Formula X

Application of a full-factorial design to the control of colloidal characteristics of Non-isocyanate polyurethane nanoparticles prepared by nanoprecipitation

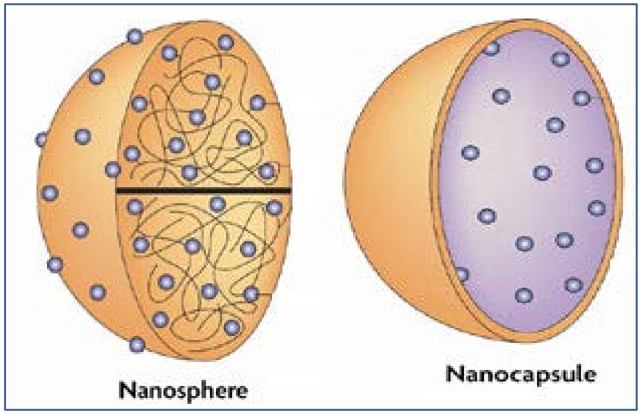
T. Quérette, C. Bordes, and N. Sintes-Zydowicz

Polymer nanoparticles

- Active agent delivery

  in cosmetics, agrochemistry, pharmaceutics

- protect and carry the active ingredient



Scheme of a NP (left) and a NC (right)

[1] Legrand, P. et al. Int. J. Pharm. 2007. **344**(1-2): p. 33-43.

Nanoparticle formation

layer-by-layer

Solvent evaporation

Supercritical fluid expansion

Salting out

Nanoprecipitation

Mini/microemulsion polymerisation

Nanoprecipitation

- ✓ Straightforward and reproducible method
- ✓ Preformed polymer
- ✓ No expensive mechanical energy input

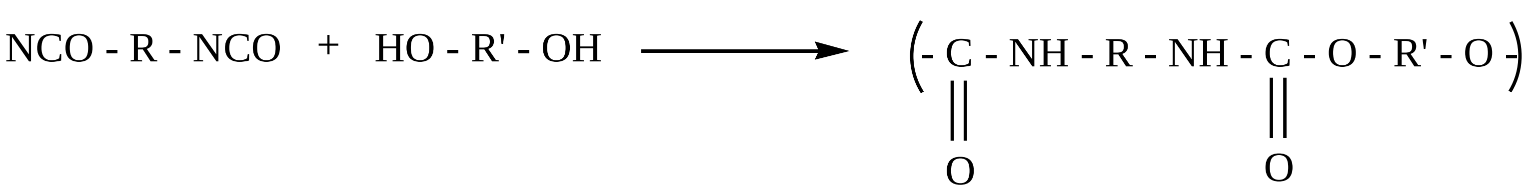
Polymers for Nanoprecipitation

PLA, PCL, PLGA, PLA-PEG, PCL-PEG, PNIPAm, PMMA

Polyurethane :
- Versatile synthetic polymer
- Biodegradability[1]
- Good blood compatibility[2]

Toxicity of the isocyanate monomers

$RNH_2 + COCl_2 \rightarrow RN{=}C{=}O + 2\ HCl$



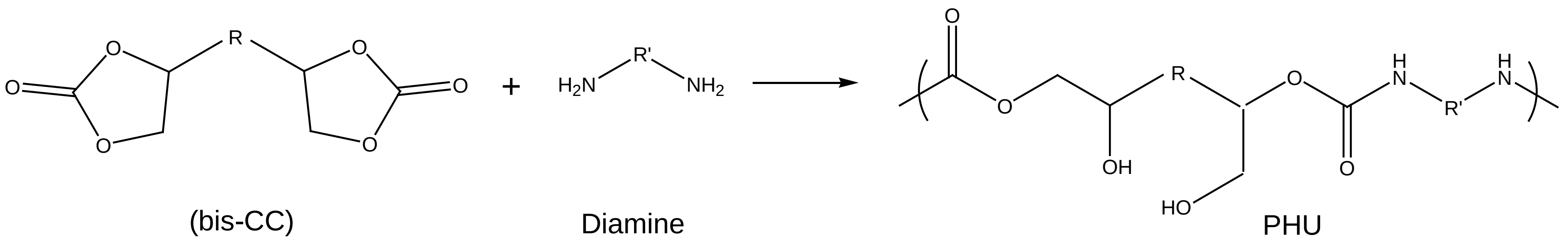
[1]. R. Chandra, R. Rustgi, Prog. Polym. Sci., 23 (1998) 1273-1335.
[2] M.D. Lelah, L.K. Lambrecht, B.R. Young, S.L. Cooper, J. Biomed. Mater. Res., 17 (1983) 1-22

