A correlation of the physical attributes of a potent anti-onychomycotic dosage form with microbiological performance.

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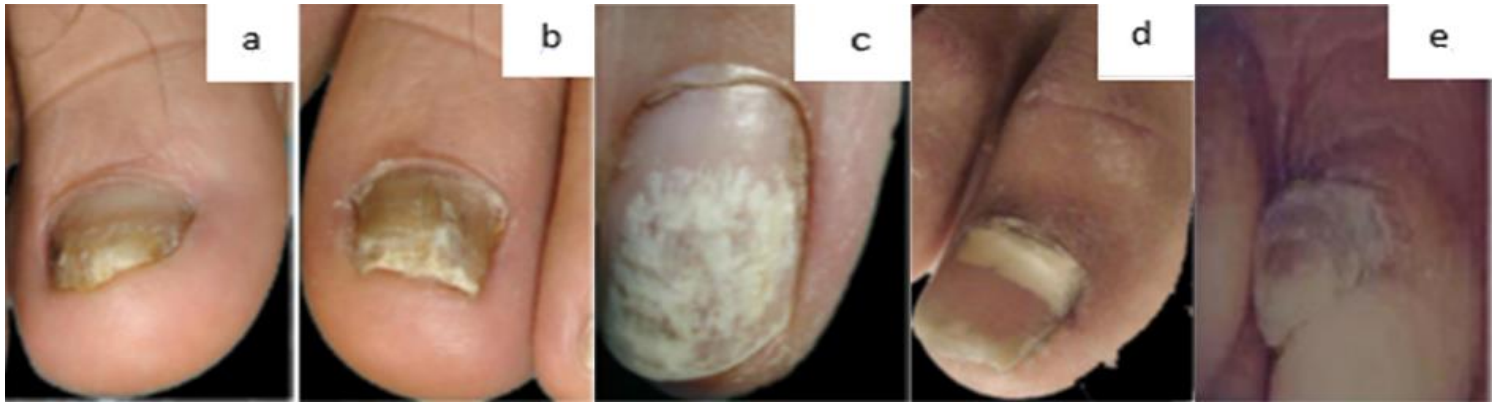
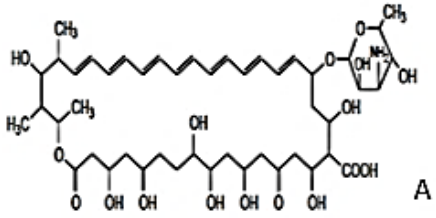
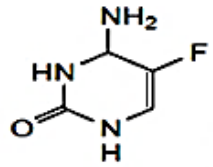
Introduction

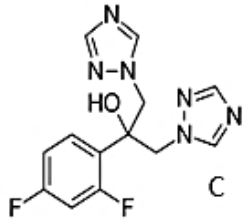
- Onychomycosis is a clinical fungal nail infection caused by *Trichophyton rubrum*.
- A prominent concurrent disease among diabetic and HIV populations.
- Prognosis may result in severe pain, sometimes disabling the infected region.

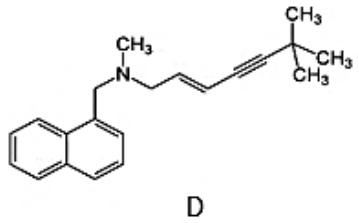


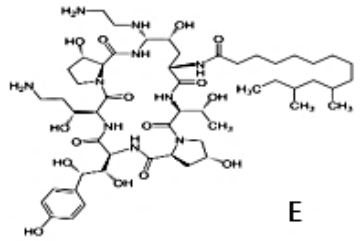
- Figure 1| **Appearance of toenails during onychomycosis.** (a) **distal subungual** – terminal part of the nail is infected (b) **lateral subungual** – paronychial margins are effected (c) **superficial white** – upper surface of the nail plate is affected with patchy appearance (d) **proximal** – lunular region of the nail is infected (e) **total dystrophic onychomycosis** – nail plate is completely eroded from the nail bed (Ameen *et al.* 2014).











Chemical structure of some classical antifungal drugs (A) amphotericin B (polyene) (B) 5 – fluorouracil (pyrimidine analogue) (C) fluconazole (azole antifungal) (D) terbinafine (allylamine) and (E) Capsofungin (echinocandin).

Current treatment strategies

- systemic → Classical antifungals
- topical
  - prescription → Classical antifungals
  - OTC → K101 formulations

Undesired effects:

- Adverse drug reactions
- Lipid depositions
- Antifungal resistance

- Slow mechanism of action

- In the process of overcoming these challenges such as adverse drug reactions and antimicrobial resistance, a team of researchers at Athlone Institute of Technology have invented a novel molecular entity (NME) with potent antimicrobial activity.
- Development of that NME into potent, non-invasive and discriminating dosage form towards an effective treatment of onychomycosis has been carried out in this research.
- Conventional method of development includes characterizing the active ingredient and its corresponding excipients according to the defined characteristics as per reference listed drug (RLD) and drug products.
- But Quality-by-design offers risk assessment based strategy with built in quality constructed on the patient orientation of the targeted treatment.
- Hence, these principles have been employed in this research to develop the dosage form with NME

Why QbD?

The final target of QbD is to incorporate the quality into the product by design but not by testing (ICH Q8(R2)).

