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Seeing the unseen; Monitoring drug precipitation by Nanoparticle Tracking Analysis

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Drug Precipitation and HTS

- HTS – Discovery compounds stored dissolved in DMSO
- Aliquots of DMSO stock then utilised in screening[1]
- Bioassays, kinetic solubility
- Majority of compounds synthesised – poorly water soluble
- Bioassays – stock added to aqueous buffer

1. Dehring, K. A.; Workman, H. L.; Miller, K. D.; Mandagere, A.; Poole, S. K., Automated robotic liquid handling/laser-based nephelometry system for high throughput measurement of kinetic aqueous solubility. Journal of Pharmaceutical and Biomedical Analysis ***2004,*** *36 (3), 447-456*

Does the compound remain in solution?

- Growing realisation it may not [2]
- Drug may precipitate due to poor aqueous solubility
- Can affect results in two ways;

1. False negative – not enough compound in solution to react
2. False positive – promiscuous inhibition [3]

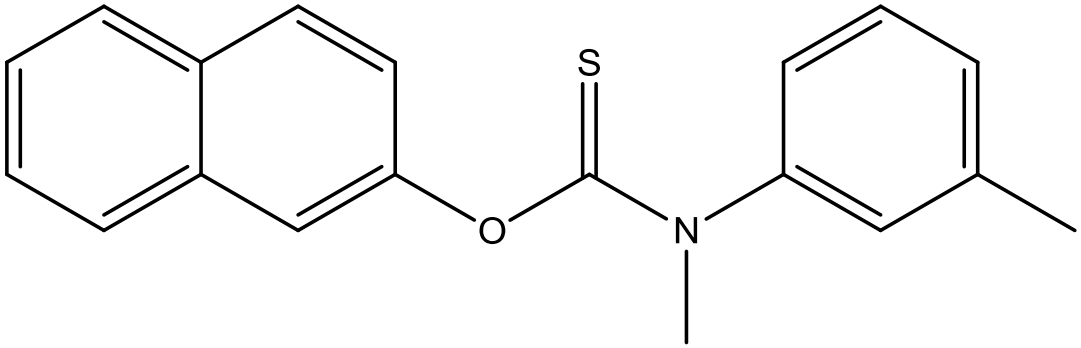
2. Di, L.; Kerns, E. H., Biological assay challenges from compound solubility: strategies for bioassay optimization. Drug Discovery Today ***2006,*** *11 (9-10), 446-451.*

3. Seidler, J.; McGovern, S. L.; Doman, T. N.; Shoichet, B. K., Identification and Prediction of Promiscuous Aggregating Inhibitors among Known Drugs. Journal of Medicinal Chemistry ***2003,*** *46 (21), 4477-4486*

Project – Overall Aims

1. Investigate the physical chemistry behind what happens when (poorly water soluble) drugs dissolved in DMSO are mixed with aqueous buffers
2. Relevant practically to pharma industry (screening)
3. Unexplored physical chemistry – precipitation/crystallisation at low concentrations

Model compound -Tolnaftate



Properties

MW – 307.4g/mol

Low aqueous solubility (<3μM /0.9μg/mL[4])

Neutral

Used as an anti fungal

4. Physchem forum solubility data, http://physchem.org.uk/solubility_data.htm, Feb 2011

Tolnaftate

- From other experiments - known to precipitate at concentrations relevant to drug screening

BUT

- Precipitation not visible to naked eye
- Utilised NanoSight and NTA to ‘see’ what was going on during precipitation process – results then compared to DLS