Objectives

- non isocyanate polyurethane[1] nanoparticles

➡ ***Poly(hydroxy)urethane*** *(PHU)* [2]

- Same advantages as PU
- *Isocyanate free* synthesis
- Possibility to introduce *stimuli-responsive* functions (OH groups)

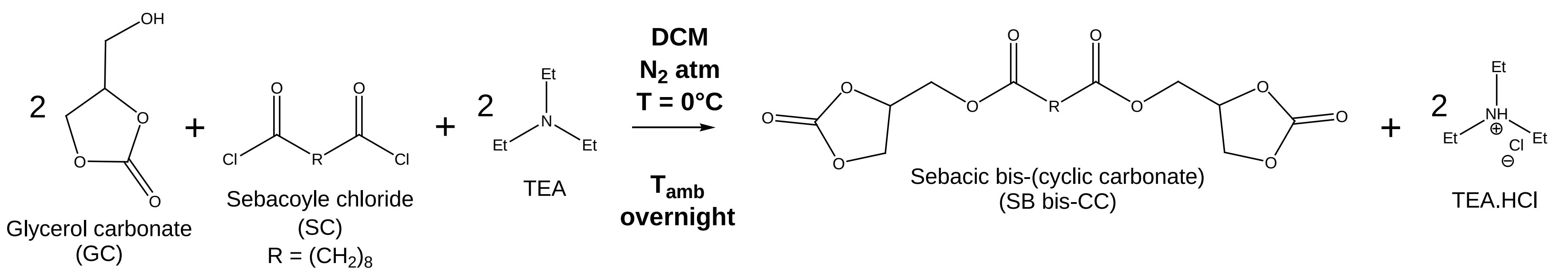


[1] Kathalewar, M.S. et al. RSC Adv. 2013. **3**(13): p. 4110-4129.
[2] Proempers, G. et al. Des. Monomers Polym., 2005. **8**(6): p. 547-569.

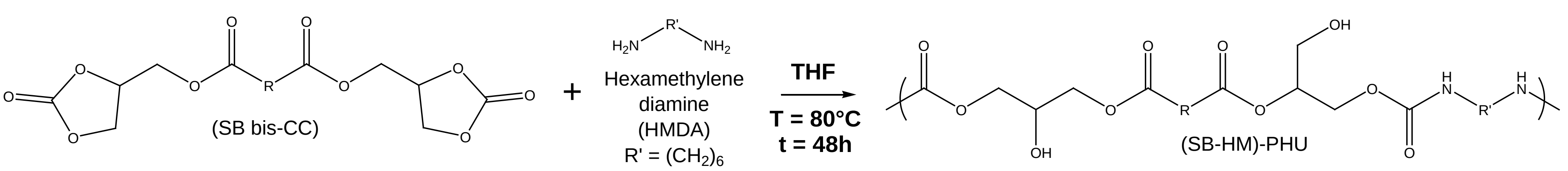
PHU Nanoparticles

PHU nanoprecipitation[1]

- SB bis-CC monomer synthesis



- Poly(hydroxy)urethane synthesis



[1] T. Quérette, E. Fleury, N. Sintes-Zydowicz, European Polymer Journal, 114 (2019) 434-445.

Nanoprecipitation method

- Preparation of a water-insoluble polymer organic solution
- The organic solvent is completey water-miscible
- Dropwise addition of the organic solution into an aqueous phase

