Breakout Session #2: *Complex Product Characterization/ Analysis:* **Sub-Session 2A:**

"Industry perspective on the gaps in Complex Gx Product characterization and future direction"

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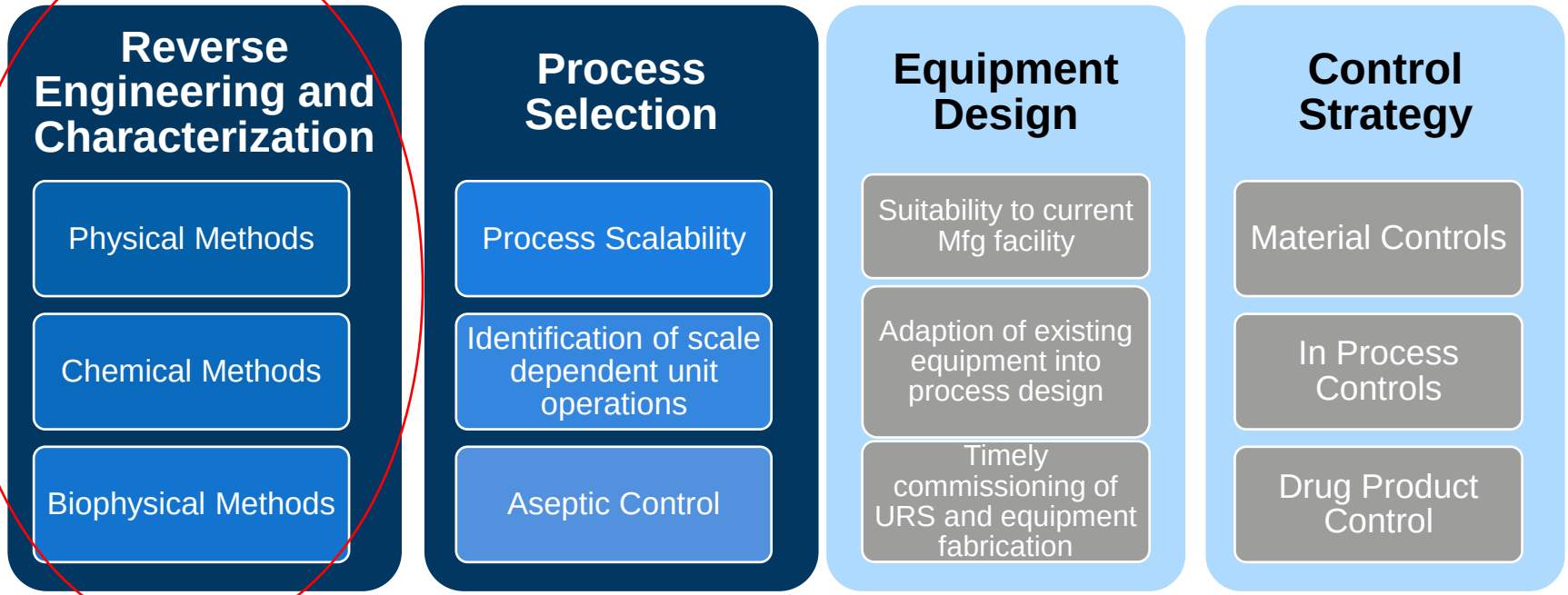
Agenda

- Complex Gx and Sources of Complexity
- Advances in Methodology for Characterization and Comparability
- Sample Preparation Considerations
- CRO Costs

Disclaimer

“The opinions expressed herein are solely those of the presenter(s) and do not represent statements or opinions of Sandoz Inc., or Novartis Pharmaceuticals.”

Critical factors in robust design and control of Complex Injectable Gx products



Maximize the benefit of FDA guidances (when available), controlled correspondences (where applicable), and Product Development and Pre-Submission Meetings to de-risk development

What are the sources of complexity and challenges?