NanoSight Instrument and NTA

❖Essentially a light scattering technique

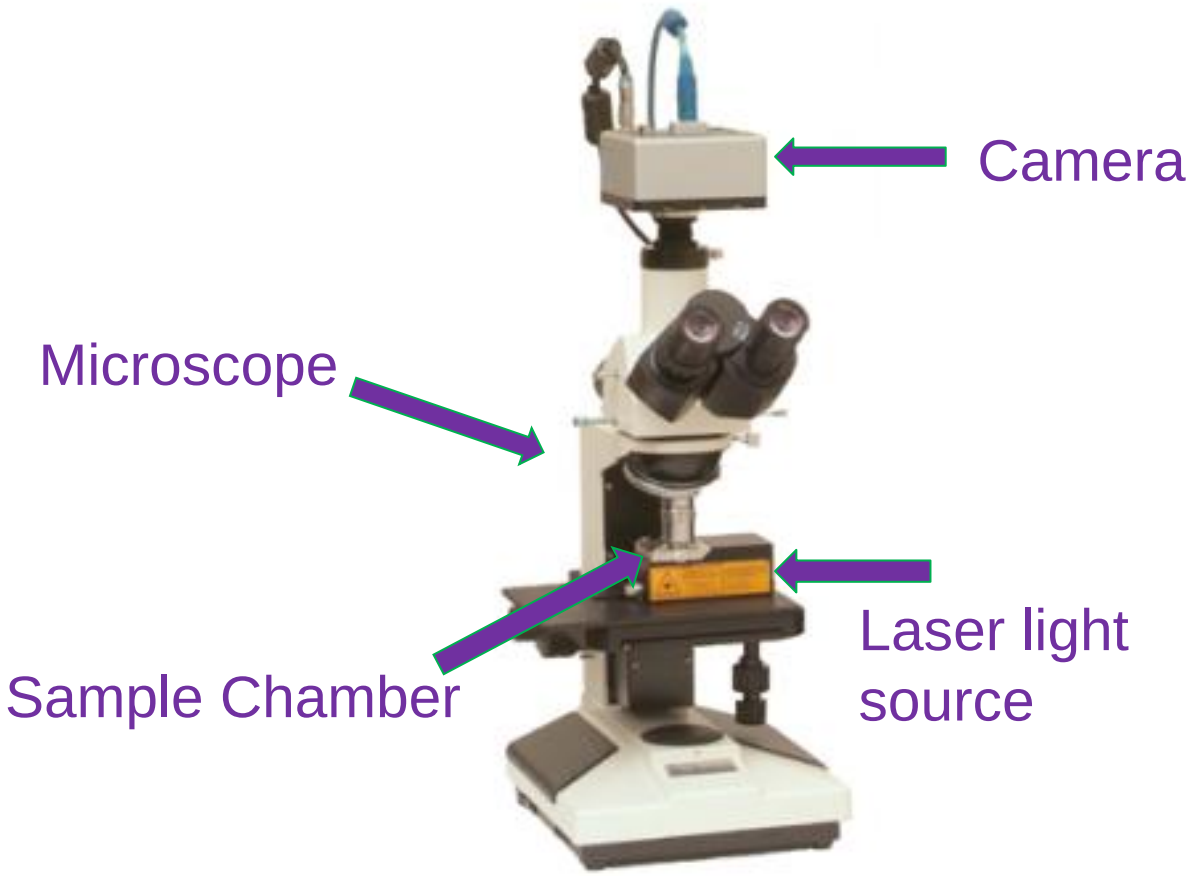
❖Instrument basically consists of

1.laser light source

2.microscope (lens)

3.CCD camera

NanoSight LM10



Sample Procedure

- Sample introduced into NanoSight chamber
- Image of particles' scattering of laser light recorded
- Video then analysed using NTA software
- Output – Mean size (diameter), concentration, particle size distribution