➡ Spontaneous water-organic solvent interdiffusion

➡ Precipitation of the polymer

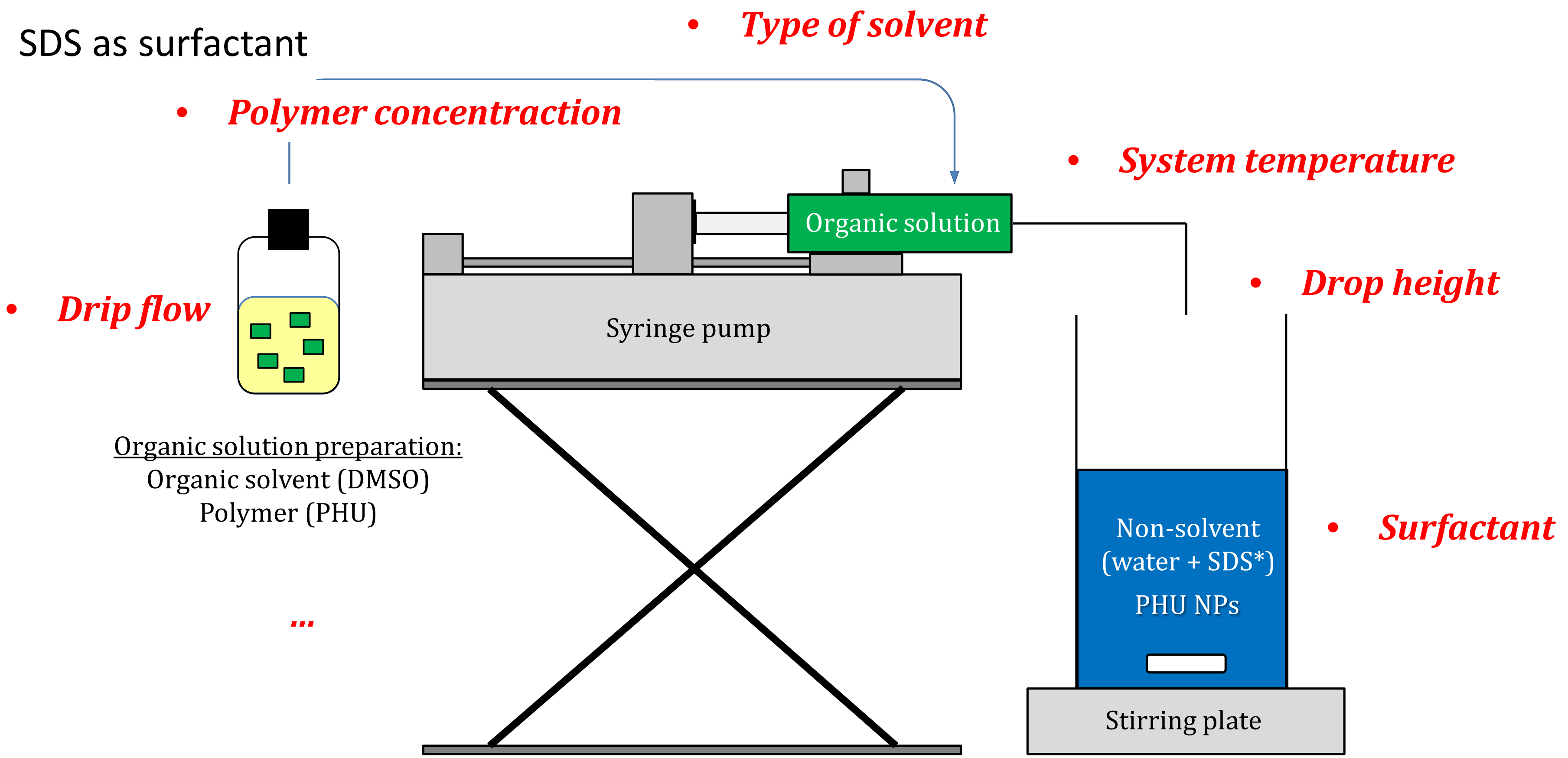
➡ Formation of monodisperse NP in the 50-300 nm range

Nanoprecipitation technique[1]

Experimental set-up scheme:

- ✓ PHU soluble in DMSO
- ✓ PHU not soluble in water
- ✓ DMSO and water fully miscible
- ✓ SDS as surfactant

$\overline{M}_n$ = 12 900 g/mol, $\overline{M}_w$ = 30 000 g/mol,
T_d = 210°C, T_g = -7°C



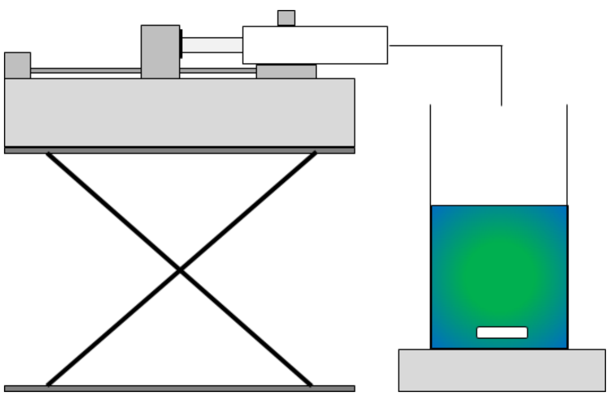
* Sodium dodecyl sulfate (SDS)

[1] T. Quérette, E. Fleury, N. Sintes-Zydowicz, European Polymer Journal, 114 (2019) 434-445.