Material Complexity – *API and Excipients*

- Difficult to characterize (*Synthetic Peptides, Oligonucleotides, Iron Colloids, Natural Products, Branched and linear polymers*)
- Stability challenges (*Lipids, peptides*)
- Difficult to Control (*Branched polymers, Mixture of polymers, Nanoparticle and Aseptic APIs*)

Formulation Complexity

- Dynamic and metastable nature of the formulation during/post processing (Emulsions, Liposomes, Microspheres, lipid nanoparticles)
- High sensitivity of Critical Process Parameters impact on Formulation CQAs (*particle size, material phase, drug encapsulation)*

Manufacturing Process and Supply Chain Complexity

- Specially designed and dedicated equipment train
- Scale up challenges (i.e. Microspheres and Liposomes) and Laborious process train set up
- Challenging aseptic processes involving multiple and discontinuous unit operations
- Long processing time
- Process operations extending beyond one facility (Drug-device combo products with manufacturing and device assembly at different sites)

Analytics Complexity

*API characterization -> Test Product -> RLDs

Physico-chemical
- pH, buffer/ionic strength
- Osmolality, Viscosity

Impurity profiling
- IP-RP-UV-MS
- RP-UV, AEX-UV, CE-UV
- LC-MS

Identity / Primary Structure
- HRMS sequencing
- Tm by Temp UV
- ICP-OES
- NMR (1H, 13C, 31P)
- Amino acid Analysis
- Edman Degradation

Higher order structure
- FTIR, Far/Near UV CD
- NMR (PCA)
- Intrinsic Fluorescence
- DSC
- Raman

Aggregates
- SEC-UV-MALS
- AF4-UV-MALS
- SV-AUC
- CG-MALS
- Fluorescence
- DLS

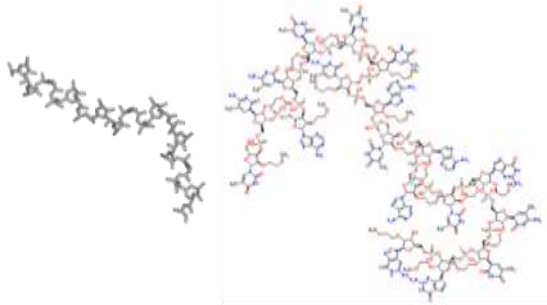
Particle Size / Shape
- DLS
- SAXS, SANS
- TEM, cryoTEM

Other
- Biological Activity
- xyz

Di-Sulfide Bridges
- Raman
- NMR
- LCMS

Comparability/characterization exercise as key activity to demonstrate Gx sameness to the RLD

- **Intense reverse engineering**
- **HA interaction** to align on acceptable in-vitro study design and including **Data driven discussions**
- **Strong scientific justification** for in-vitro study design proposal: in-depth understanding of API chemistry and interaction with formulation components and critical quality attributes affecting efficacy and safety
- **Demanding characterization methods and Orthogonal methods**



*Not exhaustive, primary and orthogonal techniques for peptides and oligonucleotides

What are the source of complexity and challenges (cont'd)

Analytical and Sourcing Complexity

- Access to multiple lots of RLD, and/or of different ages
- Lack of readily available in-house characterization tools or expertise to comply with guidance
- Complex, expensive and orthogonal Analytical Characterization tools
- Leveraging on external CRO capabilities and interpretation of data (qualitative vs. quantitative)
- Formulation Matrix Spectral Interference (Spectral Processing) and appropriateness of Sample Preparation
- Appropriateness of dissolution/release methods

Regulatory Complexity

- FDA product specific guidances (Availability and long lead times) and General guidances even when available may not provide sufficient clarity and *Replication of experiments-published literature*.
- Guidances may also evolve subject to new technical information or citizen petitions submitted by innovator companies