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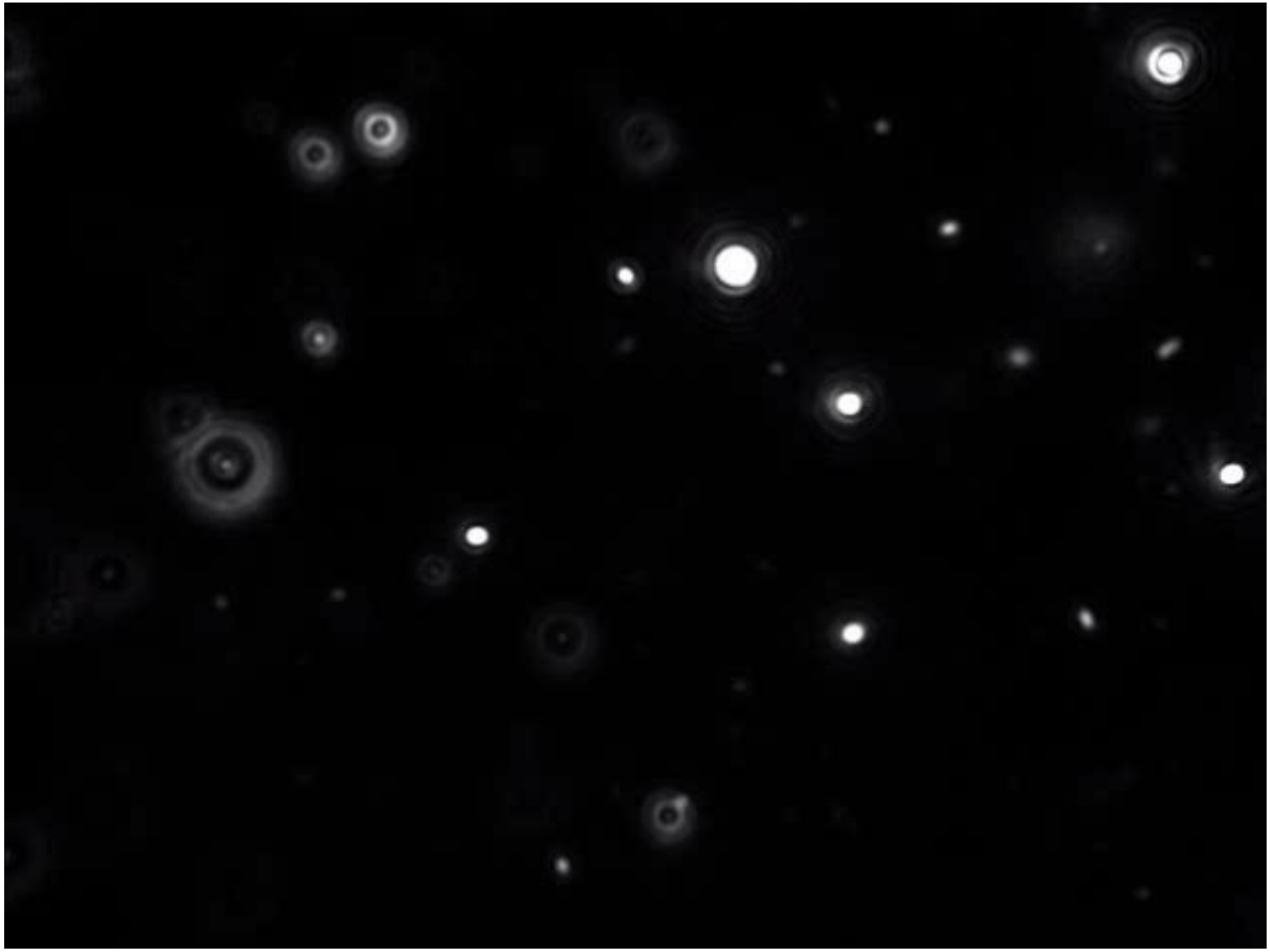
NanoSight – Recorded Video



