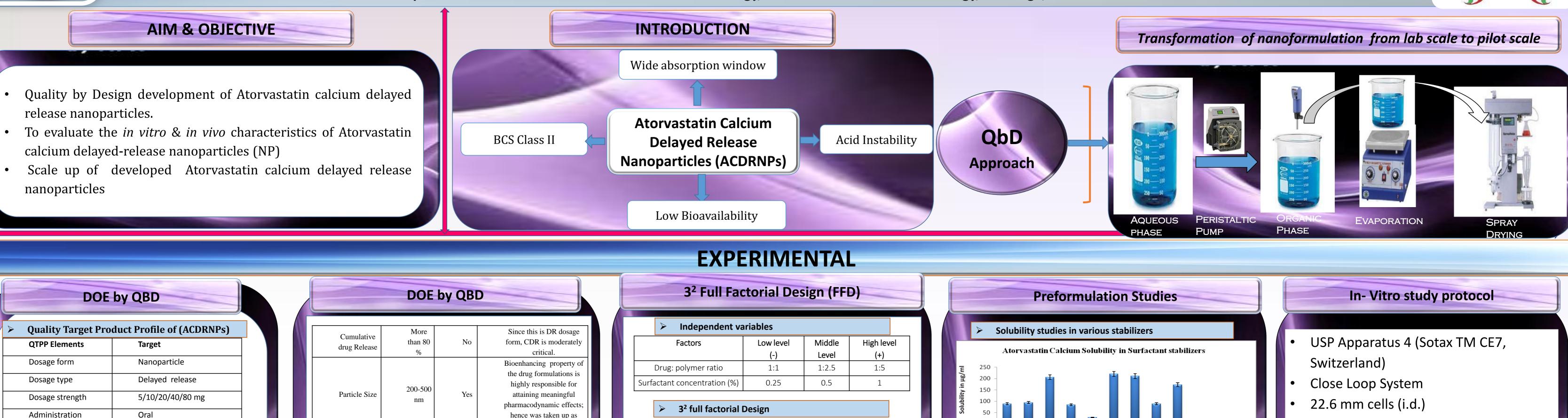# Scale up of Atorvastatin Delayed Release Nanoparticles for Treatment of Hyperlipidemia: Quality by Design (QbD) Approach

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AIM & OBJECTIVE

- Quality by Design development of Atorvastatin calcium delayed release nanoparticles.
- To evaluate the *in vitro* & *in vivo* characteristics of Atorvastatin calcium delayed-release nanoparticles (NP)
- Scale up of developed Atorvastatin calcium delayed release nanoparticles

INTRODUCTION

- Wide absorption window
- BCS Class II
- Atorvastatin Calcium Delayed Release Nanoparticles (ACDRNPs)
- Acid Instability
- Low Bioavailability
- QbD Approach

Transformation of nanoformulation from lab scale to pilot scale

AQUEOUS PHASE — PERISTALTIC PUMP — ORGANIC PHASE — EVAPORATION — SPRAY DRYING

EXPERIMENTAL

DOE by QBD

Quality Target Product Profile of (ACDRNPs)

QTPP Elements	Target
Dosage form	Nanoparticle
Dosage type	Delayed release
Dosage strength	5/10/20/40/80 mg
Administration	Oral
Packaging	Alu-Alu blister
Stability	6 months

Critical Quality Attributes of ACDRNPs

Quality Attributes of the Drug Product	Target	Is this a CQA ?	Justification
Physical attributes	Color / Odor / Appearance	No	Physical attributes of the formulation were not considered as critical, as these are not directly linked to the efficacy and safety.
Assay and Content uniformity	90-100%	No	Assay and content uniformity tend to affect safety and efficacy of formulations, variables were regarded as moderately critical.

DOE by QBD

	More than 80 %	No	Since this is a DR dosage form, CDR is moderately critical.
Cumulative drug Release			
Particle Size	200-500 nm	Yes	Bioenhancing property of the drug formulations is highly responsible for attaining meaningful pharmacodynamic effects; hence was taken up as highly critical.
Polydispersity index	0.1-0.3	Yes	Stability of nanoparticles is dependent on PDI so it is highly critical
Encapsulation efficiency (EE)	60-100%	Yes	Dosage form is DR, so it is very important that EE should be at higher side. So it was attributed as highly critical parameter

Risk Assesment analysis

Drug Product CQA's	Drug: polymer ratio	Stabilizer conc.	Stirring rate	Mixing time
Assay and CU	Low	Low	Low	Low
CDR	Low	Low	Low	Low
Particle Size	High	High	Low	Low
PDI	Medium	Medium	Medium	Medium
EE	High	High	Medium	Medium