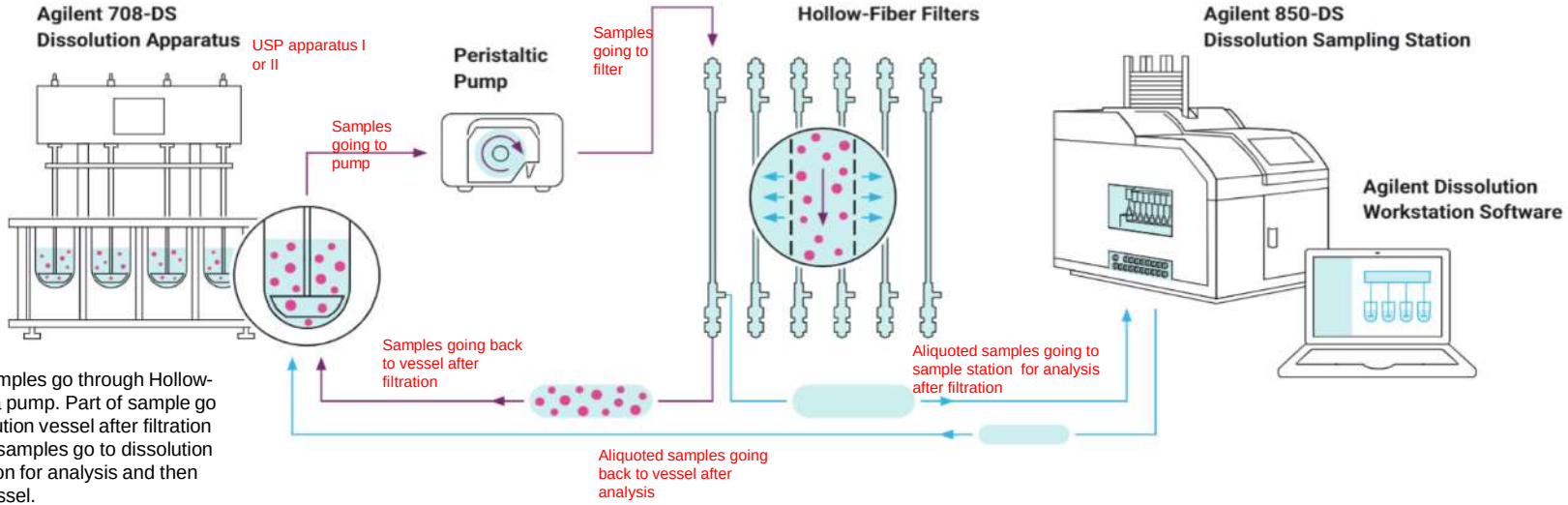
"Bio"– Complexity

- Difficult to establish Bioequivalence due **to high inter or intra-subject variability** (*Suspensions, Microspheres, Liposomes*)
- Long and expensive pharmacokinetic studies, or clinical endpoint studies in healthy volunteers / patients, for long-acting formulations (*Suspensions, Microspheres*)

Advances in Methodology for Characterization and Comparability

NanoDis System - Dissolution for Nanoparticle formulations

- **Agilent NanoDis System** is based on cross flow filtration (CFF) combined with conventional dissolution apparatus and with the aim to automate the filtration process. Nanoparticle formulations, e.g., Liposomes, Emulsions, Suspensions.
- Can address insufficient separation of nanoparticles from the dissolution medium during the sampling process.
- Advantages include CFF to separate the nanoparticles from the dissolution medium and can prevents the blockage of filters in a dead-end filtration.
- USP Apparatus I, II or IV (open or closed loop) dissolution



Dissolution samples go through Hollow-Fiber filters via pump. Part of sample go back to dissolution vessel after filtration and aliquoted samples go to dissolution sampling station for analysis and then back to the vessel.

