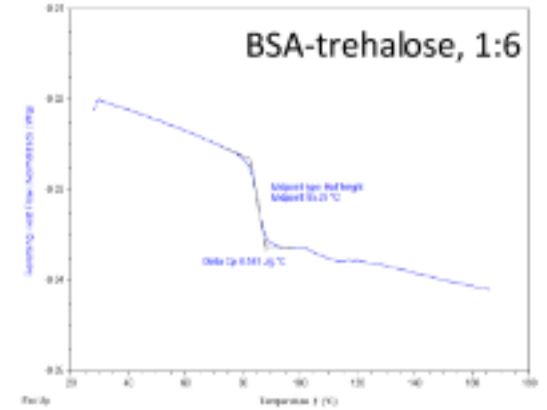
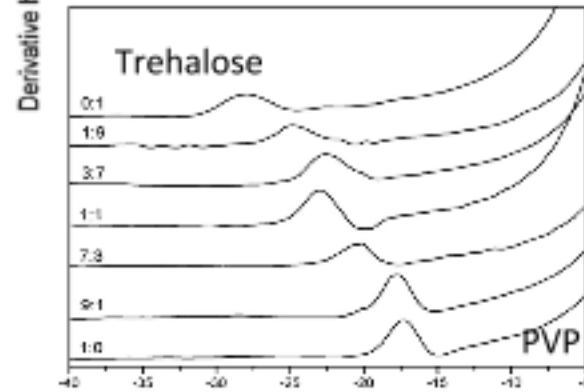
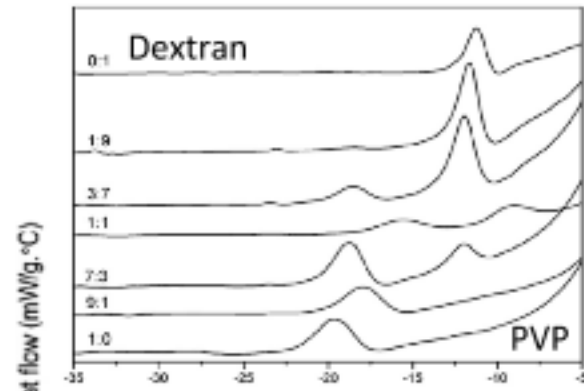
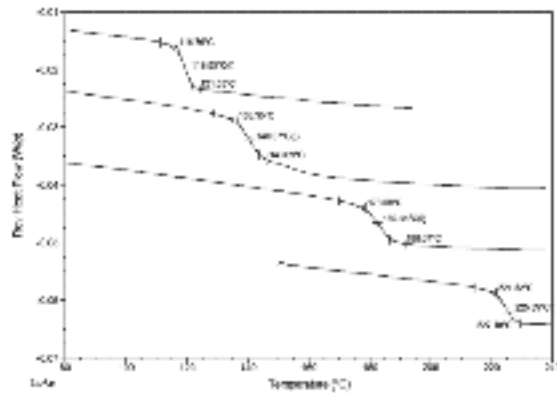
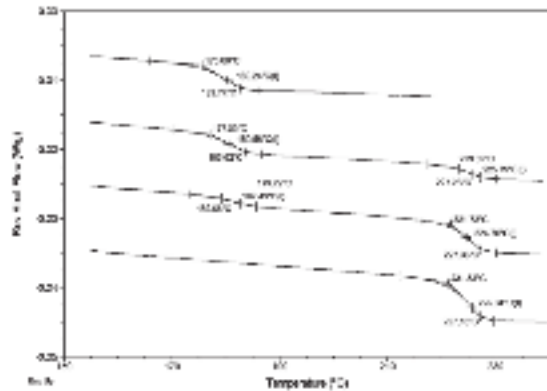
Background

- Formulations are becoming more complex
 - Multi-stabilizers: sugars, polyols, polymers etc
- A miscible system is desirable
- Lyophilization can induce phase separation
 - Freezing: ice-solute separates
 - Drying: thermodynamics/kinetics ... crystallization
- Formation of drug-rich and excipient-rich regions
- **Phase separation = undesirable effect**
- Ideally, to detect phase separation as early as possible