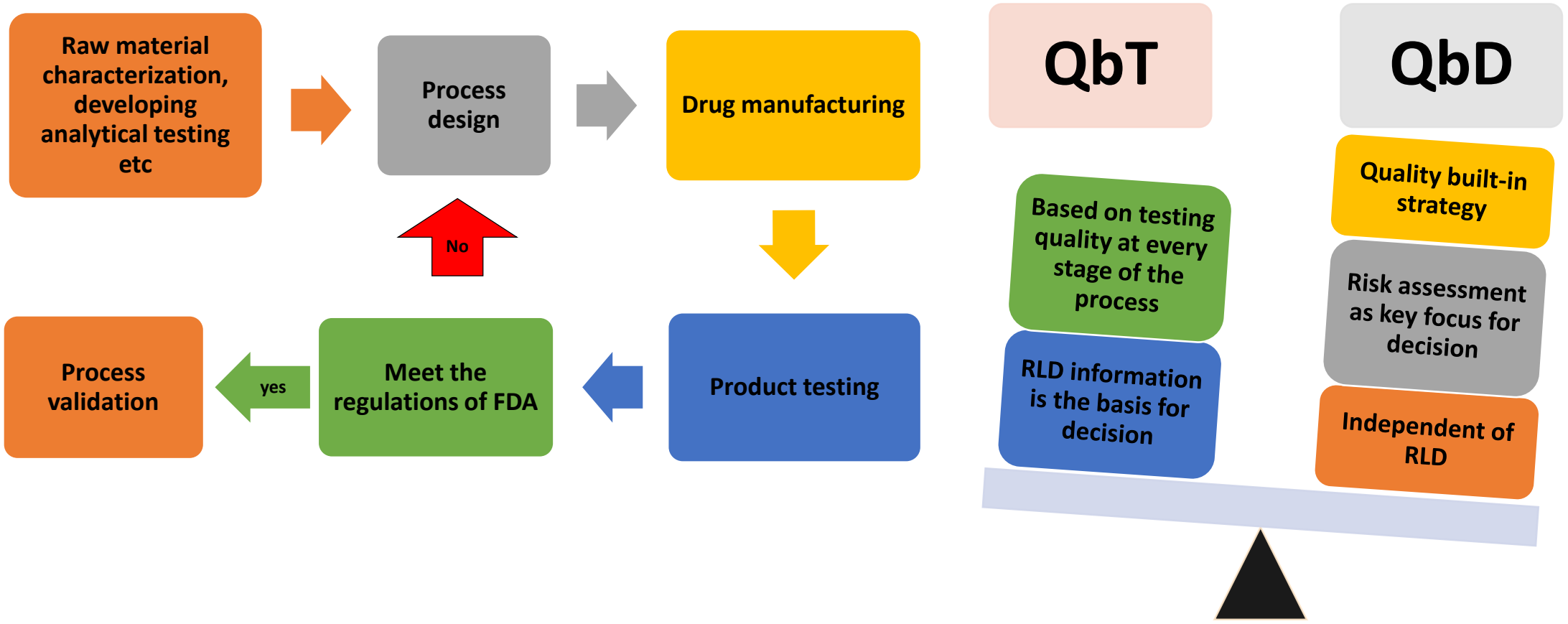
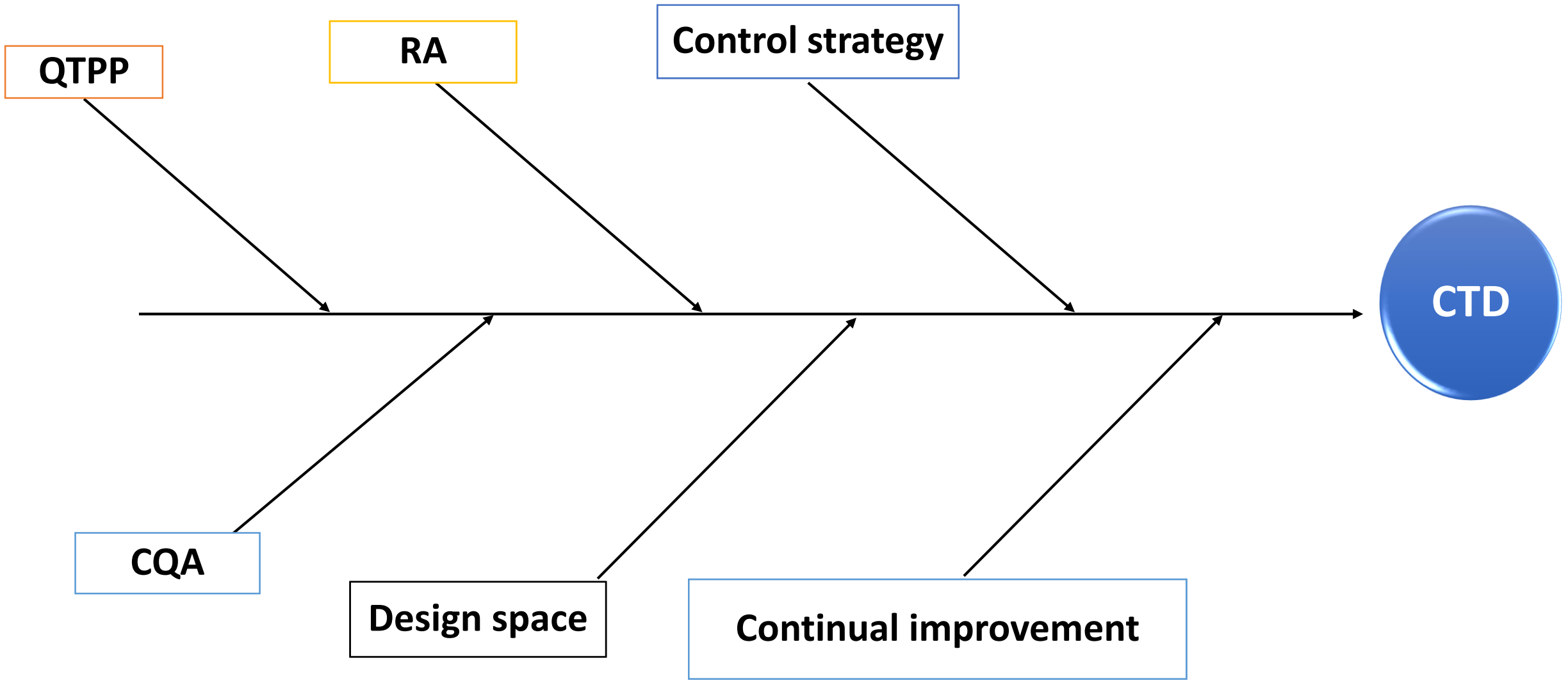


Figure 2| **Scheme of QbT approach** (adapted from: Zhang and Mao, 2016).

How QbD works?