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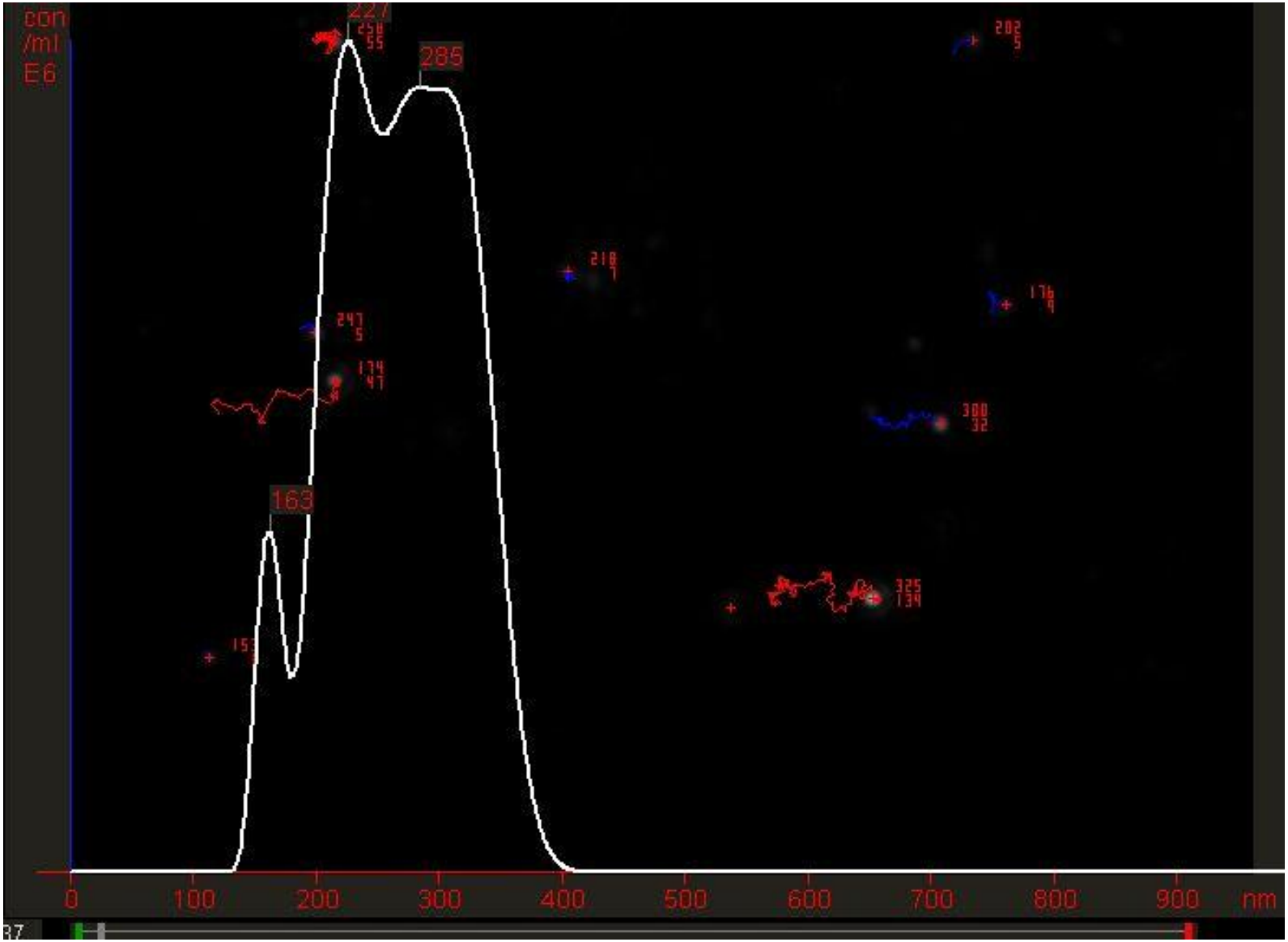
NanoSight – Tracking Example