Analysis methods

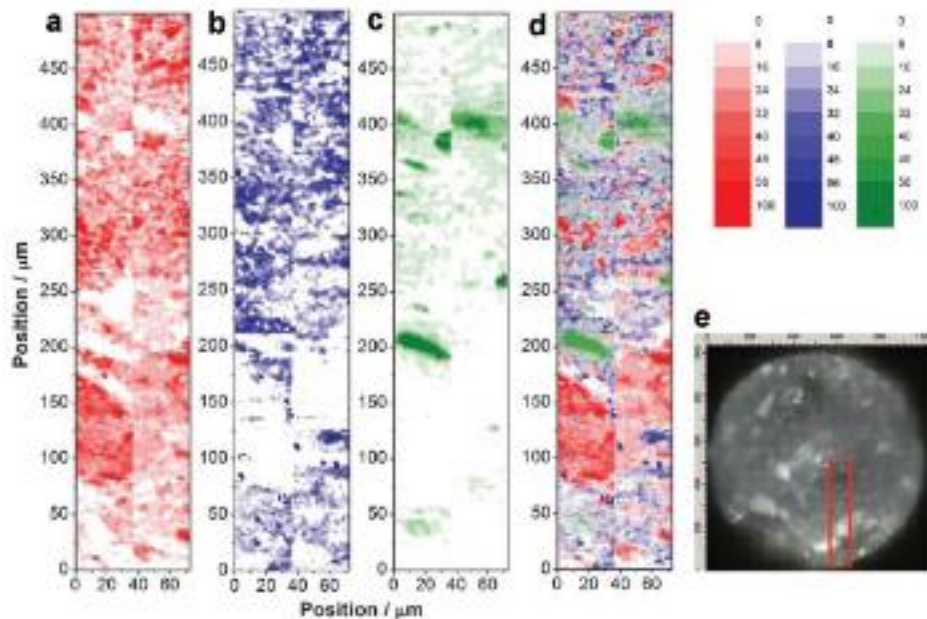
1. DSC (goldstandard)
 - Probing the number of glass transition temperatures (T_g s)
2. Spectroscopic imaging (Raman)
 - Visual map
 - Statistical analysis
3. SEM
 - High magnification images

DSC (goldstandard)

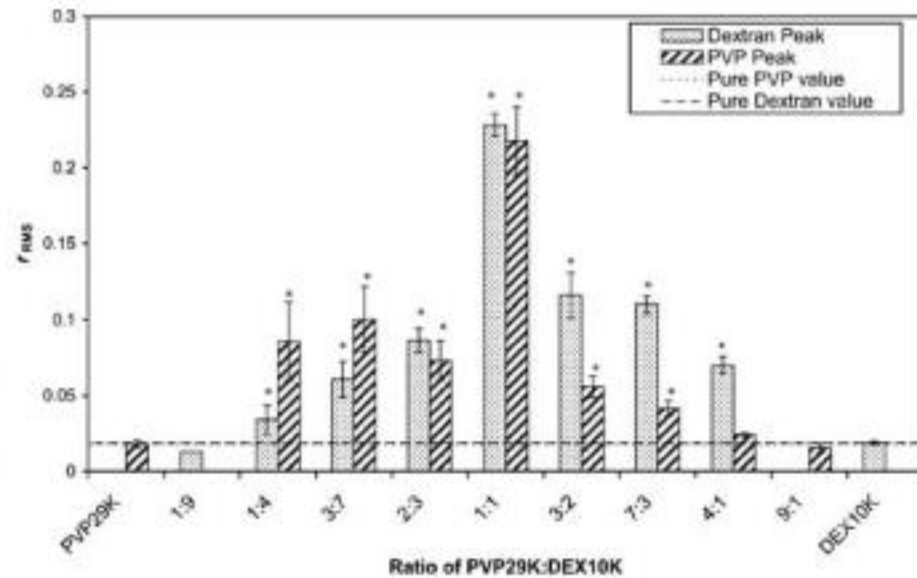


1. Newman *et al.*, Journal of Pharmaceutical Sciences, 2008, 97:11 , p4840-4856
2. Chieng *et al.*, International Journal of Pharmaceutics, 2013, 454, p167-173
3. Unpublished results(BSA-trehalose systems)