Agilent White Paper – Agilent NanoDis system method development guide

Sedimentation Velocity-Analytical Ultracentrifugation (SV-AUC) in the development of nanoparticle drug products

Sedimentation velocity applications:

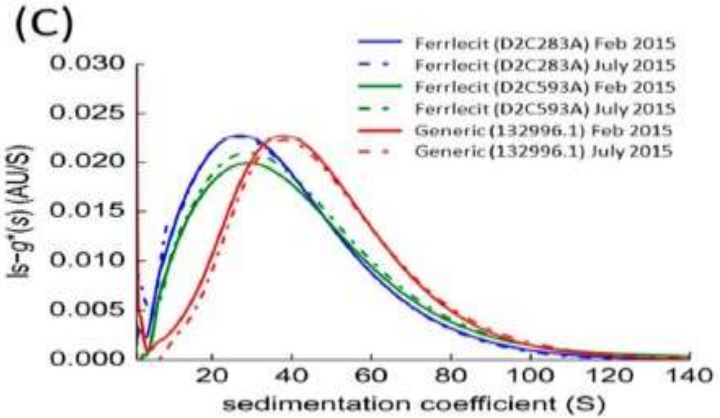
- Size distributions by SV-AUC analysis is a very sensitive analytical technique to characterize nanoparticles (i.e., iron colloids).
- Sedimentation coefficient is proportional to molecular weight and hydrodynamic radius (assuming that samples have a similar hydrodynamic shape).
- Allows for comparative characterization of iron colloids.

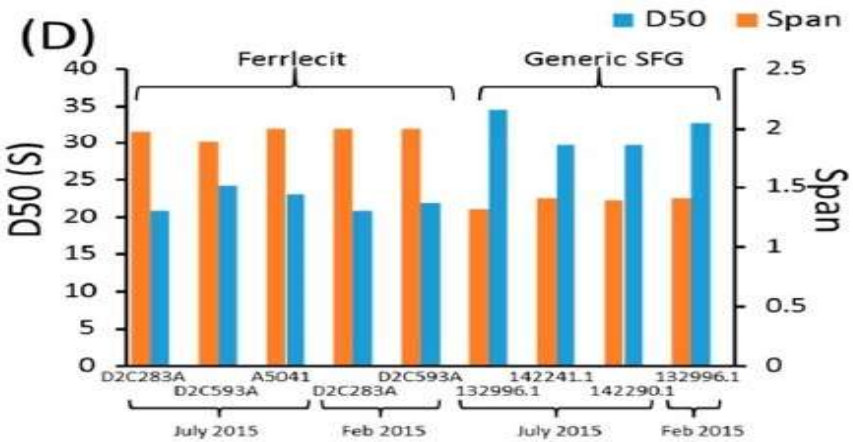
$$Span = \frac{D_{v0.9} - D_{v0.1}}{D_{v0.5}}$$

2. Type of study: Particle size distribution
Design: In vitro testing on at least three lots of both test and reference products

Parameters to measure: D_{10}, D_{50}, D_{90}

Bioequivalence based on: D50 and SPAN [i.e. $(D_{90}-D_{10})/D_{50}$] or polydispersity index using the population bioequivalence statistical approach.