NanoSight and NTA – Particle Sizing

- From the Brownian motion, particle diameter can be calculated using the Stokes-Einstein equation
- The Diffusion Coefficient (D_t) from the mean squared displacement of the particle tracked is calculated
- All other parameters in equation are known, and so r_h can be calculated

$$D_t = k_B T / 6\pi\eta r_h$$

NanoSight and NTA – Particle Concentration

Estimation of the concentration of the particles based on

- - assumed scattering volume calculated from the dimensions of the field of view (at a given magnification)
- -depth of the laser beam.
- Average number of particles per millilitre of sample is then extrapolated from the assumed scattering volume [5]

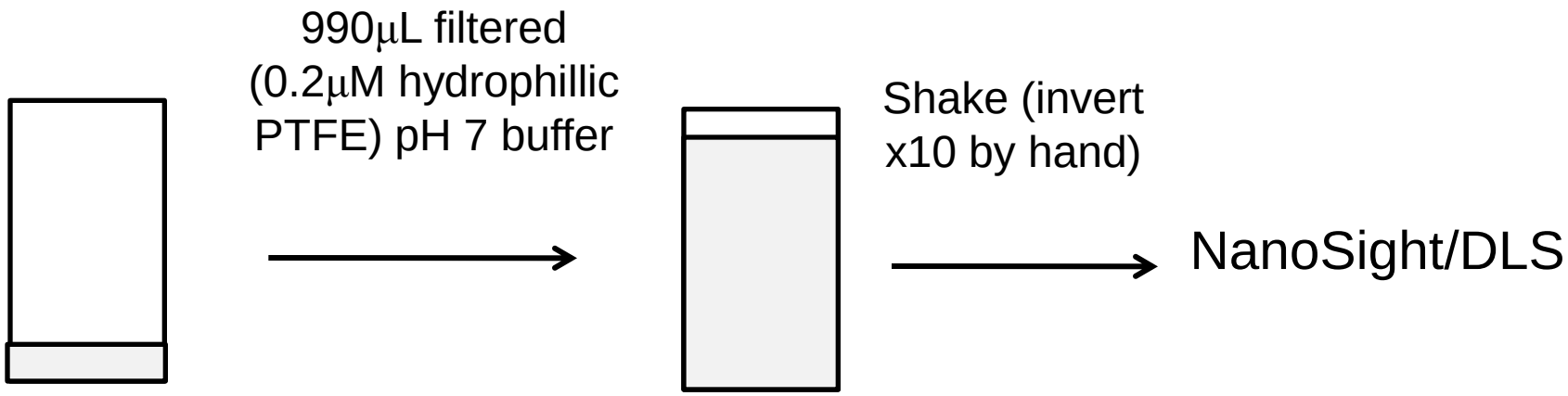
5.NanoSight, L., NanoSight LM20 & NTA 2.0 Analytical Software Operating Manual, Version 2.1. 2009.

Main Differences – NTA and DLS

1. Image of the particles' scattering recorded in NTA
2. NanoSight gives an estimate of particle concentration
3. Calculated diameter based on individual particles [5]