2^3 Full-factorial design[1]

Critical parameters:

- Polymer concentration in the organic solution
- Volume of water in the beaker
- Amount of surfactant in the non-solvent



- Effect of these parameters on the quality of the suspension
- Nanoprecipitation Process optimization

[1]: M. Sabapati et al., Drug development and industrial pharmacy, 45 (2019) 577-586

2^3 Full-factorial design

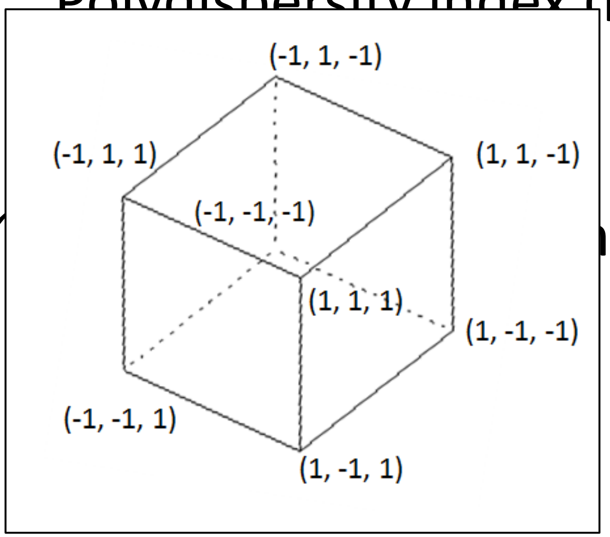
✓ 2 **responses** (DLS measure

Experimental matrix:

Z-average diameter / mea

Polydispersity index (PDI)

✓ …nts



	Run	[PHU]	V_{water}	[SDS]	X_1	X_2	X_3
		Natural variables			Coded variables		
3D cube edges	1	1	50	0	-1	-1	-1
	2	5	50	0	1	-1	-1
	3	1	150	0	-1	1	-1
	4	5	150	0	1	1	-1
	5	1	50	25	-1	-1	1
	6	5	50	25	1	-1	1
	7	1	150	25	-1	1	1
	8	5	150	25	1	1	1
3D cube centre	A	3	100	12.5	0	0	0
	B	3	100	12.5	0	0	0
	C	3	100	12.5	0	0	0
	D	3	100	12.5	0	0	0
		mg/ml	ml	mmol/l			

, 1 , 2

REGRESSION (runs 1–8)

DEV (runs A–D)

✓3 **factors** & 3 **coded variables** [1] :

[PHU] (1, 3, 5 ; mg/ml)* ⇔ X_1 (−1, 0, +1)

V_{water} (50, 100, 150 ; ml) ⇔ X_2 (−1, 0, +1)

[SDS] (0, 12.5, 25 ; mmol/l)** ⇔ X_3 (−1, 0, +1)

** Concentration of PHU in DMSO . ** Concentration of SDS in water*

[1] C.E. Mora-Huertas, H. Fessi, A. Elaissari, Int J Pharm, 385 (2010) 113-142

Regression model

Main effect Coefficients

2- & 3-factor **interaction** Coefficients

$$\hat{Y} = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_{12} X_1 X_2 + b_{13} X_1 X_3 + b_{23} X_2 X_3 + b_{123} X_1 X_2 X_3$$

Regression model for responses ($\hat{Y}_1$ and $\hat{Y}_2$)

- $\hat{Y}: predicted\ response$
- Multiple linear regression analyses performed by NEMRODW®

Experimental Results

	Run	Coded variable			Natural variable			Response	
		X_1	X_2	X_3	[PHU] g/l	V_w ml	[SDS] Mmol/l	**Y_1 (PDI)**	**Y_2 (d) nm**
Cube edges	1	-1	-1	-1	1	50	0.0	**0.28**	**103**
	2	1	-1	-1	5	50	0.0	**0.27**	**110**
	3	-1	1	-1	1	150	0.0	**0.15**	87
	4	1	1	-1	5	150	0.0	**0.26**	**101**
	5	-1	-1	1	1	50	25.0	**0.15**	55
	6	1	-1	1	5	50	25.0	**0.10**	88
	7	-1	1	1	1	150	25.0	**0.52**	**179**
	8	1	1	1	5	150	25.0	**0.20**	81
Centre points	A	0	0	0	3	100	12.5	**0.12**	102
	B	0	0	0	3	100	12.5	**0.13**	86
	C	0	0	0	3	100	12.5	**0.13**	93
	D	0	0	0	3	100	12.5	**0.13**	93
Repeated runs	3’	-1	1	-1	1	150	0.0	**0.20**	88
	3’’	-1	1	-1	1	150	0.0	**0.25**	**118**
	4’	1	1	-1	5	150	0.0	**0.28**	**137**
	4’’	1	1	-1	5	150	0.0	**0.29**	**110**
	5’	-1	-1	1	1	50	25.0	**0.14**	55
	6’	1	-1	1	5	50	25.0	**0.10**	90
	6’’	1	-1	1	5	50	25.0	**0.12**	86