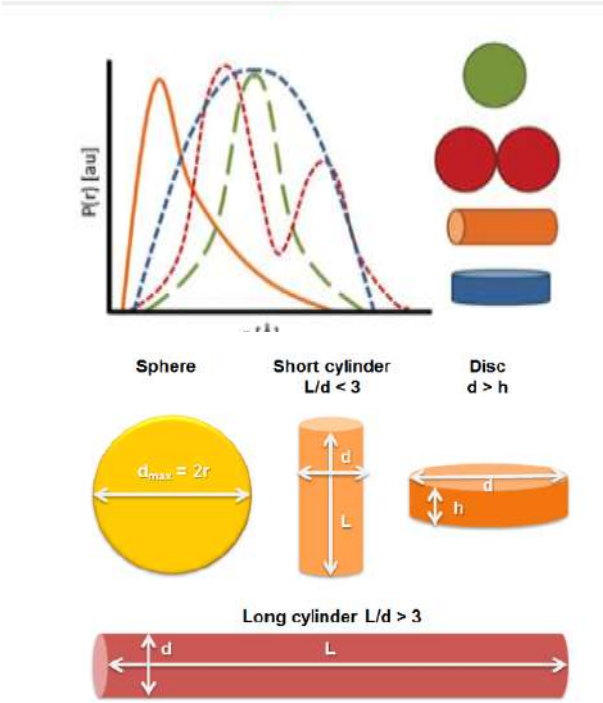


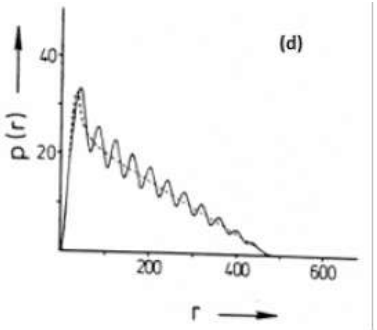
Nanomaterials ***2018***, *8(1), 25; https://doi.org/10.3390/nano8010025*

Small-Angle X-ray Scattering (SAXS) : Ferric Nanoparticles

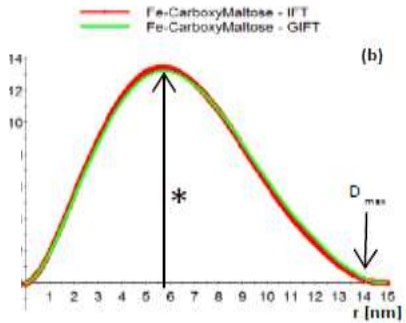
- *DLS measurements* captures size of the whole particle, including the oligosaccharides shell of the ferric nanoparticles, while *SAXS* determines the core shape and particle size.

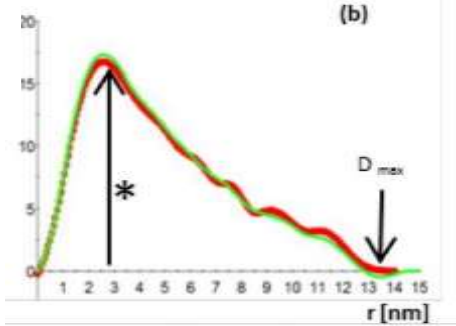
- These complementary techniques enable differentiation between the core and shell dimensions of the particle.