Spectroscopic imaging by Raman



(a) theophylline anhydrate, (b) tripalmitin, (c) polyethylene glycol

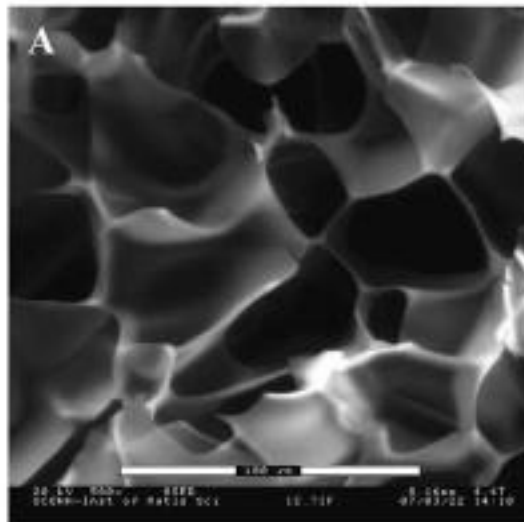


“Root mean square” method

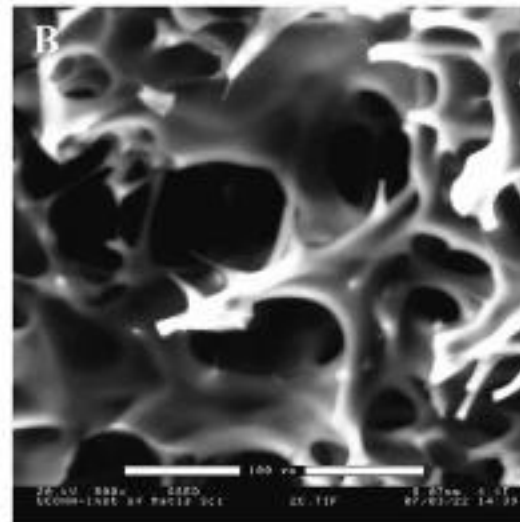
4. Windbergs *et al.*, Journal of Pharmaceutical Sciences, 2010, 99:3, p1464-1475
5. Padilla *et al.*, Journal of Pharmaceutical Sciences, 2011, 100:1, p206-222

SEM

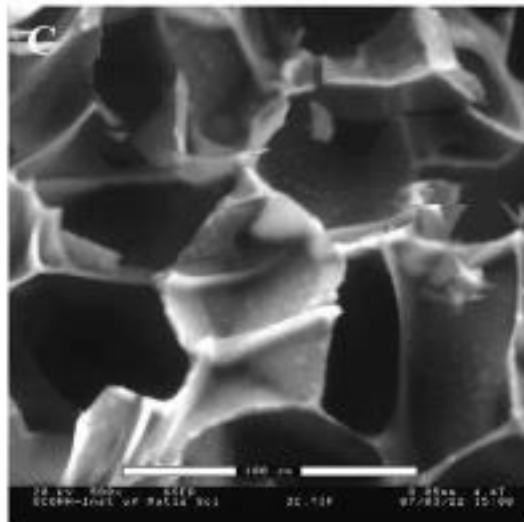
Dextran



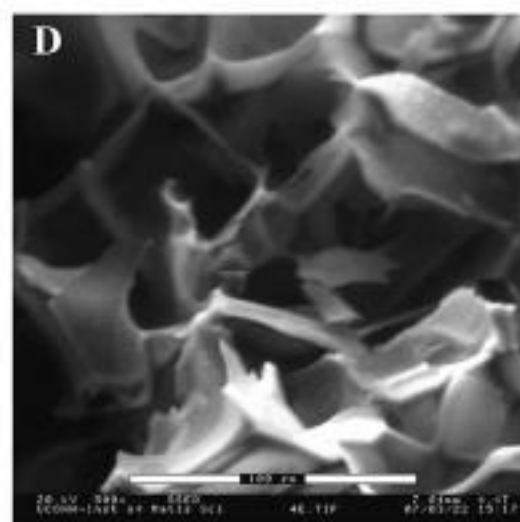
PVP



PVP:Dex
3:2



PVP:Dex
2:3



Analysis methods – Limitation(s)

1. DSC

- Missed interpretation, two (very) close proximity T_g s
 - End ratios, i.e. 1:9
- Protein (strong glasses) is hard to differentiate