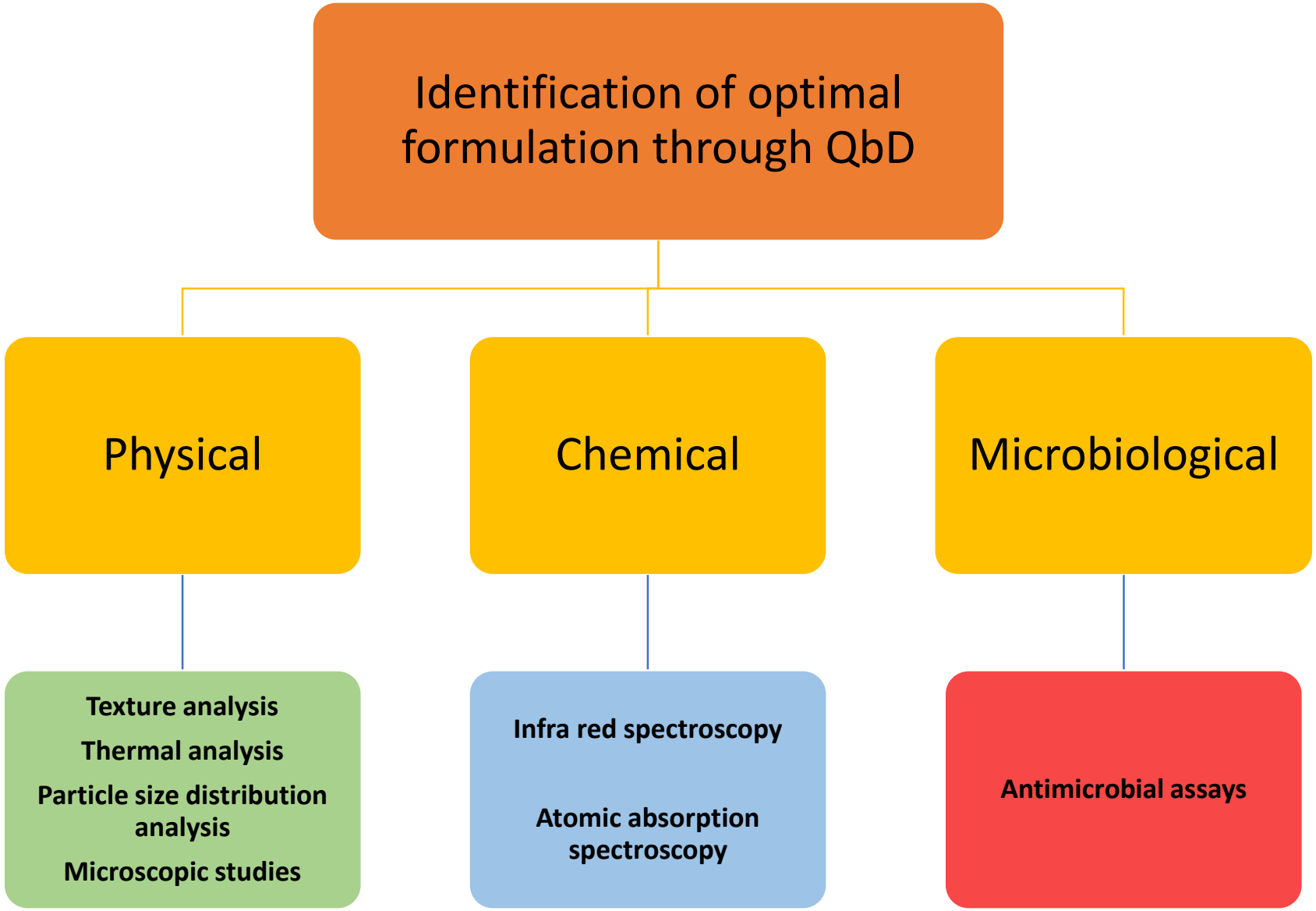


Quality targeted product profile towards an effective treatment

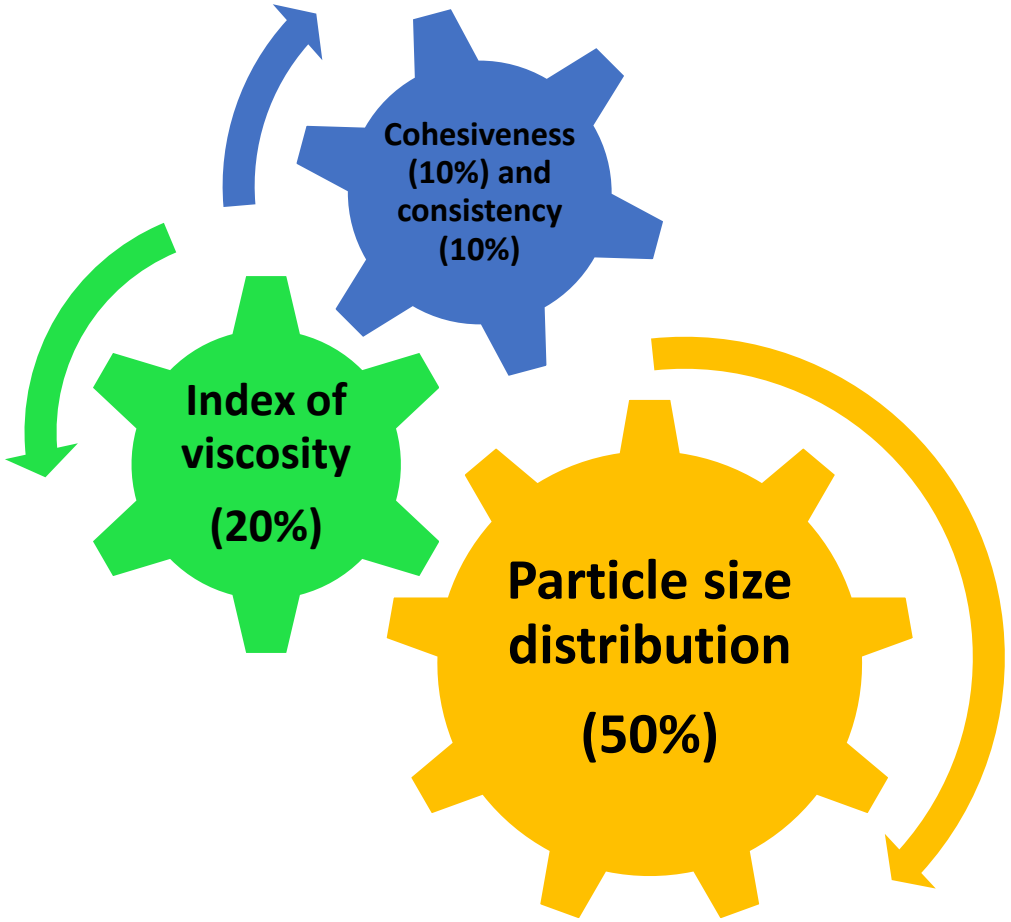
Elements	Target	Justification
Dosage form	**Ointment**	**To provide prolonged and direct effect on the infected area**
Route of administration	**Topical**	**Hypo and paronychia regions of nail plates of fingers**
Dosage strength	**Clinically effective**	**To have an effective concentration of MCO**
Dosage design	**Oil-in-water emulsion dispersed in aqueous base**	**To enhance the solubility of drug for the essential action**
Appearance	**Colourless or cloudy**	**Elegance**
Identification	**For the API (MCO)**	**To meet the standards**
Impurities	**Within the limits**	**For safety**
Physical attributes	**Consistency, viscosity, cohesiveness etc.**	**To facilitate extrusion from the container and application**
Microbial limits	**Within the limits as per USP <61>**	**To avoid interference with API and enhance stability**

Quality targeted product profile towards an effective treatment cont'd

Elements	Target	Justification
Residual solvents	**Within the limits as per USP <467>**	**For safety**
Container closure system	**Appropriate materials**	**Proper support for the storage and extrusion**
Package integrity	**No failure**	**Needed for stability and clinical efficacy**
Stability	**Not less than 24 months**	**Match the shelf-life of reference listed drugs**
In vitro release test	**Using Franz apparatus**	**For appropriate release over the target site of application**
Assay	**To quantify the amount of API in a unit dosage form**	**To provide information for the quality reviewer**
Homogeneity	**Uniform release of API**	**To have consistent activity and uniformity**


Identification of optimal formulation through QbD
Physical
Chemical
Microbiological
Texture analysis
Thermal analysis
Particle size distribution analysis
Microscopic studies
Infra red spectroscopy
Atomic absorption spectroscopy
Antimicrobial assays

Contribution of risk by the physical parameters



Particle size distribution determines the extent of sedimentation and phase separations as the QTPP targeted includes oil-in-water emulsion

Viscosity is the integral property which effects the emulsion extrubability and spreadability over the targeted site of application

Cohesiveness and consistency are combined results of ingredients and process performances

Strategy employed to optimise the formulation

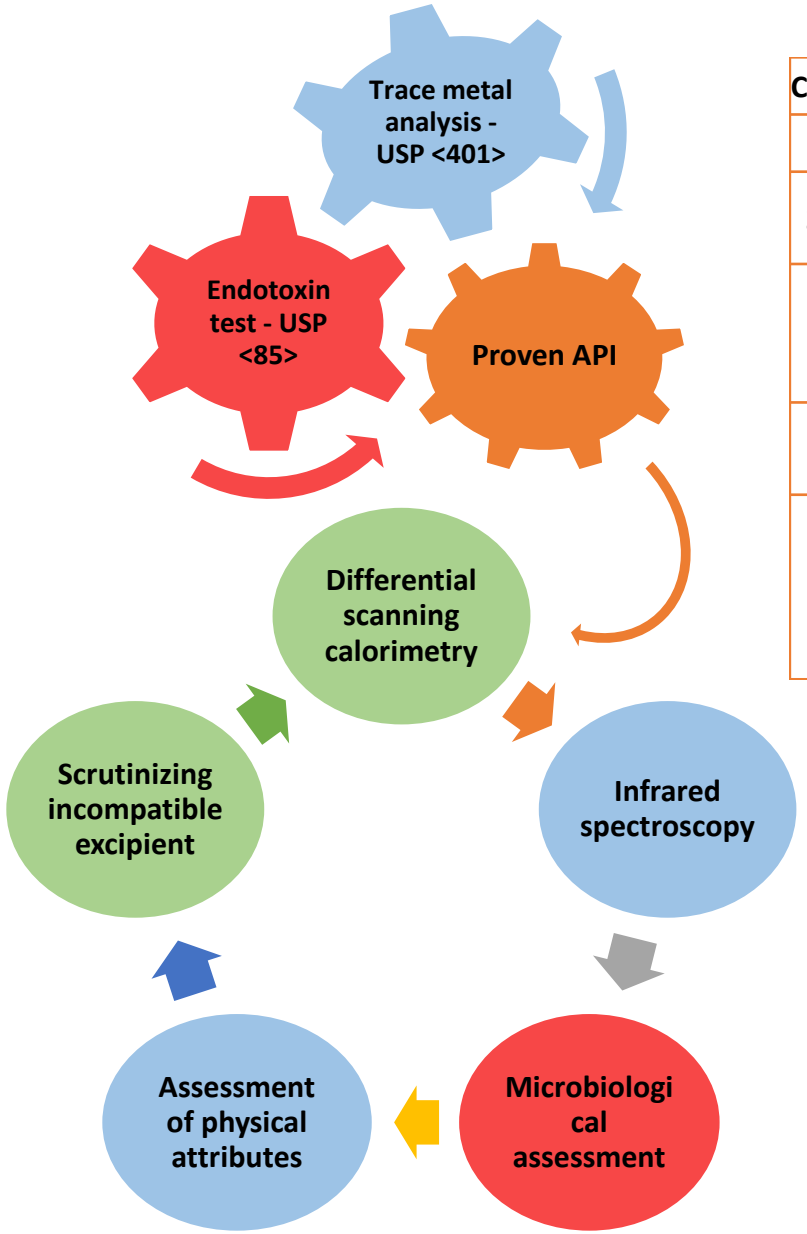


Table 1| **Elemental Impurity Classification (ICH Q3D)**

Class	*Included Elemental Impurities*	*Include in Risk Assessment ?*
1	Arsenic, lead, cadmium, mercury	Yes
2A	Vanadium, molybdenum, selenium, and cobalt	Yes
2B	Silver, gold, thallium, palladium, platinum, iridium, osmium, rhodium, and ruthenium	Yes only if intentionally added
3	Antimony, barium, lithium, chromium, copper, tin and nickel	Dependent upon route of administration
4	Boron, iron, zinc, potassium, calcium, sodium, manganese, magnesium, tungston and aluminium	No