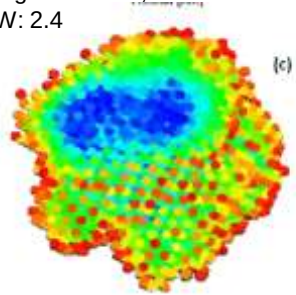


Pair-distance distribution functions

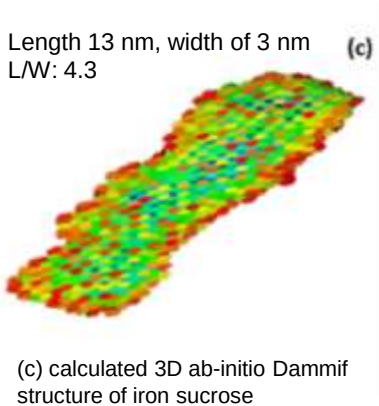




Length 14.5 nm, width of 6 nm
L/W: 2.4



Length 13 nm, width of 3 nm
L/W: 4.3

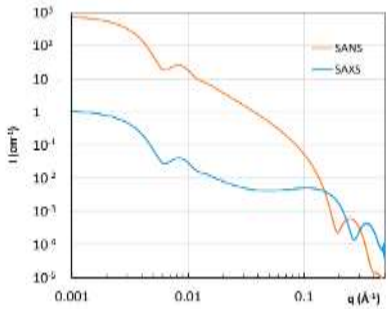


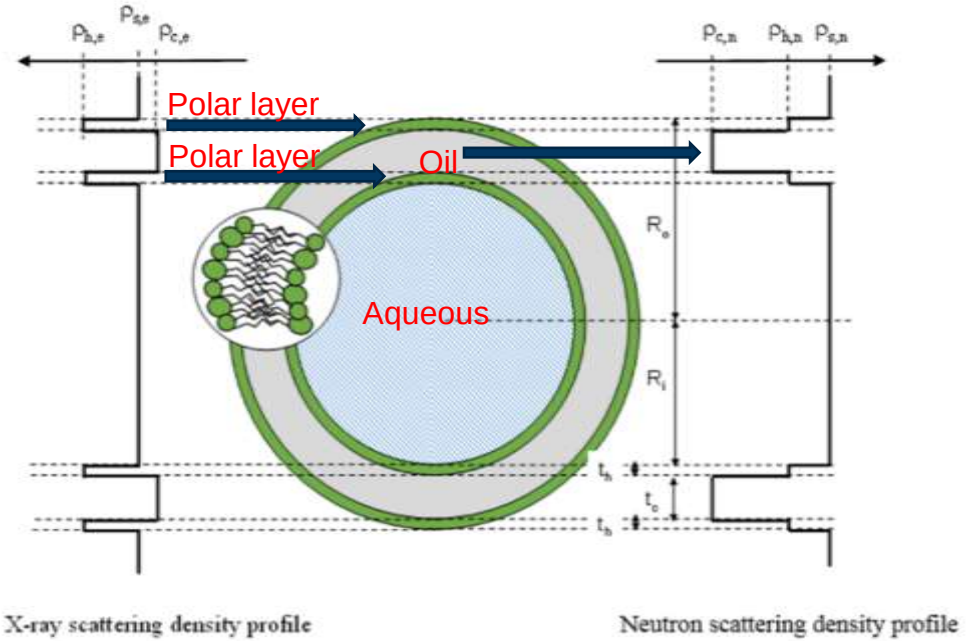
(c) calculated 3D ab-initio Dammif structure of iron sucrose

*application report D51IA051EN-B, Anton-Parr.com

SAX and SANS for the characterization of liposomes

- Small-angle X-ray (SAXS) and neutron (SANS) scattering techniques provide information at the nanoscale.
- Techniques with higher space and time resolution for in-depth structural characterization of liposomes.
- SAXS / SANS when combined, allow a fine structural description of the particles and allow to characterize internal aqueous environment of liposomes.
- SAXS more sensitive to the polar layer, whereas SANS provides information on the hydrophobic layer.
- Drug molecules, according to their water affinity, will preferentially be inserted into one liposome compartment, modifying its avg scattering length density and thus the scattered intensity.





Calculated small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS) intensity for a suspension of 1% of liposomes.

Emanuela Di Cola et al, Pharmaceutics 2016, 8, 10, doi: 10.3390/pharmaceutics8020010

Characterization of Liposomal formulation for size-based distribution of drug and excipients using AF4 and LC-MS

- AF4 promising technique to study size-based separation of liposomes and investigating drug/excipient distributions.
- Liposome size fractions collected from AF4 were analyzed for *particle size using NTA and DLS* and Lipids / Dox composition using LC-MS.
- Lipid compositions (Drug / lipid ratio) was close to be constant as a function of particle (Liposomal) size suggesting drug loading proportional to membrane surface area of any given liposome particle.
- Liposomal based drug development tool for comparison of generic versions against RLD.

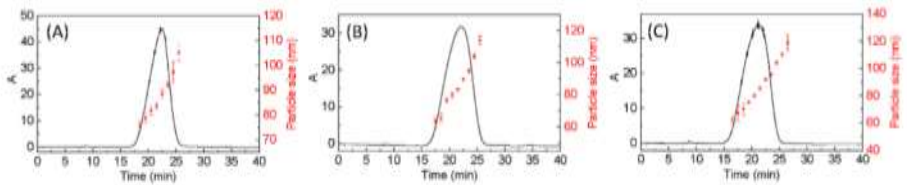


Fig. 1. Fractogram of doxorubicin liposomal formulations (A) DLF-1, (B) DLF-2, and (C) DLF-3 and average hydrodynamic diameter of each fractions as determined by NTA on the fractions.