2. Spectroscopy (Raman)

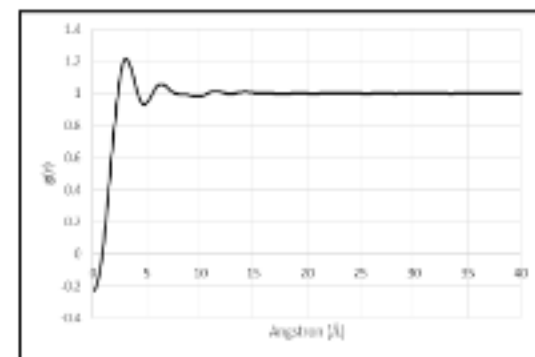
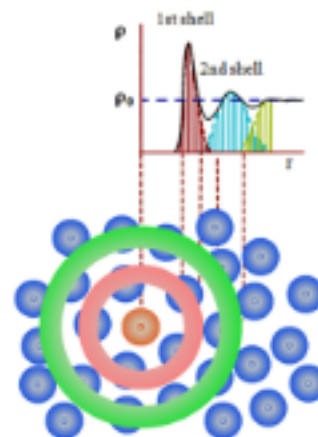
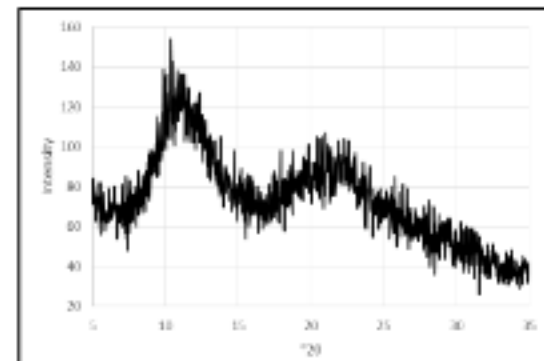
- Long acquisition time (used to be)
- Mapping is great, but how to interpret the map?
- Statistical analysis: Requires a 'baseline'

3. SEM

- Subjective

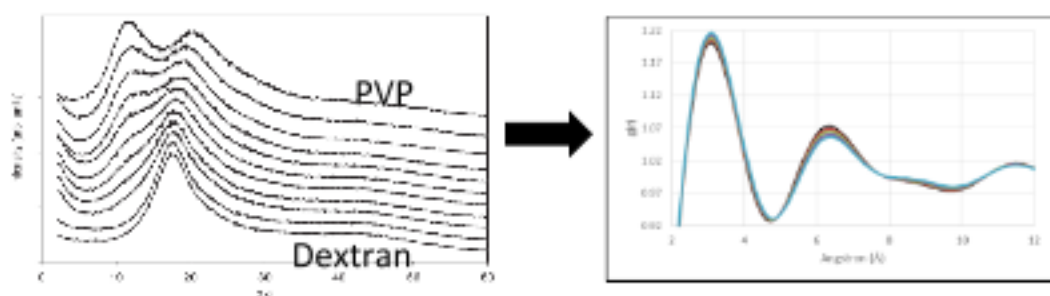
XRPD and PDF

- XRPD
 - Method used to measure crystallinity (or the lack of it)
- PDF (PairDistribution Function)
 - Probability of finding two atoms separated by a distance “r”.

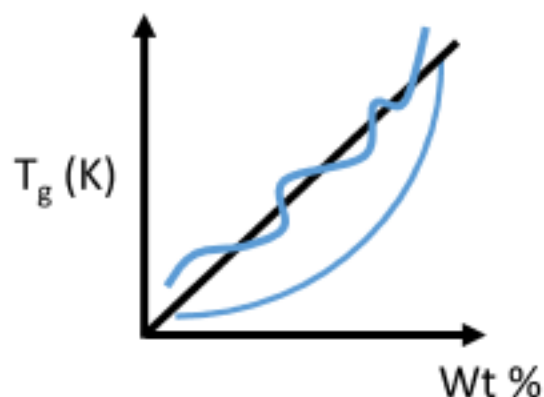


XRPD-PDF approach

- Converting a 'halo' into a 'distance plot'