$\sigma_1 = 0.024$

$\sigma_2 = 13$ nm

$Y_1 \pm 0.05$
$Y_2 \pm 25$ nm

95%

Bold figures: unimodal and polydisperse (PDI > 0.2) size distributions

Red figures: multimodal and polydisperse size distributions

PDI ≤ 0.2 ⇨ d = z-ave ; PDI > 0.2 ⇨ d = d_{moy}

Effects of parameters

$$\hat{Y} = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_{12} X_1 X_2 + b_{13} X_1 X_3 + b_{23} X_2 X_3 + b_{123} X_1 X_2 X_3$$

Effects	b_0	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}	b_{123}
Y_1	0.24	-0.03	0.04	0.00	-0.02	-0.06	0.07	-0.05
Y_2	101	-6	12	0	-15	-11	18	-17

- Coefficients determined using ordinary least square regression on 8 design points

$$\sigma_{bi(Y1)} = \frac{\sigma_1}{\sqrt{N}} \sim 0.01$$

$$\sigma_{bi(Y2)} = \frac{\sigma_2}{\sqrt{N}} \sim 4.3$$

N = 8

$$\Delta b_i = t_{theo} \times \sigma_{b_i}$$

$\Delta b_{i(Y1)}$= 0.02

$\Delta b_{i(Y2)}$= 9.5

95%

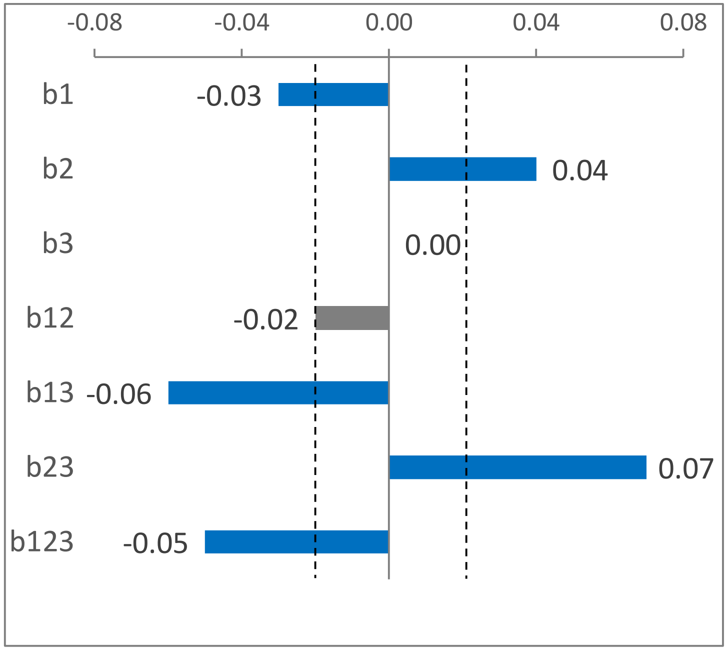
t_{theo} : the theoretical value of the Student t statistic which is equal to 2.22 with a risk of 0.05 and a degree of freedom equal to 10

G.E. Box, J.S. Hunter, W.G. Hunter, Statistics for experimenters, Wiley Series in Probability and Statistics, Wiley Hoboken, NJ2005