5. NanoSight, L., NanoSight LM20 & NTA 2.0 Analytical Software Operating Manual, Version 2.1. 2009.

Tolnaftate Experiments

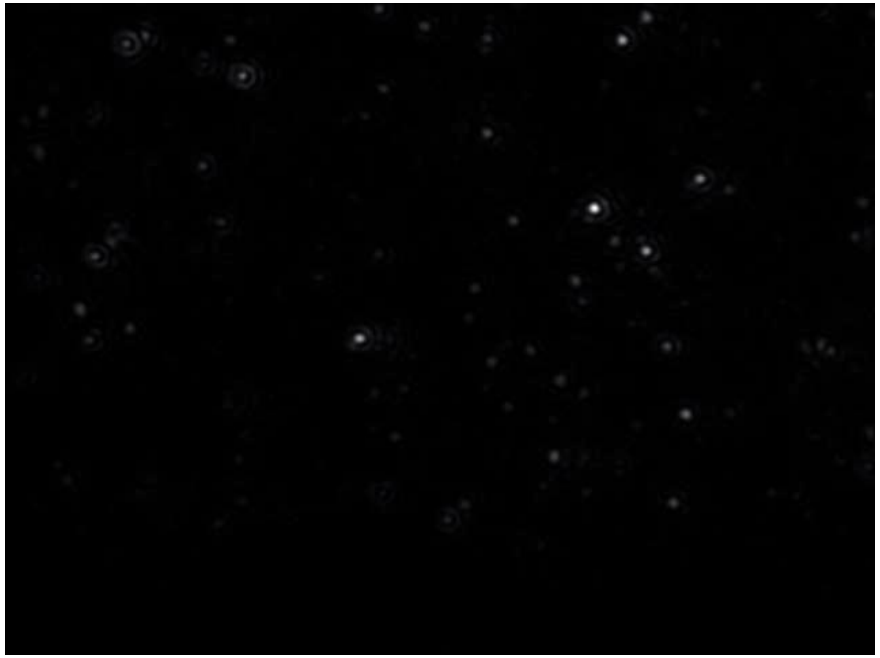


- Samples monitored over a 3 hour time period

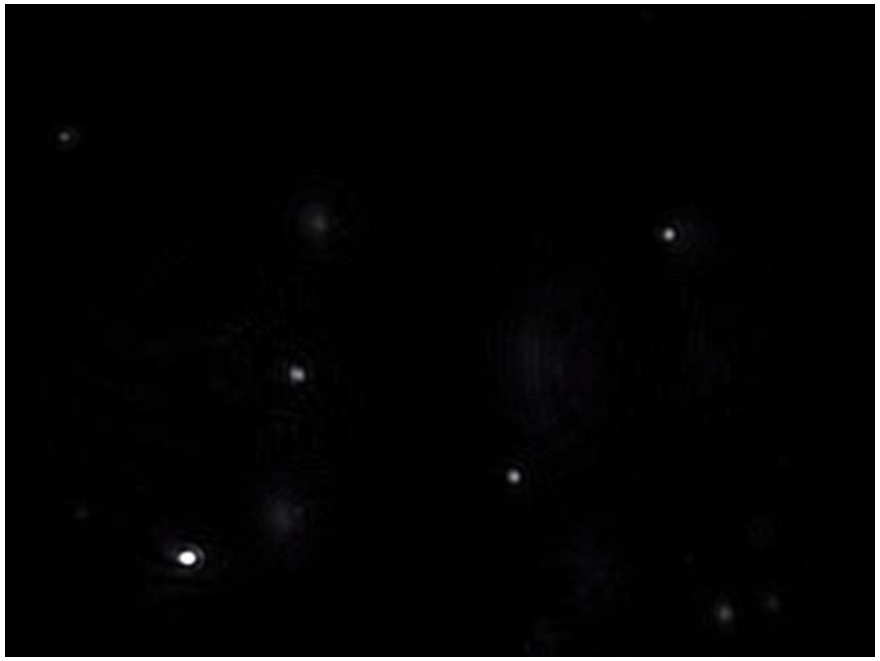
NanoSight Results – Mean Size

- Growth over time and decreases in concentration (expected)