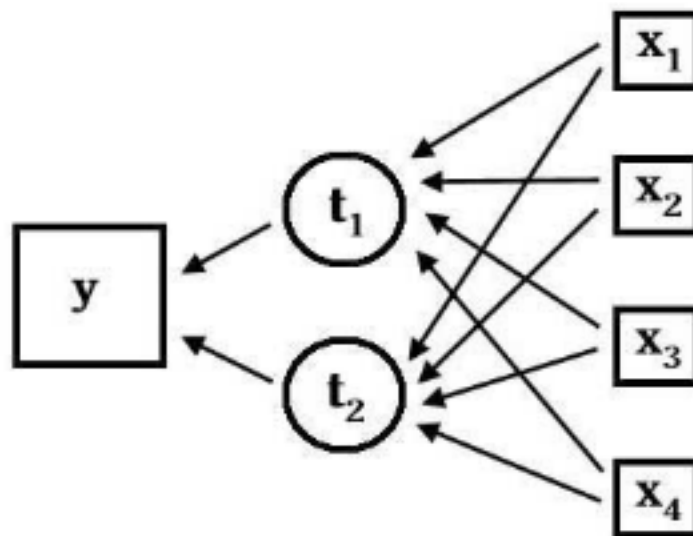
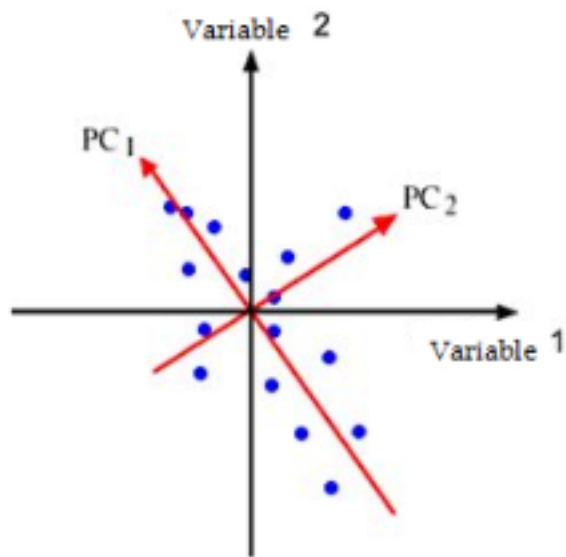


- Calculate the linear combination using pure PDF data for a specific ratio
 - Theoretical PDF data = $(A \times 0.1) + (B \times 0.9)$
- Compare experimental with theoretical PDF data



Chemometrics – PCA

- Principal component analysis
- Dimension reduction (while retaining the variability of the data)



Can we improve XRPD-PDF analysis?

7. dos Santos *et al.*, Chemometrics: Theory and Application. IntechOpen 2013
8. Martens and Næs, Multivariate calibration. John Wiley & Sons Inc., Chichester 1989.

Aim of study

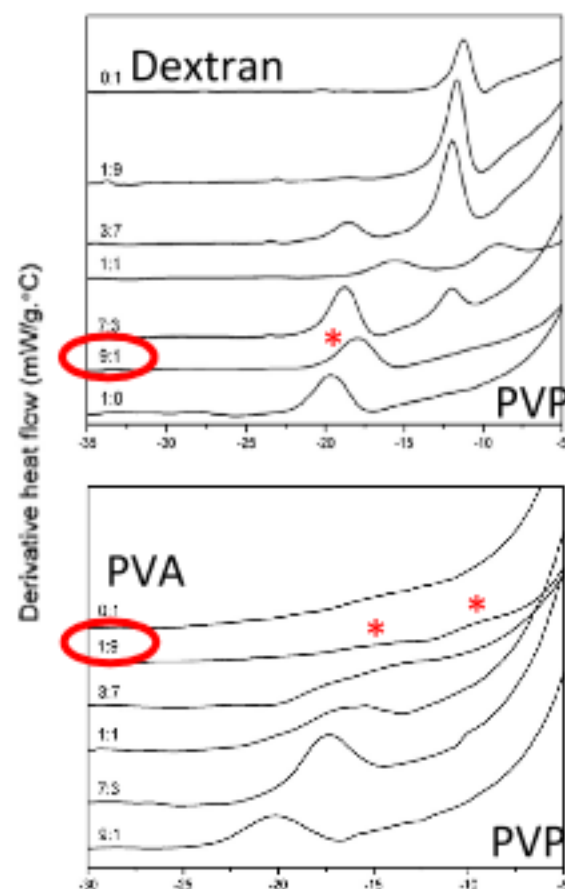
To explore the use of PCA combined with XRPD-PDF for detecting phase separated (freeze dried) samples