New formulations designed

Excipients chosen were:

- External phase
- Emulsifiers
- Viscosity modifying agents

Scrutinising the formulations

Physical characterisation

- Texture analysis
- Particle size distribution
- Conductivity
- Turbidimetry

Chemical compatibility assessment

- IR spectroscopy

Antimicrobial studies in the presence of keratin

Bulk production of the optimised formulation

- Standard manufacturing method as has been established from the attributes determined during the characterisation.
- Optimised method has been documented as per the cGMP regulations.

Degradation studies under induced stress conditions and quality control

- Three batches with 15 samples each have been produced for the stability examination.
- Stress conditions: 75 ± 5% RH at 25 degrees centigrade for 90 days.
- Samples were collected from a fresh container and already opened container from previous time point after 0, 15, 30, 60 and 90 days.

Assessment

All samples were tested asper the physical, chemical and antimicrobial studies performed in the characterisation stage.

Data determined from the above analysis was evaluated by constructing Six Sigma based QC charts

Results from physical assessment

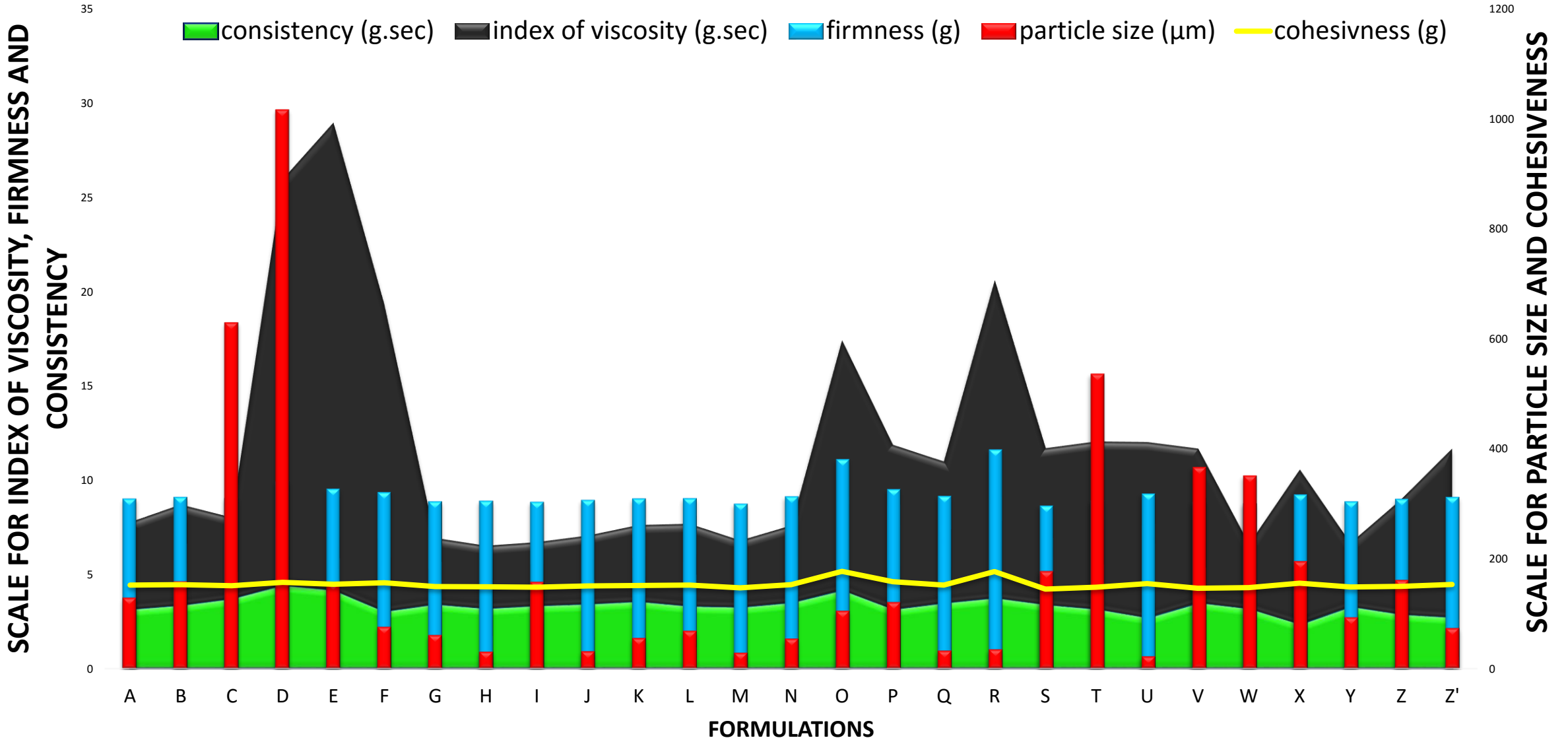


Figure 2| **Graphical representation of cumulative data from the physical characterisation of formulations A to Z'**

Results from physical assessment contd’