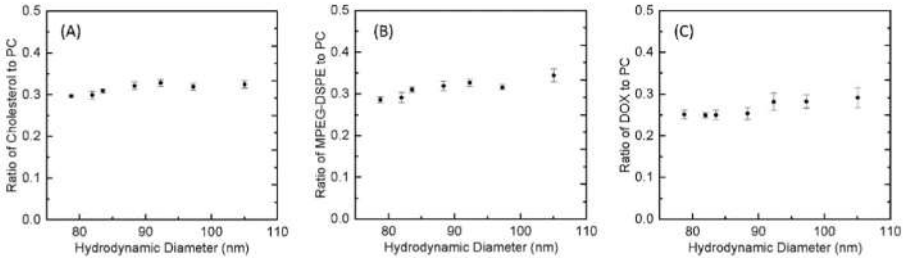
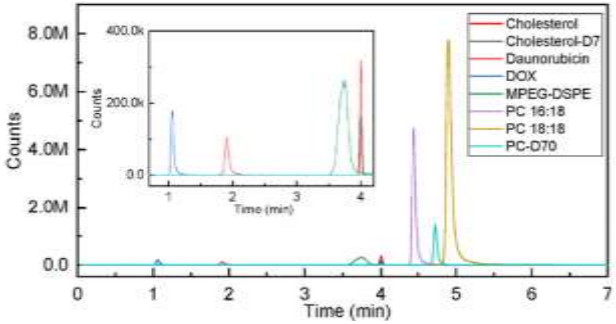


Fig. 3. The mass ratio of (A) cholesterol to total PC, (B) MPEG-DSPE to total PC, and (C) DOX to total PC as a function of the number averaged hydrodynamic diameter of DLF-1.

Siyam M. Ansar et al, International Journal of Pharmaceutics 574 (2020), 118906

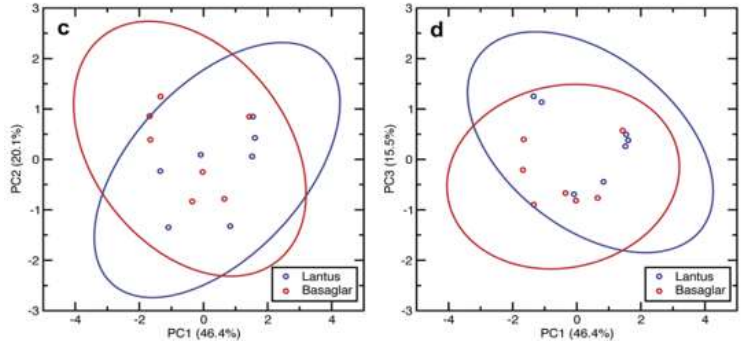


Lipids and DOX compositions in liposomal fractions from AF4 were determined using LC-MS

- Mass ratio of cholesterol / total PC, MPEG-DSPE / total PC and DOX / total PC are constant as a function of liposome size.
- Similar trend found for DLF-2 and DLF-3 that the ratios between the excipients held constant regardless of liposome size

Similarity evaluation of Higher order structure by NMR-PCA (principal component analysis)

- Peptide higher order structure (HOS) is a quality attribute that could affect therapeutic efficacy and safety.
- Establishing quantitative HOS similarity for Reference / Generic Peptides is valuable as part of characterization strategy. Techniques for Proteins can be leveraged upon.
- NMR-PCA derived unitless Mahalanobis distance (**DM)** values can be used as a robust and sensitive measure of HOS similarity.
- **DM** values can represent realistic and achievable similarity metrics for developing generic drugs, quality assurance, or control.