Model systems

- PVP-DEX (phase separated)
- PVP-PVA (miscible)
- PVP-Trehalose (miscible)

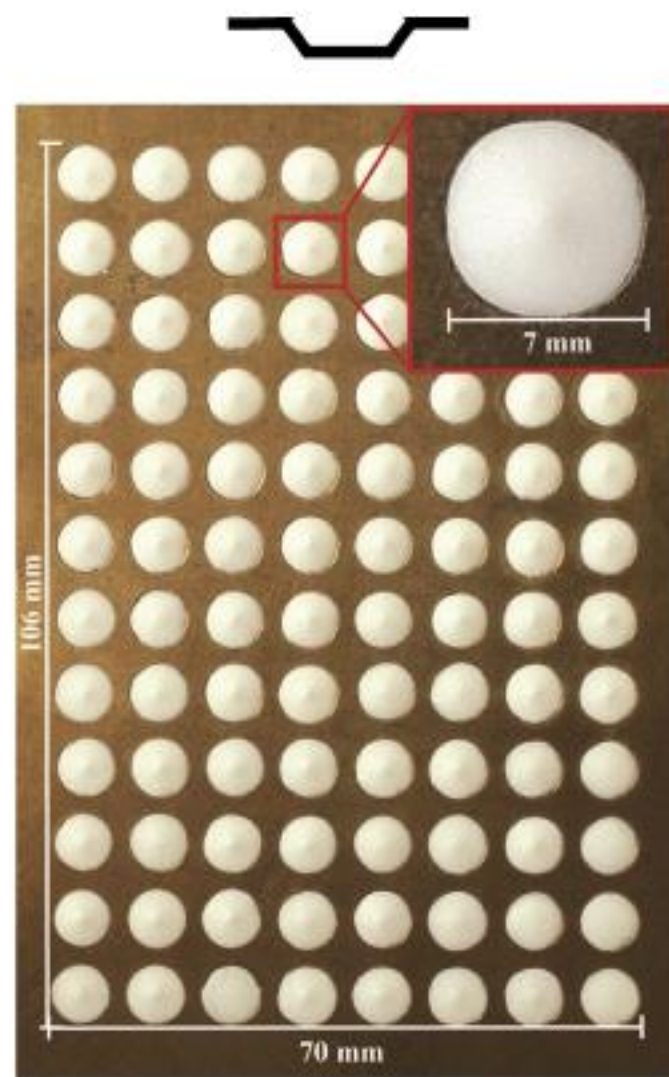
Freeze-drying

- Freezing at 1 °C/min
- 1° at -30 to -35 °C for 12-13h
- 2° at +40 °C for 4h



Sample preparation

- Well-plate method
 - Simple to set up
 - Brass plate
 - Glue on foil – good seal
- Each well takes ~60 μL
- Ideal for formulation screening