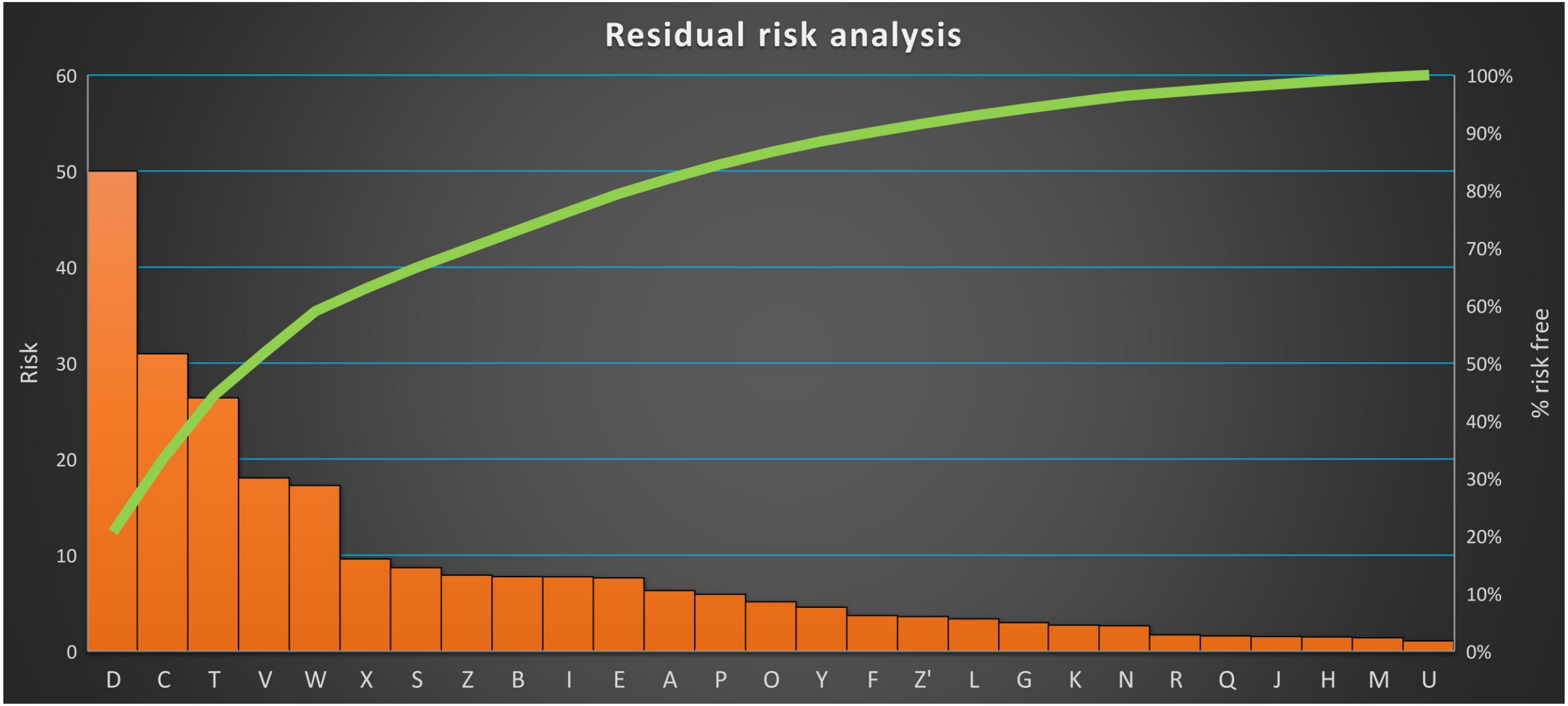


Figure 3| **Graphical representation of risk analysis of cumulative data from the physical characterisation of formulations A to Z’**

Results from physical assessment contd’

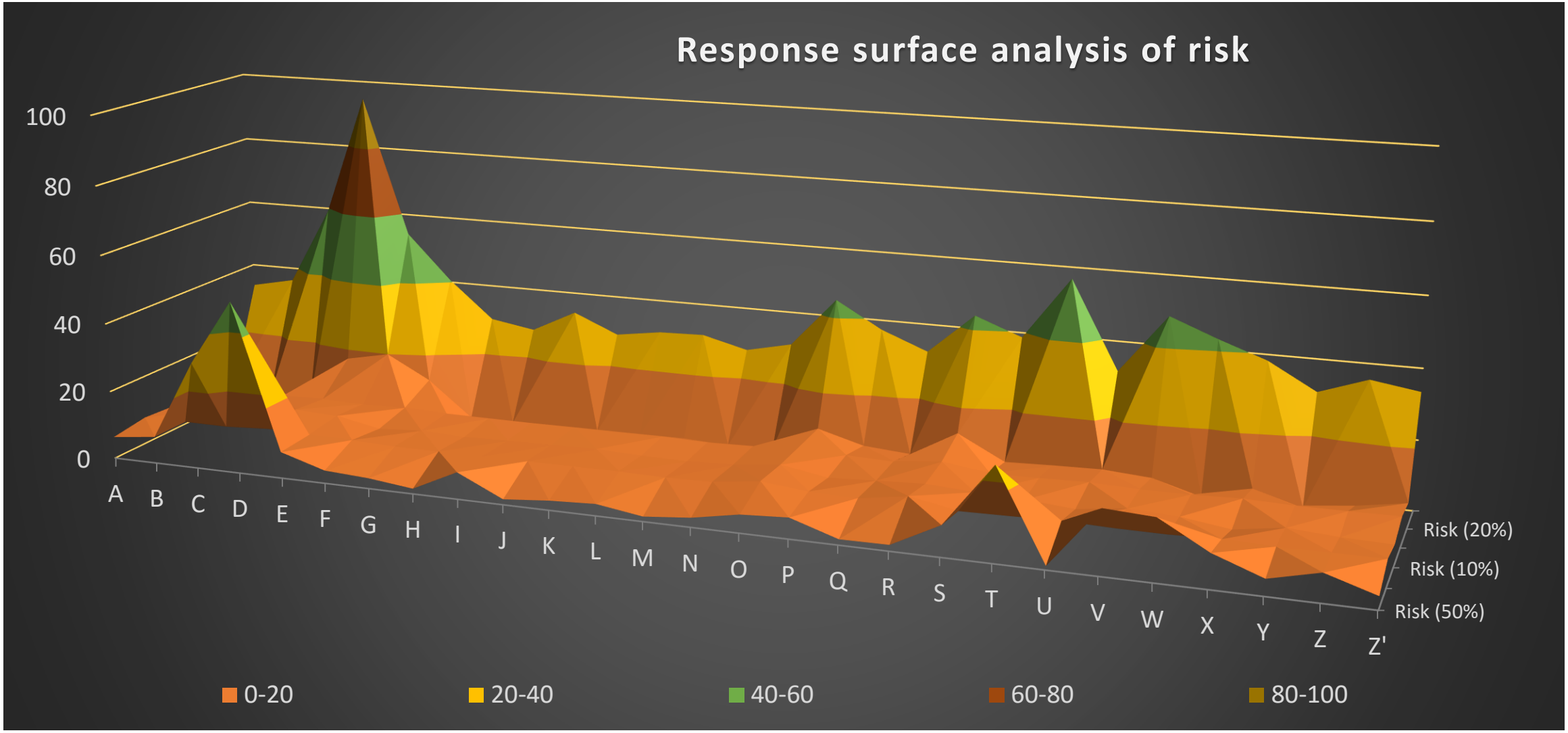
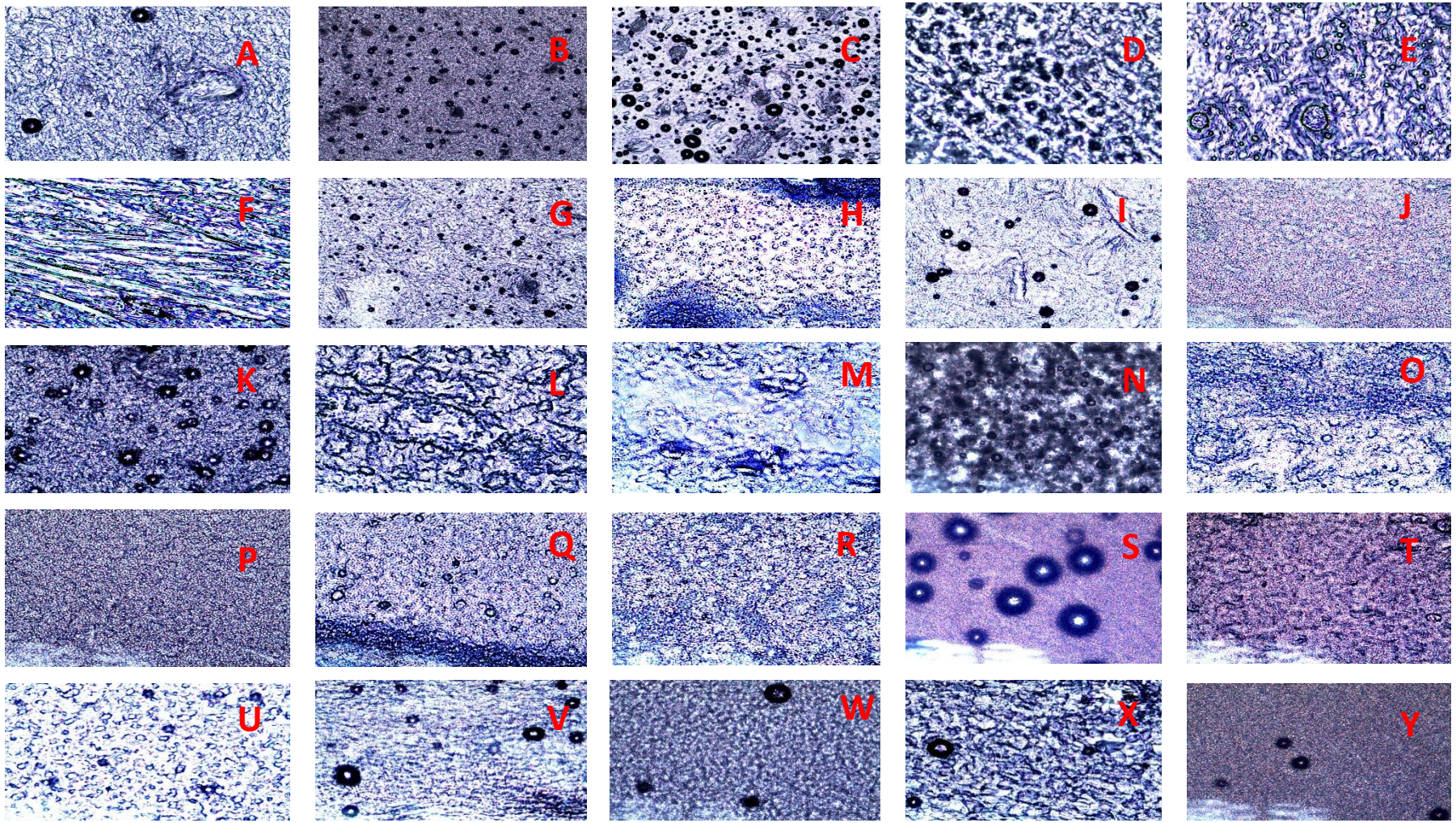