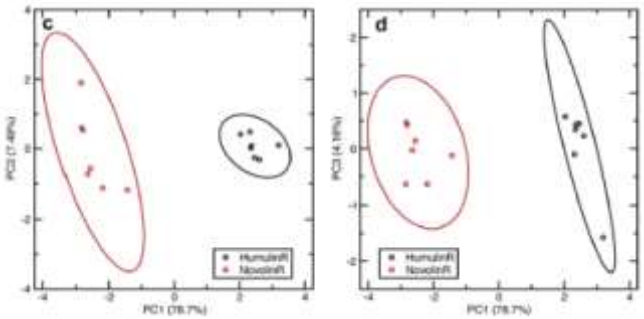


RLD Lantus® (blue)
Basaglar® (red)
The 90% confidence ellipses are drawn for each brand of insulin glargine.
D_M=1.58

Drug Product	Interbrand D_M
Lantus®	1.58
Basaglar®	
Humalog®	3.29
Admelog®	
HumulinR®	20.5
NovolinR®	

RLD Humalog® (blue) Admelog® (red)

D_M=3.29



RLD HumulinR® (black) NovolinR® (red).

D_M= 20.5

* Journal of Pharmaceutical Sciences, Volume 109, Issue 4, April 2020, Pages 1519-1528
https://www.sciencedirect.com/science/journal/00223549

Sample preparation considerations

Sample preparation procedures are product specific. Below are some examples.

Instrument/Test	Comment
Particle size DS	Type of cuvettes evaluated for suitability
Aggregation studies SEC-UV-MALS, SV-AUC, AF4	• At line dilution with formulation buffer (dilution series). • Sample dilution may disassociate non-covalent aggregates that may be present. • Beware: On-line Dilution (as result of instrument analysis).
Higher order structure studies :CD, FTIR, NMR	• Excipients may interfere w/ the measurement; • Dialysis/TFF to remove interfering components. • Buffer exchange generally involves dilution with the desired formulation buffer first, then followed by gentle overnight dialysis into the same buffer using a certain membrane. (Membrane type, size, etc.)
AFM	Dilution and dialysis with formulation buffer in order to observe and be able to measure xyz, ie the iron core
Cryo-TEM	Dilution with formulation buffer in order to observe and be able to measure xyz, ie the iron core
Xray	Dilution and dialysis with formulation buffer followed by lyophilization in order to observe and be able to measure xyz, ie the iron core.

CRO Testing Costs

Instrument/Test	*CRO cost $ per sample (USD) , For comparison purposes only
AF4	$1000 – $3000 (triplicate analysis)
SAXS	$500 – $700 (includes report)
AUC	1st sample $2500 and subsequent $800
NMR / PCA	NMR (1D, 2D, PCA) 1H NMR $1000 – 1500 for 1st sample in triplicate. PCA analysis is costed based on an hourly basis. (apx $100–$200 per hour)
Mossbauer **EPR**	$200 /run $400 – $500 / run
AFM	$200 – $225 /run
Cryo-TEM	$3000 /run

*May not be inclusive of method development costs for optimization of method and sample preparation development, and special post acquisition data processing development.

Advancing Complex Gx

Significant number of off-patent complex product drugs for which there are few or no generic versions and larger number of newer complex drugs where no PSGs exist.

Bringing new complex generic drugs to market requires access to:

- *Appropriate physicochemical characterization methods,*
- *Access and availability to instruments (either in-house or qualified CROs, Universities),*
- *Experience (Execution, Interpretation and Comparison to orthogonal techniques),*
- *RLD availability to enable rapid de-formulation,*
- *RLD to demonstrate adequate comparability to the innovator and assess whether Q3 equivalence (ie, topical drug products and orally inhaled and nasal drug products (OINDPs)) has been achieved as part of in vitro bioequivalence testing.*

US FDA actively encouraging drug companies to take on the challenge of developing and launching complex generics by releasing PSG describing in vitro BE approaches which may be used in lieu of clinical studies, and requesting of Product development / Pre-Submission meetings

- Active FDA research and publication of research articles
- Center for Complex Generics research program

Opportunities:

- Product Dev. Meetings: Easier Process to submit and request meeting, Questions only in initial request?
- PSG: Reduction in lead time, issuance of PSG after *x* time from complex product NDA approval.
- Mid-Cycle Review meetings: Dialogue enhancement to address outstanding scientific questions

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Thank you