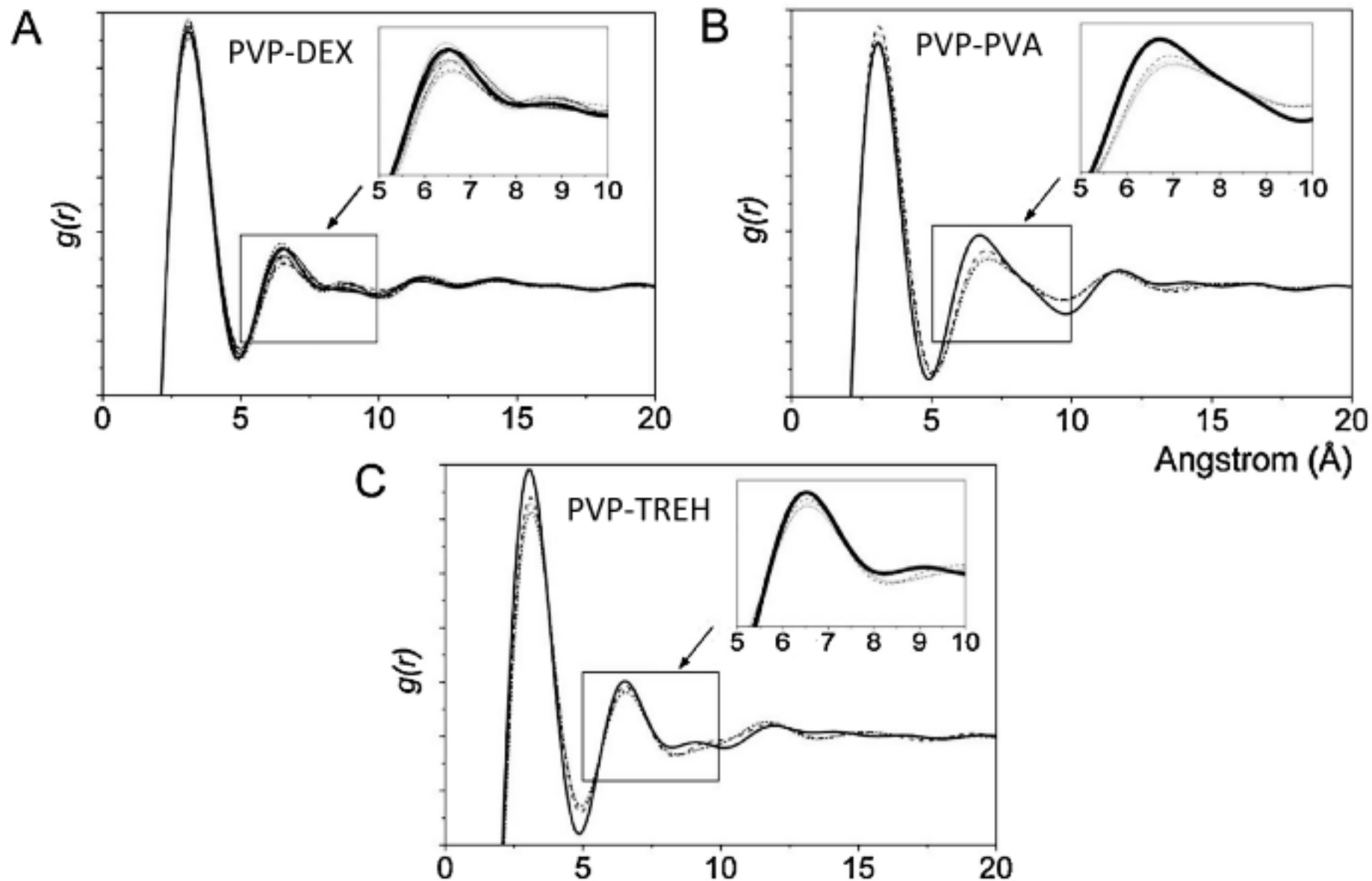
9. Trnka *et al.*, *Pharmaceutical Development and Technology*, 2015, 20:1, 65-73
10. PANalytical Empyrean Technical Specification