Figure 4| **Graphical representation of response surface analysis of cumulative data from the physical characterisation of formulations A to Z’**

Results from physical assessment contd’



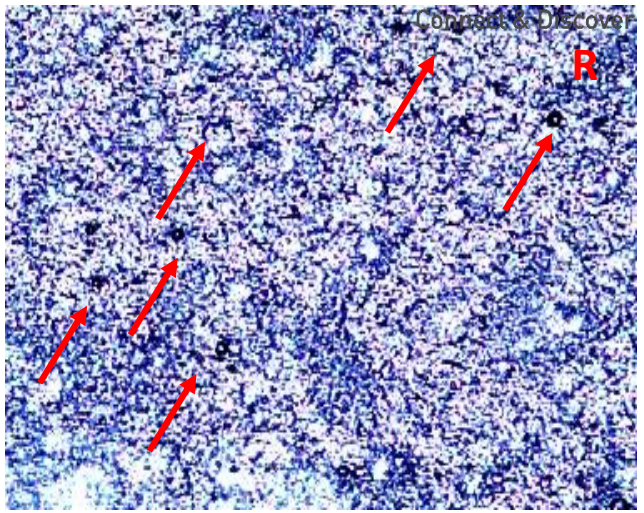
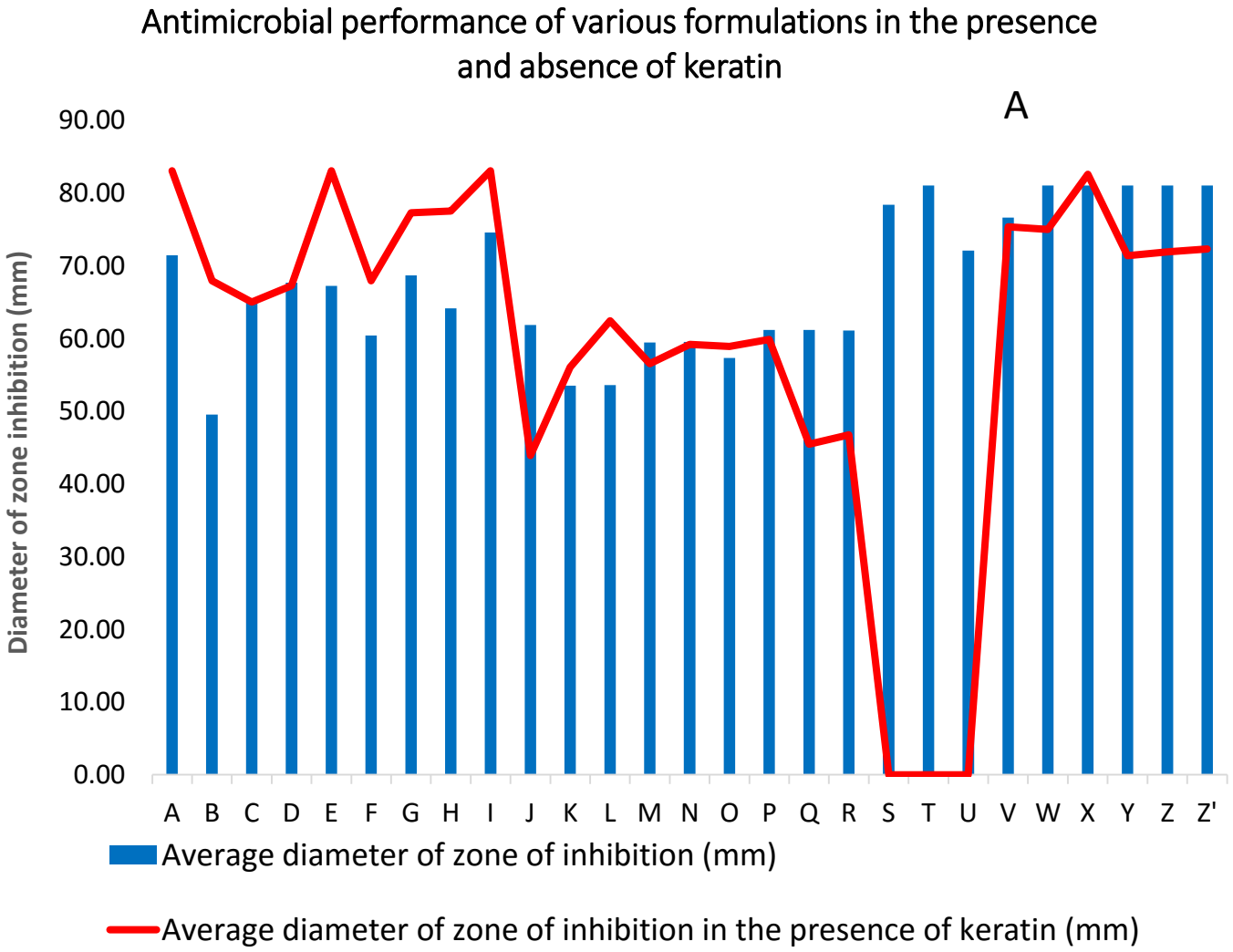


Figure 5| **(A to Z’): Light microscopic images of formulations at 100x magnification.**