Analysis of DOE

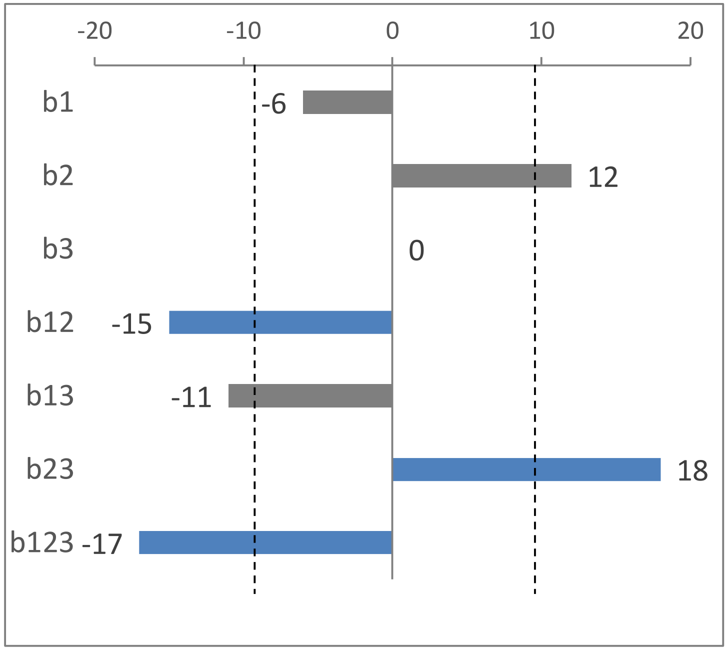
$\Delta b_{i(Y1)}$ = 0.02

PDI



$\Delta b_{i(Y2)}$ = 9.5

Size



$$\hat{Y}_1 = 0,24 - 0,03X_1 + 0,04\,X_2 - 0,06\,X_1X_3 + 0,07\,X_2X_3 - 0,05\,X_1X_2X_3$$

$$\hat{Y}_2 = 101 + 12\,X_2 - 15\,X_1X_2 - 11\,X_1X_3 + 18\,X_2X_3 - 17\,X_1X_2X_3$$

Analysis of DOE results obtained for PDI

$$\widehat{Y}_1 = 0,24 - 0,03X_1 + 0,04\,X_2 - 0,06\,X_1X_3 + 0,07\,X_2X_3 - 0,05\,X_1X_2X_3$$

- Minimal PDI value

[PHU] = 5g/l
V_w = 50 ml
[SDS] = 25 mM

	Run	Coded variable			Natural variable			Response	
		X_1	X_2	X_3	[PHU] g/l	V_w ml	[SDS] Mmol/l	Y_1 (PDI)	Y_2 (d) nm
Cube edges	1	-1	-1	-1	1	50	0.0	**0.28**	**103**
	2	1	-1	-1	5	50	0.0	**0.27**	**110**
	3	-1	1	-1	1	150	0.0	**0.15**	87
	4	1	1	-1	5	150	0.0	**0.26**	**101**
	5	-1	-1	1	1	50	25.0	**0.15**	55
	6	1	-1	1	5	50	25.0	**0.10**	88
	7	-1	1	1	1	150	25.0	**0.52**	**179**
	8	1	1	1	5	150	25.0	**0.20**	81
Centre points	A	0	0	0	3	100	12.5	**0.12**	102
	B	0	0	0	3	100	12.5	**0.13**	86
	C	0	0	0	3	100	12.5	**0.13**	93
	D	0	0	0	3	100	12.5	**0.13**	93
Repeated runs	3’	-1	1	-1	1	150	0.0	**0.20**	88
	3’’	-1	1	-1	1	150	0.0	**0.25**	**118**
	4’	1	1	-1	5	150	0.0	**0.28**	**137**
	4’’	1	1	-1	5	150	0.0	**0.29**	**110**
	5’	-1	-1	1	1	50	25.0	**0.14**	55
	6’	1	-1	1	5	50	25.0	**0.10**	90
	6’’	1	-1	1	5	50	25.0	**0.12**	86

✓

Analysis of DOE results obtained for the diameter

$$\hat{Y}_2 = 101 + 12\,X_2 - 15\,X_1X_2 - 11\,X_1X_3 + 18\,X_2X_3 - 17\,X_1X_2X_3$$

- Minimal diameter (55 nm)

[PHU] = 1g/l
V_w = 50 ml
[SDS] = 25mM

Run	X_1	X_2	X_3	[PHU]	V_w	[SDS]	PDI	Z-avr
5	**-1**	**-1**	**1**	**1**	**50**	**25.0**	**0.15**	**55**

✓

Predictive performance of the models

- Calculated centre points $\boldsymbol{\widehat{Y}_1} = 0.24$ $\boldsymbol{\widehat{Y}_2} = 101$ nm

- Experimental centre points

	Run	Coded variable			Natural variable			Response	
		X_1	X_2	X_3	[PHU] g/l	V_w ml	[SDS] Mmol/l	Y_1 (PDI)	Y_2 (d) nm
Centre points	A	0	0	0	3	100	12.5	**0.12**	102
	B	0	0	0	3	100	12.5	**0.13**	86
	C	0	0	0	3	100	12.5	**0.13**	93
	D	0	0	0	3	100	12.5	**0.13**	93
Mean value								**0.13**	**94**