Medium risk factors are optimized on the basis of preliminary batches

3² Full Factorial Design (FFD)

Independent variables

Factors	Low level (-)	Middle Level	High level (+)
Drug: polymer ratio	1:1	1:2.5	1:5
Surfactant concentration (%)	0.25	0.5	1

3² full factorial Design

Batch code	Drug: Polymer ratio	Surfactant concentration (%)
ACNP1	1.00	0.25
ACNP2	1.00	0.50
ACNP3	1.00	1.00
ACNP4	2.50	0.25
ACNP5	2.50	0.50
ACNP6	2.50	1.00
ACNP7	5.00	0.25
ACNP8	5.00	0.50
ACNP9	5.00	1.00

Experimental procedure for preparation of of Nanoparticle

Drug+Polymer in Organic solvent → Aqueous surfactant solution → Spray Drying

Preformulation Studies

Solubility studies in various stabilizers

Atorvastatin Calcium Solubility in Surfactant stabilizers

Polymer drug compatibility studies using FTIR and DSC

Fig. FTIR Spectra of AC — Fig. FTIR Spectra of physical mixture

Fig.DSC thermogram of AC — Fig. FTIR Spectra of physical mixture

In- Vitro study protocol

- USP Apparatus 4 (Sotax TM CE7, Switzerland)
- Close Loop System
- 22.6 mm cells (i.d.)
- Piston pump (Sotax CY7–50)
- Dissolution Medium- 1.2 pH HCL followed by pH 6.8 Phosphate buffer 900ml at 37±0.5°C
- Flow rate- 8 ml/min
- Time- 2 hr in 1.2pH HCl and then 5,15,30,45,60, 120 min

In- Vivo study protocol

- Male Albino rats (6–7 weeks old) weighing between 180 and 200 g
- Dose: 9mg/kg
- Route of administration: Oral
- Time: 0.5, 1, 1.5, 2, 3,4, 6, 8 and 12 h

RESULT AND DISCUSSION

Analysis of Data using Design Expert

Fig. 3D-Response and Model diagnostic plots of particle size

The "Pred R-Squared" of 0.9663 is in reasonable agreement with the "Adj R-Squared" of 0.9910. "Adeq Precision" measures the signal to noise ratio. A ratio greater than is desirable. Here ratio of 41.531 indicates an adequate signal. This model can be used to navigate the design space.
ANOVA Equation: P Size =+356.60+10.66 * A-36.58 * B-44.20 * A * B-49.45 * A²+24.19 * B²

Fig. 3D-Response and Model diagnostic plots of Encapsulation efficiency

The "Pred R-Squared" of 0.5463 is in reasonable agreement with the "Adj R-Squared" of 0.7258. "Adeq Precision" measures the signal to noise ratio. A ratio greater than is desirable. Here ratio of 9.487 indicates an adequate signal. This model can be used to navigate the design space.
ANOVA Equation: EE=+63.03+10.55 * A-9.66*

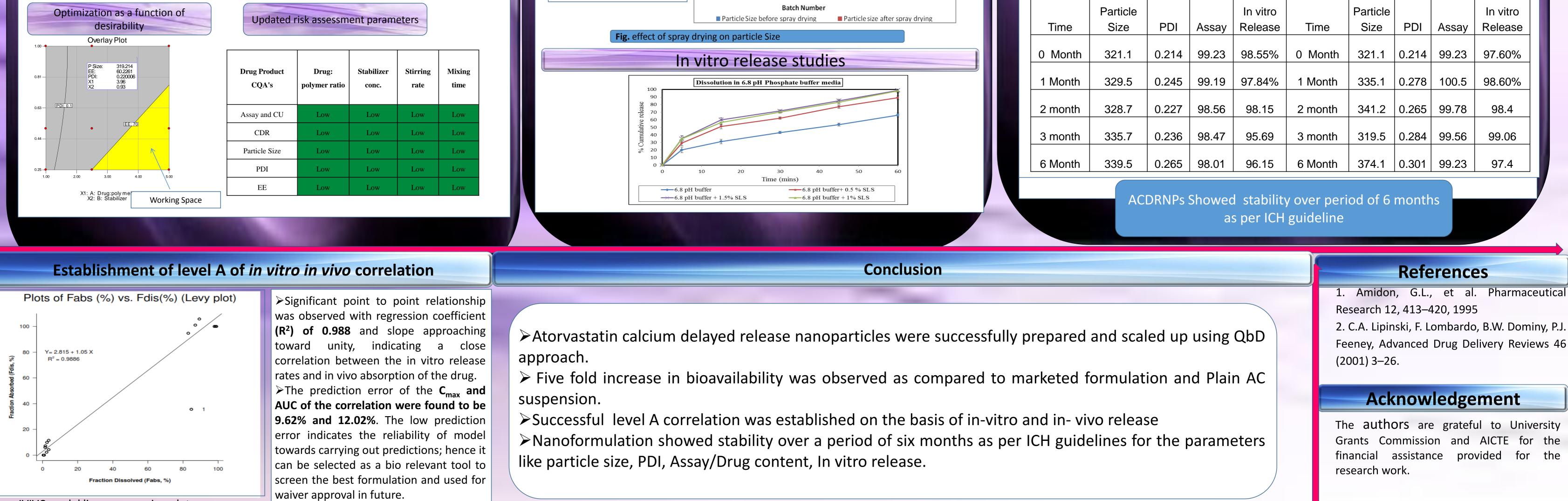**Fig. 3D-Response and Model diagnostic plots of polydispersity index**