XRPD to PDF conversion

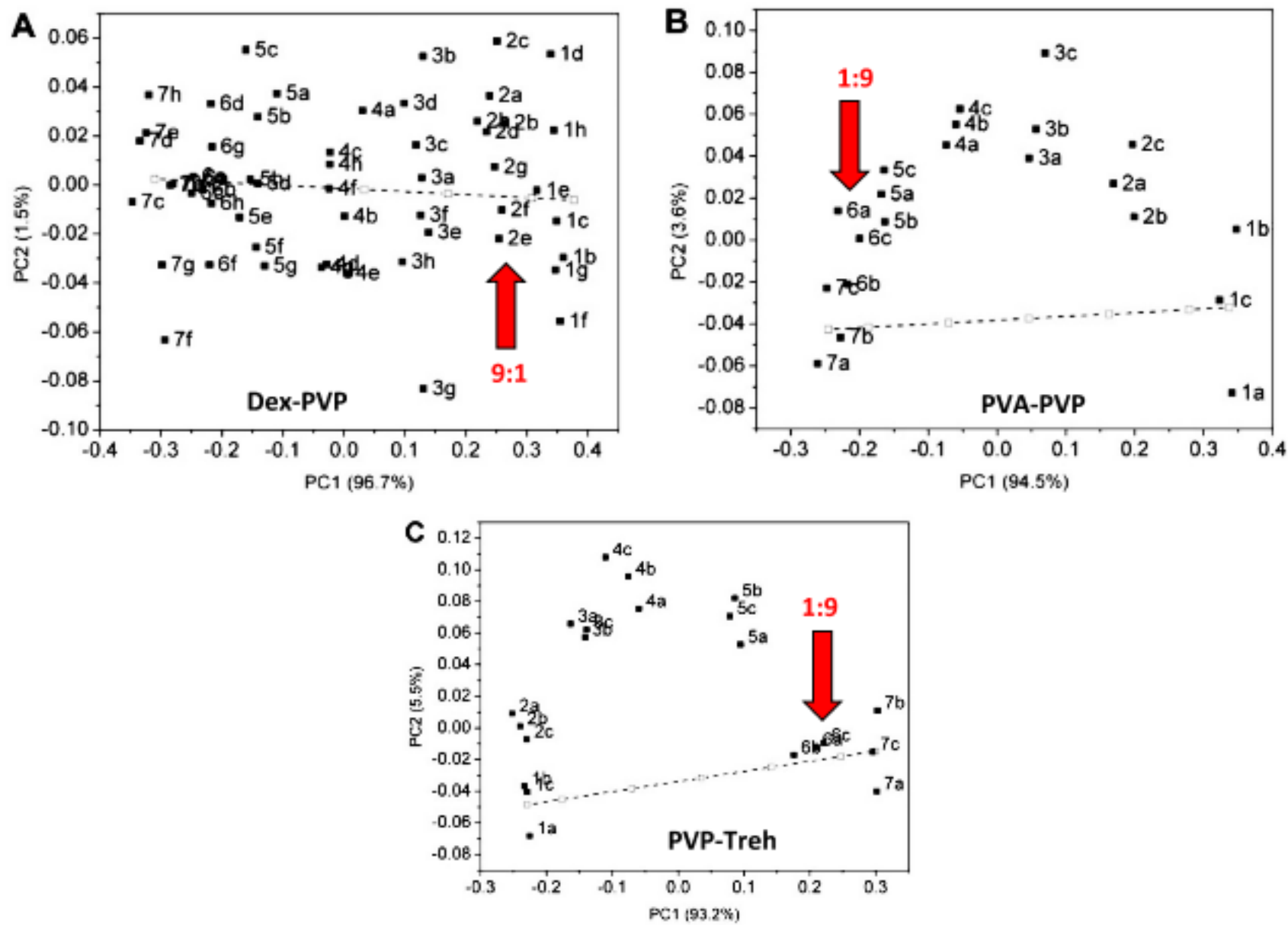
- Using a freeware program called RAD (by V. Petkov)
- Dos based program, multiple steps to process one XRPD data
- Takes a LONG time to convert!(back then)
- Calculate the linear combination using the pure PDF data



PDF data (1:1 samples)



PCA scatter plots



Comparison: DSC vs XRPD-PDF-PCA

Formulations	Ratio	Is the sample phase separated?	
		DSC (T_g')	PXRD (PDF and PCA)
PVP30-DEX10	9:1	No	Yes
	7:3	Yes	Yes
	1:1	Yes	Yes
	3:7	Yes	Yes
	1:9	Yes	Yes
PVP30-PVA	9:1	No	No
	7:3	No	No
	1:1	No	No
	3:7	No	No
	1:9	Yes	No
PVP90-TREH	9:1	No	No
	7:3	No	No
	1:1	No	No
	3:7	No	No
	1:9	No	No

Summary

- XRPD + PDF + PCA seems to work
- May be an alternative analysis
- Consider using multivariate analysis – e.g. big data, subtle changes
- 96 well-plate: quick, high-throughput, small batch
- Great for formulation screening

Limitations

- Need a full model of ratios
- Does PDF processing(still) takes a long time?
- Multiple software required – XRPD, PDF,PCA
- Cost: XPRD + the stage + 96 well-plate
- Need to use brain juice for analysis/interpretation
- No information on chemical distribution
- Not tested on strong glasses (i.e. protein-stabilizer system)

Thank you.