$\varepsilon_0 (Y_1) = |\boldsymbol{\widehat{Y}_0} - \boldsymbol{Y_0}| = 0.11$ **> 0.05**

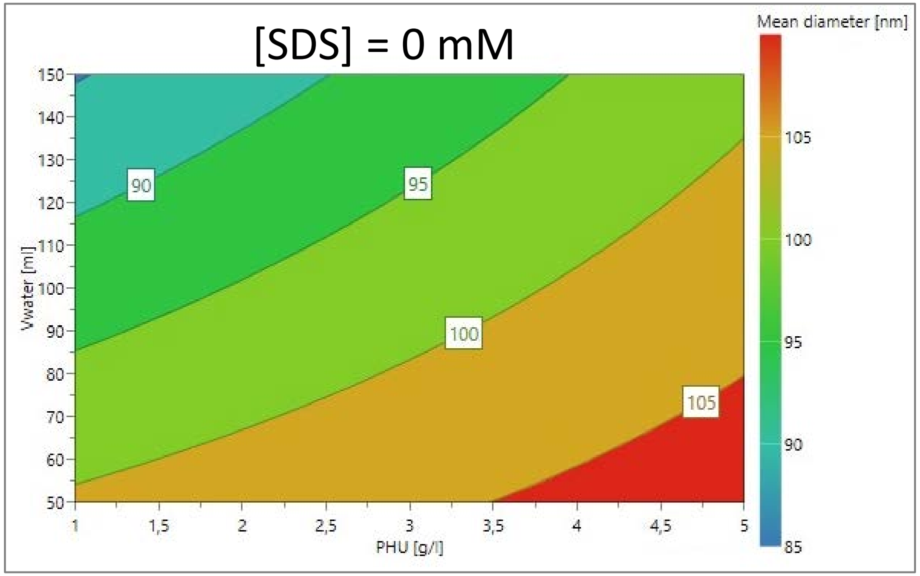
$\varepsilon_0 (Y_2) = |\boldsymbol{\widehat{Y}_0} - \boldsymbol{Y_0}| = 7$ nm **< 25 nm**

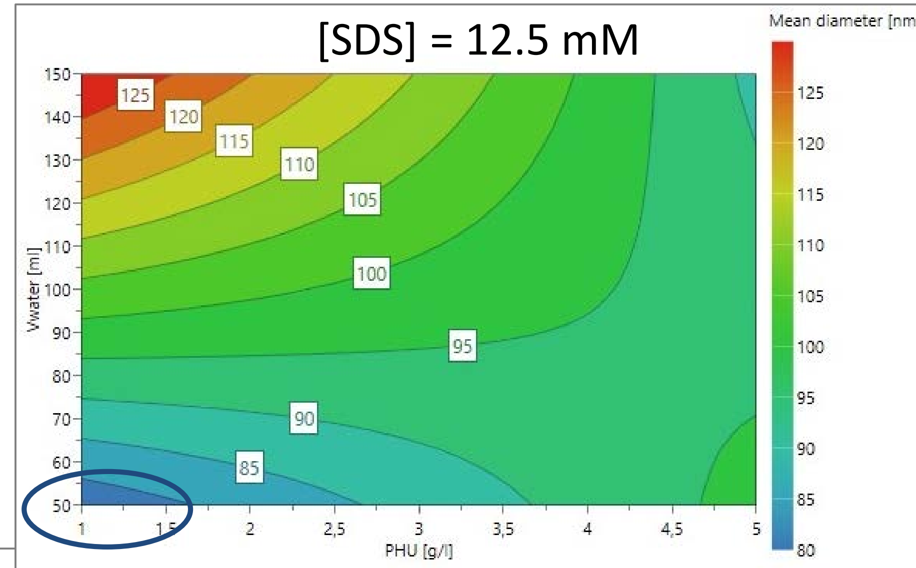
- ANOVA (p-value < 0.05) ; R^2-adjusted coefficient ≈ 0.92