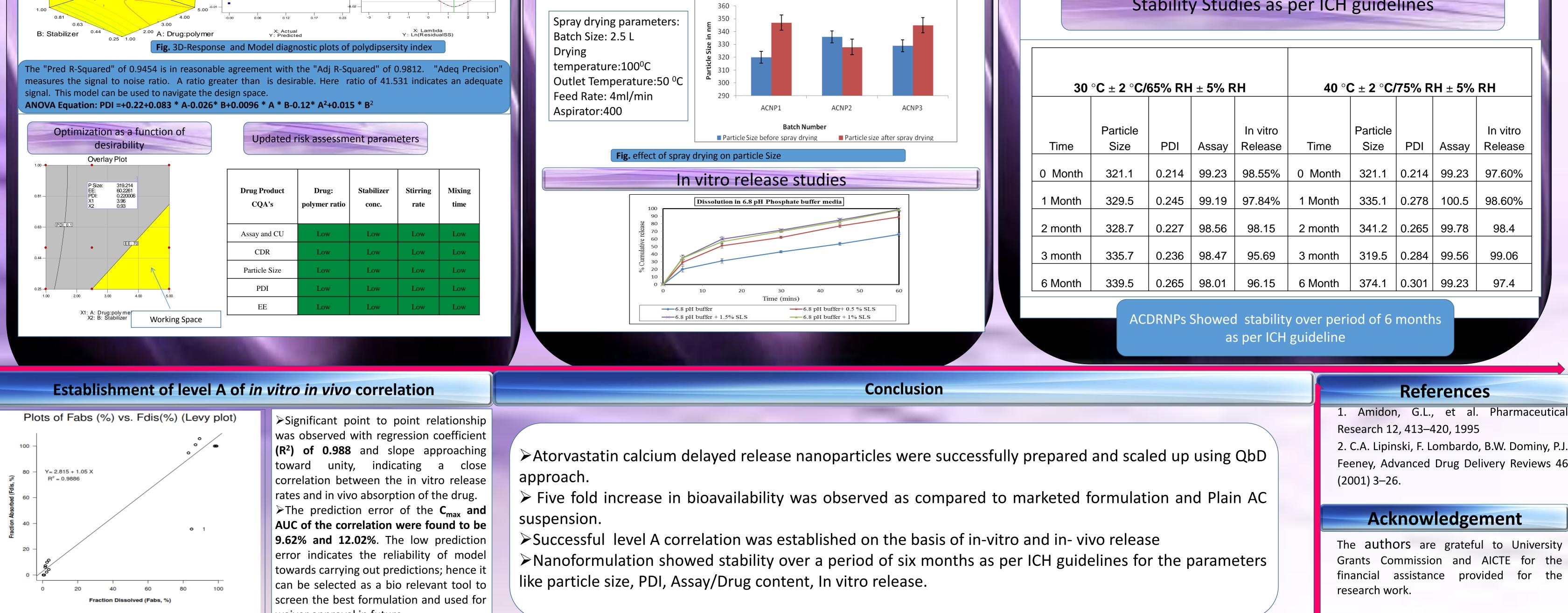The "Pred R-Squared" of 0.9454 is in reasonable agreement with the "Adj R-Squared" of 0.9812. "Adeq Precision" measures the signal to noise ratio. A ratio greater than is desirable. Here ratio of 41.531 indicates an adequate signal. This model can be used to navigate the design space.
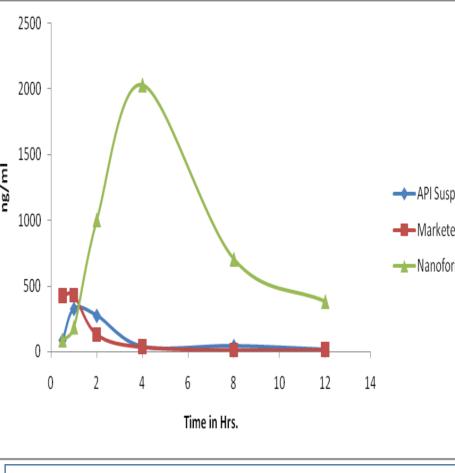**ANOVA Equation: PDI =+0.22+0.083 * A-0.026* B+0.0096 * A * B-0.12* A²+0.015 * B²**