Results from biosassay



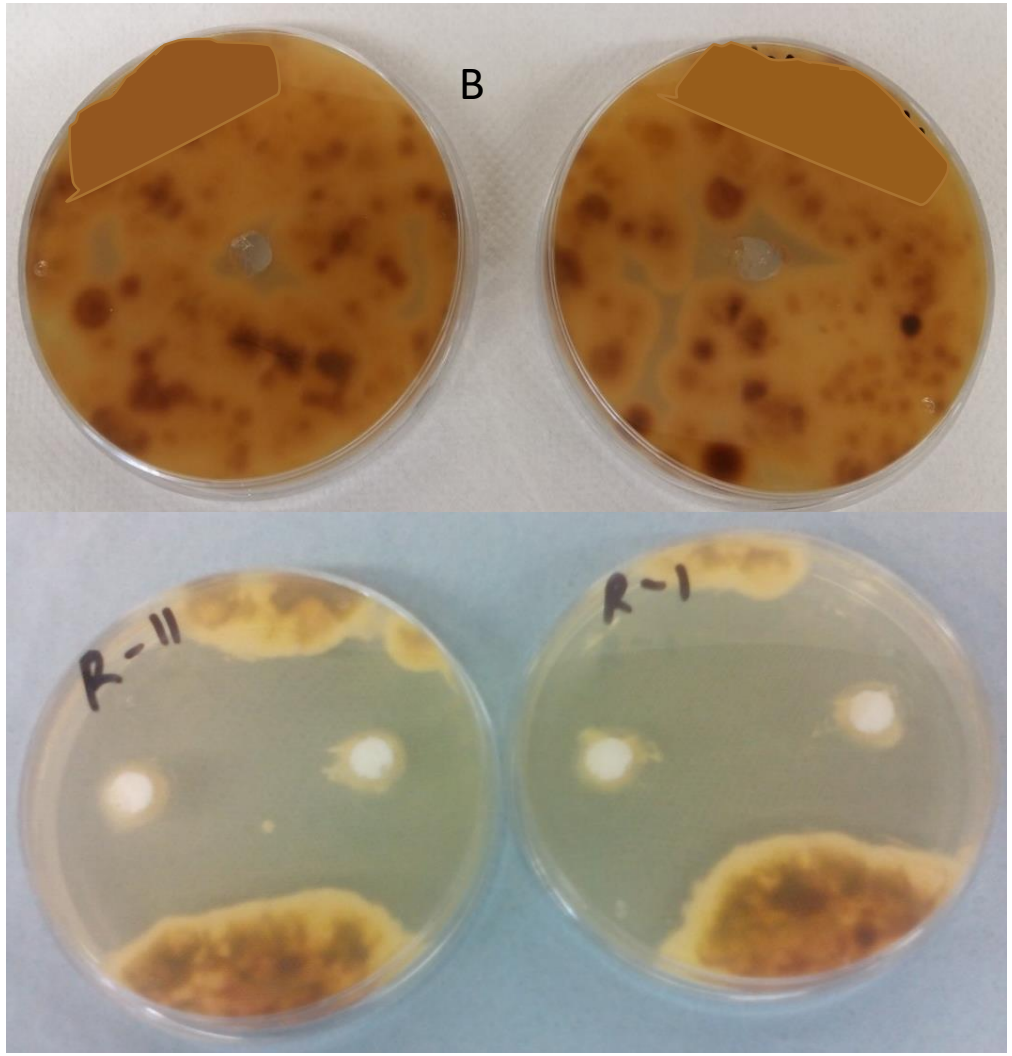


Figure 6| (A): **Antimicrobial performance determined of designed formulations in the presence of keratin on Sabouraud Dextrose agar. (B top) Antimicrobial activity of commercial positive control, (B bottom) Antimicrobial performance of formulation R.**

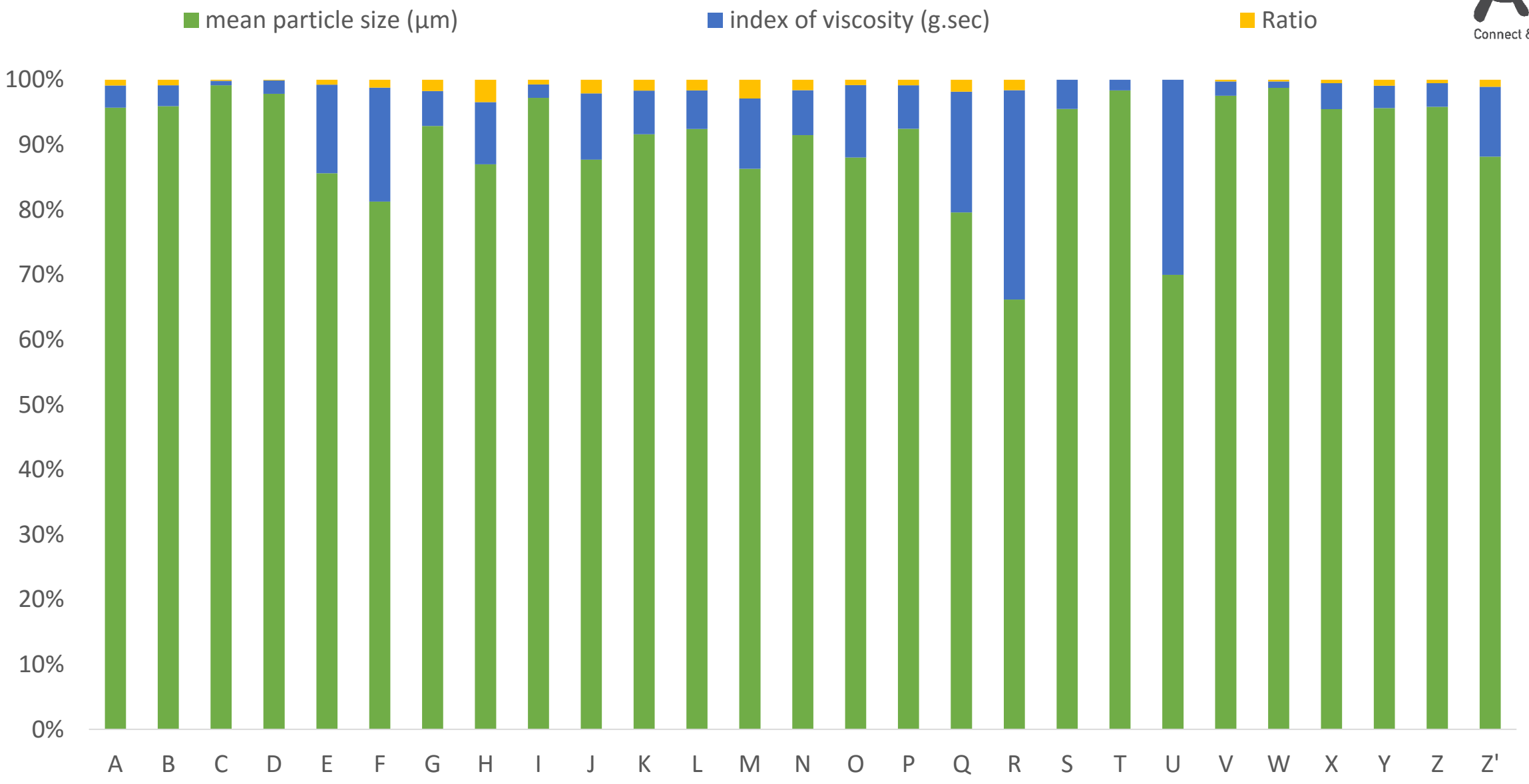
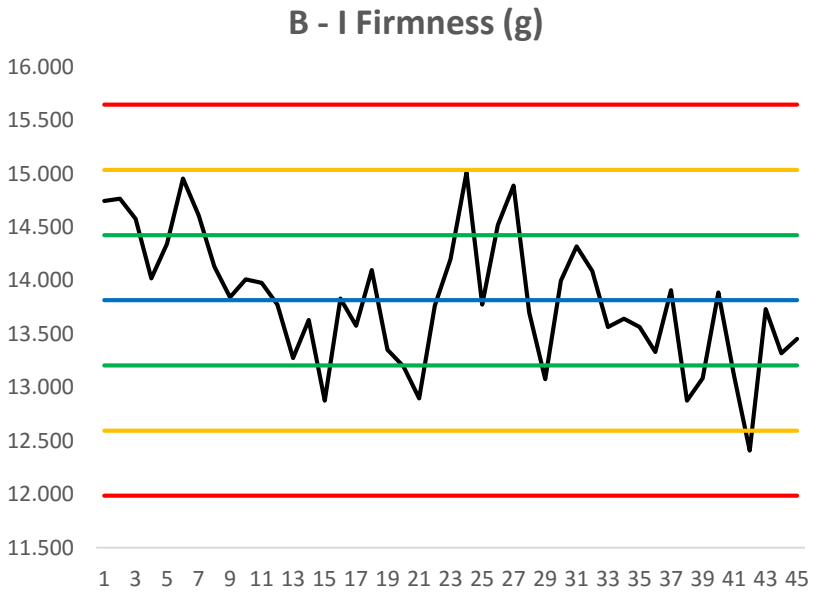
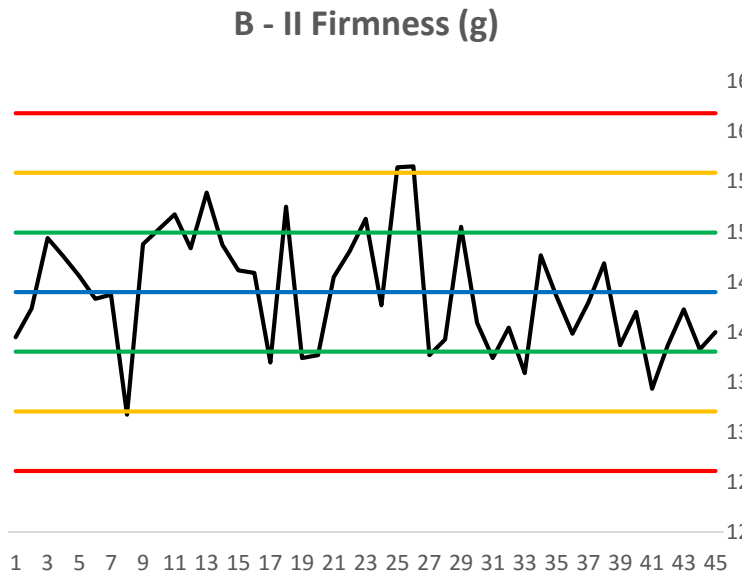
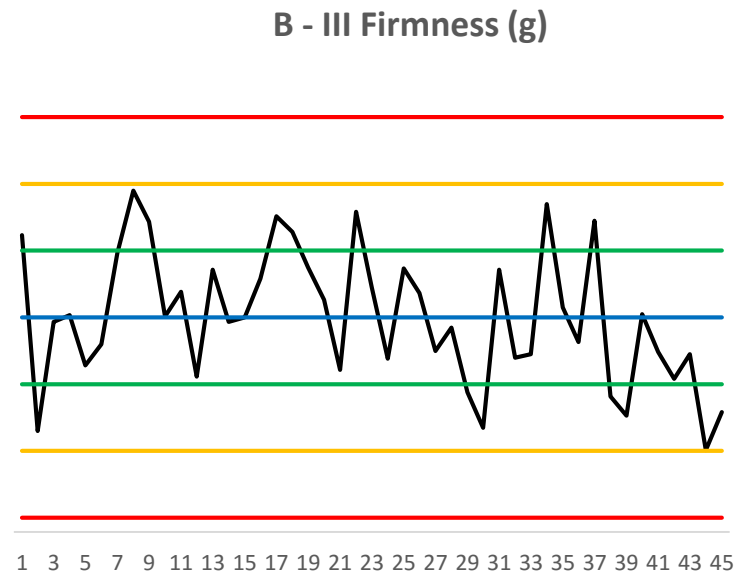