30μM Tolnaftate t=0 mins



30μM Tolnaftate =180mins



NanoSight Results – utilising concentration estimate

Calculation of amount of drug precipitating per timepoint

1. Calculate volume mean from particle size distribution

2. Volume fraction = $\pi/6d^3(cm^3)$ x conc (no.particles/mL)

3. Mass concentration (g/mL) = vol. fraction x ρ

❖ This can then be converted to a μM concentration

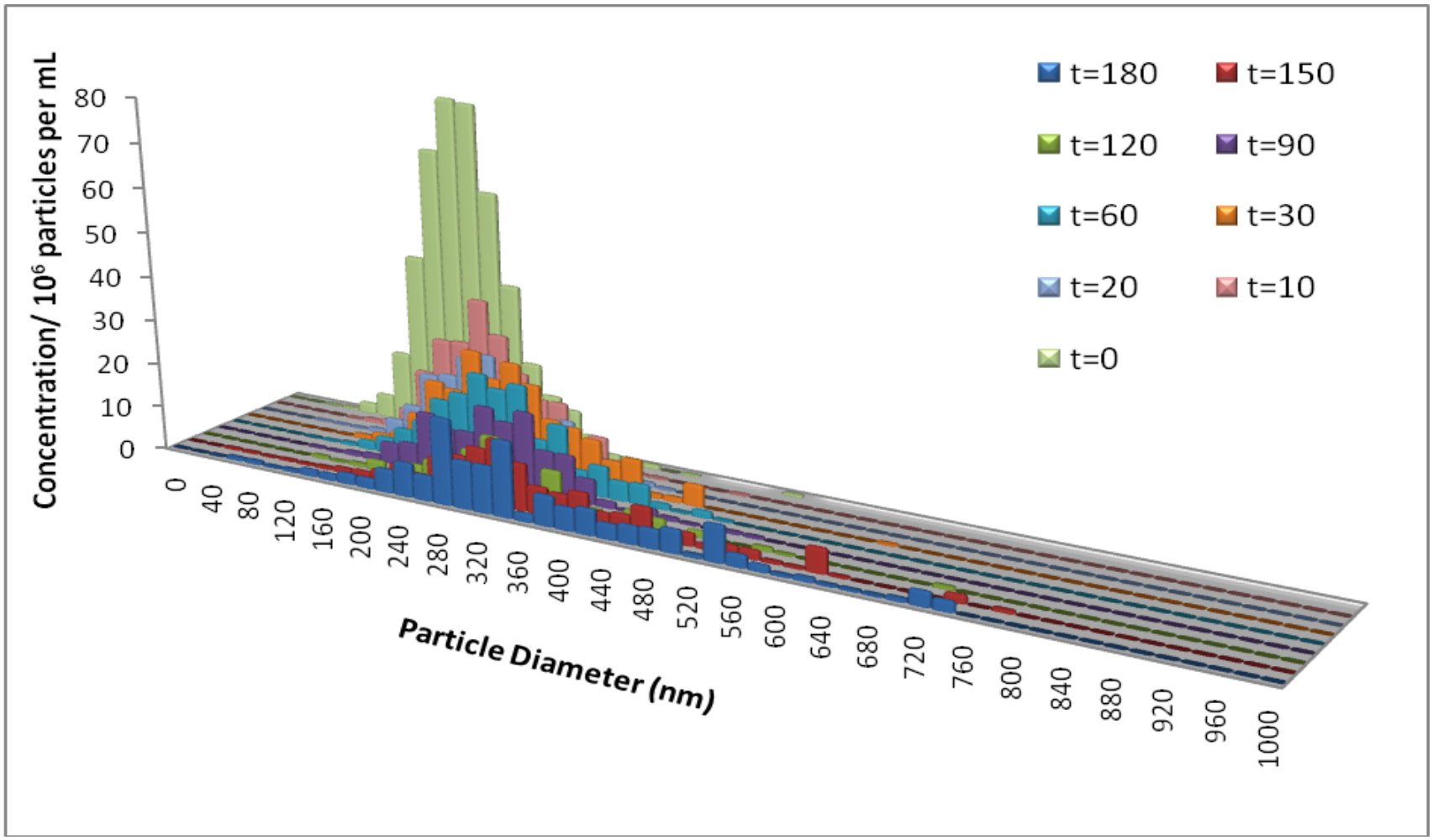
NanoSight Results – Conc. Calc.