Optimization as a function of desirability

Overlay Plot

Updated risk assessment parameters

Drug Product CQA's	Drug: polymer ratio	Stabilizer conc.	Stirring rate	Mixing time
Assay and CU	Low	Low	Low	Low
CDR	Low	Low	Low	Low
Particle Size	Low	Low	Low	Low
PDI	Low	Low	Low	Low
EE	Low	Low	Low	Low

X1 : A: Drug:poly mer
X2 : B: Stabilizer

Working Space

Validation and scale up of nanoformulation

Particle size and EE of ACDRNPs

Predicted Vs Experimental Y2 — $y = 1.120x - 47.28$, $R^2 = 0.988$

Predicted Vs Experimental Y1 — $y = 0.722x + 20.59$, $R^2 = 0.980$

All the predicted response lie within 95% CI, and gave correlation coefficient value near to 0.999. Hence, predicted responses are validated

Scale up of nanoformulation: Optimized batches were formulated and subjected to spray drying process

Spray drying parameters:
Batch Size: 2.5 L
Drying temperature:100°C
Outlet Temperature:50 °C
Feed Rate: 4ml/min
Aspirator:400

Fig. effect of spray drying on particle Size

In vitro release studies

Dissolution in 6.8 pH Phosphate buffer media

- 6.8 pH buffer
- 6.8 pH buffer 0.5 % SLS
- 6.8 pH buffer 1.5% SLS
- 6.8 pH buffer 1% SLS

Particle Size and zeta potential

In vivo pharmacokinetics Studies

Parameters	Unit	Observed values
C_{max}	µg/ml	3.99
T_{max}	h	3.00
$T_{1/2}$	h	3.85
MRT	h	8.02
$AUC_{(0-t)}$	µg/ml h	5.78
$AUC_{(0-\infty)}$	µg/ml h	36.91
AUMC	µg/ml h	289.02
Ka	h	0.17
Kel	h	0.18
Vd	L	1.33

Fig. In vivo absorption

- API Suspension
- Marketed/tablet
- Nanoformulation

Stability Studies as per ICH guidelines

30 °C ± 2 °C/65% RH ± 5% RH					40 °C ± 2 °C/75% RH ± 5% RH				
Time	Particle Size	PDI	Assay	In vitro Release	Time	Particle Size	PDI	Assay	In vitro Release
0 Month	321.1	0.214	99.23	98.55%	0 Month	321.1	0.214	99.23	97.60%
1 Month	329.5	0.245	99.19	97.84%	1 Month	335.1	0.278	100.5	98.60%
2 month	328.7	0.227	98.56	98.15	2 month	341.2	0.265	99.78	98.4
3 month	335.7	0.236	98.47	95.69	3 month	319.5	0.284	99.56	99.06
6 Month	339.5	0.265	98.01	96.15	6 Month	374.1	0.301	99.23	97.4

ACDRNPs Showed stability over period of 6 months as per ICH guideline

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Establishment of level A of *in vitro in vivo* correlation

Plots of Fabs (%) vs. Fdis(%) (Levy plot)

$Y = 2.615 + 1.05$ X, $R^2 = 0.9666$

> Significant point to point relationship was observed with regression coefficient (R²) of 0.988 and slope approaching toward unity, indicating a close correlation between the in vitro release rates and in vivo absorption of the drug.
> The prediction error of the C_{max} and AUC of the correlation were found to be 9.62% and 12.02%. The low prediction error indicates the reliability of model towards carrying out predictions; hence it can be selected as a bio relevant tool to screen the best formulation and used for waiver approval in future.

IVIVC model linear regression plot

Conclusion

> Atorvastatin calcium delayed release nanoparticles were successfully prepared and scaled up using QbD approach.
> Five fold increase in bioavailability was observed as compared to marketed formulation and Plain AC suspension.
> Successful level A correlation was established on the basis of in-vitro and in- vivo release
> Nanoformulation showed stability over a period of six months as per ICH guidelines for the parameters like particle size, PDI, Assay/Drug content, In vitro release.

References

1. Amidon, G.L., et al. Pharmaceutical Research 12, 413–420, 1995
2. C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Advanced Drug Delivery Reviews 46 (2001) 3–26.

Acknowledgement