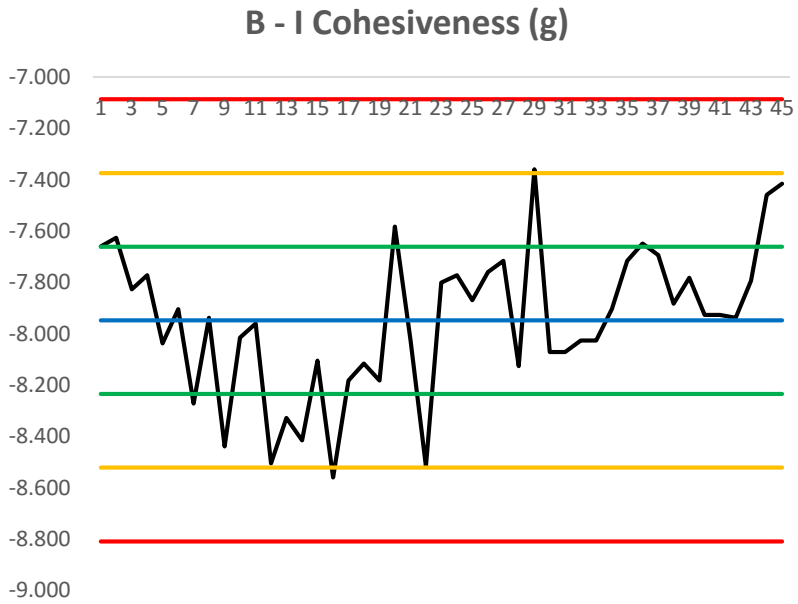
Figure 7| **Stacked values of physical and microbiological performances of the formulations developed.**

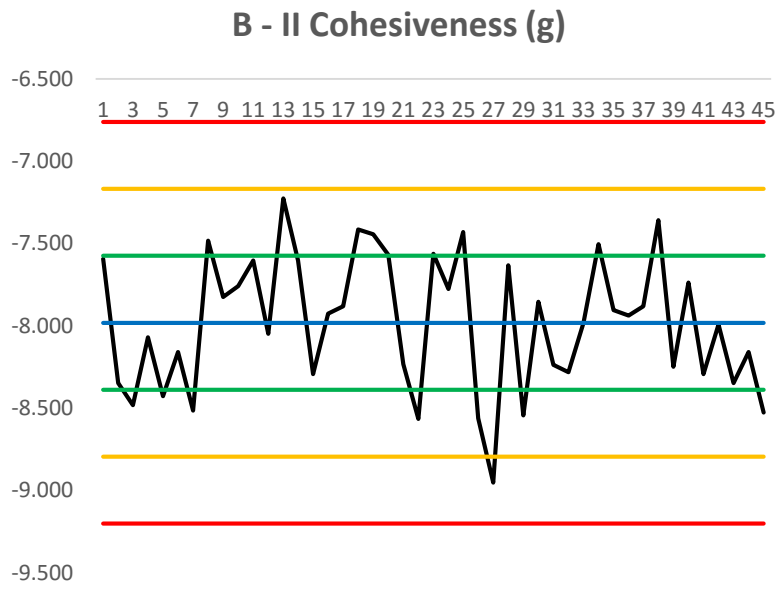
Some QC charts plotted from the data of samples exposed to stress conditions

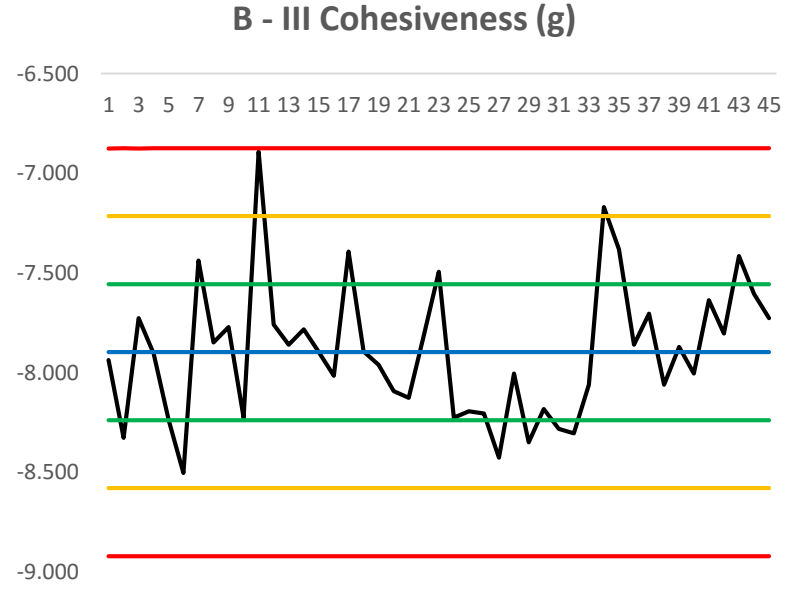












Conclusions:

- QbD strategy enables a formulation scientist to develop the formulation through risk assessment based strategy so as to position the formulation to submit a CTD.
- In this research, this channel of formulation development has directed the selection of best-in-class formulation to achieve the targeted QTPP.
- Among those low risk formulations from the residual and response surface analysis, formulation R has been proven to be effective thereby validating QbD principles.
- Although from the risk analysis, other formulations have been identified which were at low risk, microbial performance and distribution of dispersed phase remain insignificant.
- Selected R formulation has been proven stable under induced stress conditions of 25 degree centigrade and 75 percent relative humidity.
- Hence QbD serves as promising technology towards the development of RLD independent and novel formulations.

- References:

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- Zhang, L. & Mao, S., 2016. Applications of quality by design (QbD) and its tools in drug delivery. *Asian Journal of Pharmaceutical Sciences*, 11(1), pp.144–145. Available at: http://dx.doi.org/10.1016/j.ajps.2015.11.084.

- Acknowledgement:
- This project was funded by “President’s Seed Fund – 2013”, Athlone Institute of Technology.

Thank You