- Amount of drug precipitating at each timepoint

Time (Min)	Volume Mean (Adjusted not smoothed)/ nm	Concentration/10^8 particles per mL	no of nanomoles of tolnafate present in the particles	% Of Drug Added
0	218	4.41	9.5	32
10	253	1.92	6.5	22
20	264	1.57	6.0	20
30	307	1.99	12.0	40
60	308	1.72	10.5	35
90	314	1.33	8.6	29
120	353	0.76	7.0	23
150	415	1.27	18.9	63
180	428	1.52	24.8	83

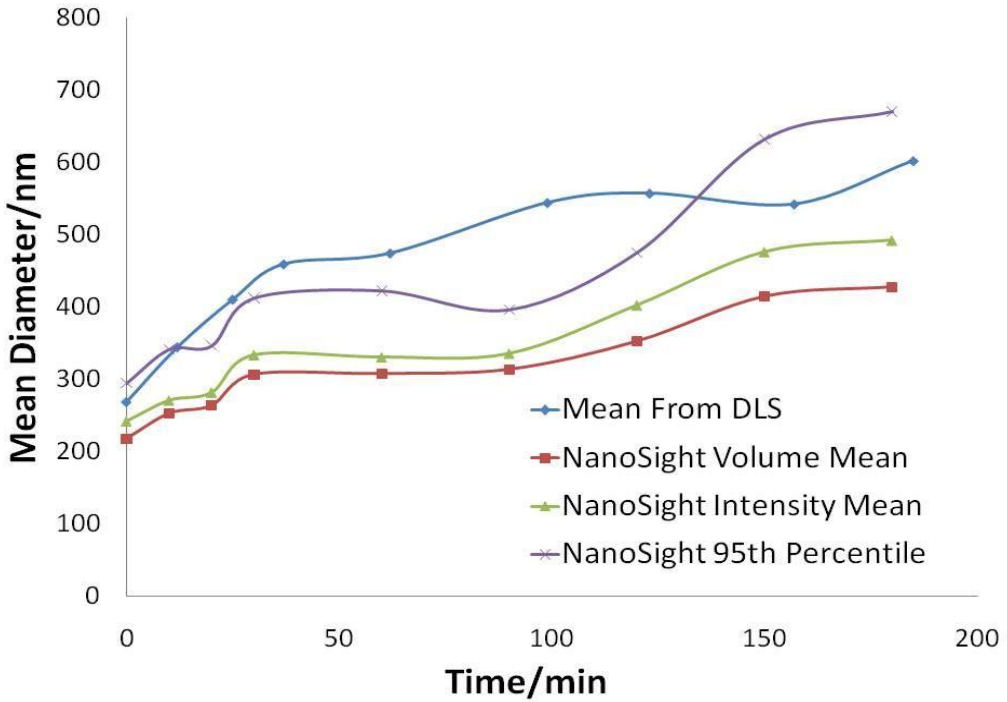
NanoSight Results - Distribution

- Changes in particle size distribution



DLS Results – Mean Size

- Similar trend seen with regards to increasing particle diameter with time



DLS Results – Conc./Distribution

- No estimate of conc. – can't perform calculation
- Could not detect changes in particle distributions
- No trend in changes in PDI over time
- Extensive comparison of techniques made by Filipe et al[6]

6. Filipe, V.; Hawe, A.; Jiskoot, W., Critical Evaluation of Nanoparticle Tracking Analysis (NTA) by NanoSight for the Measurement of Nanoparticles and Protein Aggregates. Pharmaceutical Research ***2010,*** *27 (5), 796-810.*

Conclusions

- Technique can be used to monitor precipitation of poorly soluble drugs
- Additional information obtained compared to that from DLS
- Precipitation at this concentration relevant to drug screening

Future Work - Tolnaftate

- Investigate form of particles i.e. crystalline/ amorphous
- Explore whether factors that affect HPLC solubility data for drug can be see to affect particle size/distributions
- From this link NanoSight data to HPLC solubility data

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