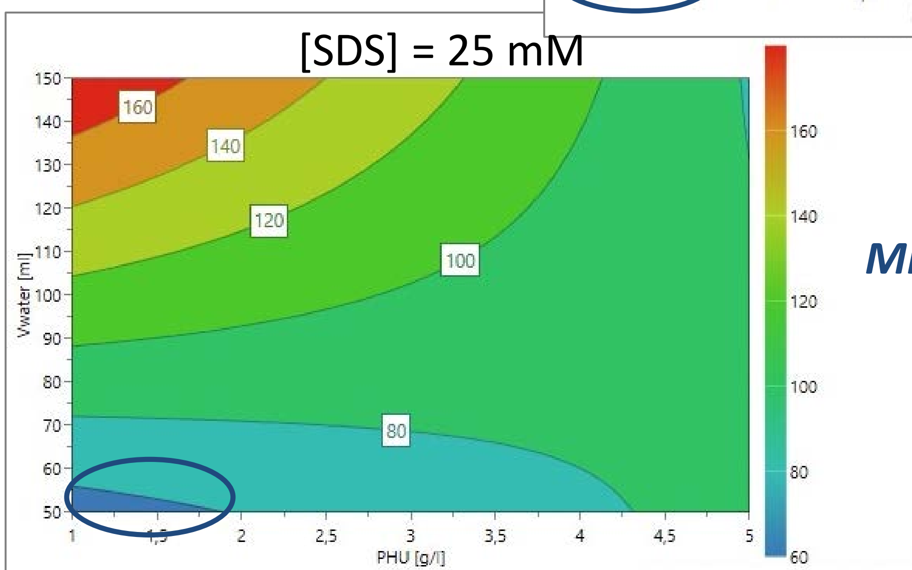
$\boldsymbol{\widehat{Y}_2}$ for prediction purpose in the whole experimental domain

G.E. Box, J.S. Hunter, W.G. Hunter, Statistics for experimenters, Wiley Series in Probability and Statistics, Wiley Hoboken, NJ2005.

Response surfaces







Minimal diameter = 75 -80 nm

Influence of SDS and PHU amounts

[SDS] = 25 mM

Run	Coded variable			Natural variable			Response	
	X_1	X_2	X_3	[PHU] g/l	V_w ml	[SDS] Mmol/l	Y_1 (PDI)	Y_2 (d) nm
7	-1	1	1	1	150	25.0	**0.52**	**179**
8	1	1	1	5	150	25.0	**0.20**	81

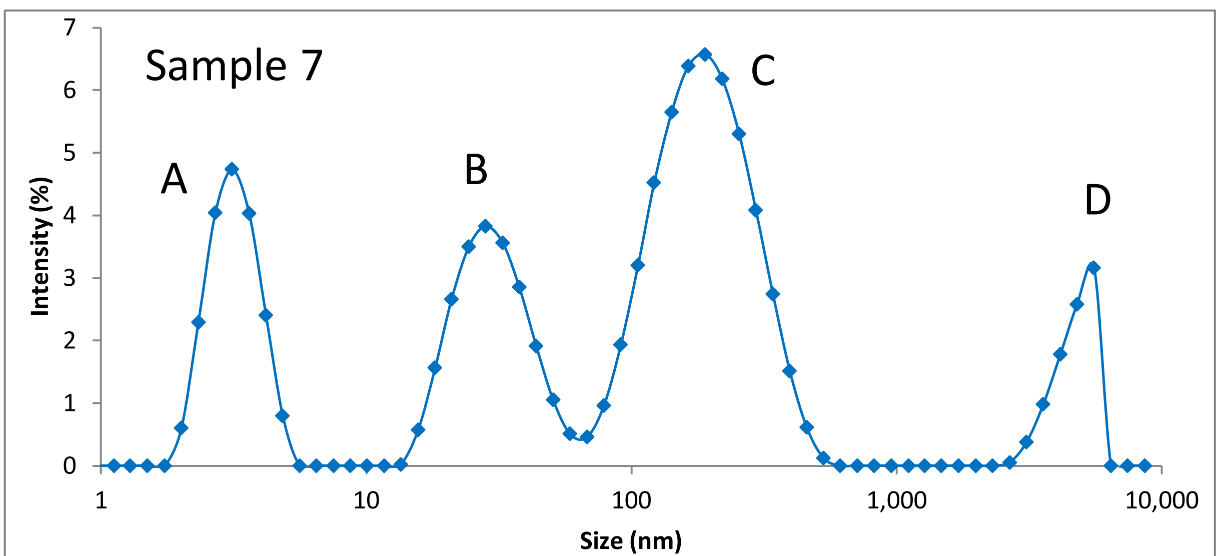
$$\hat{Y}_2 = 101 + 12\,X_2 - 15\,X_1X_2 - 11\,X_1X_3 + 18\,X_2X_3 - 17\,X_1X_2X_3$$

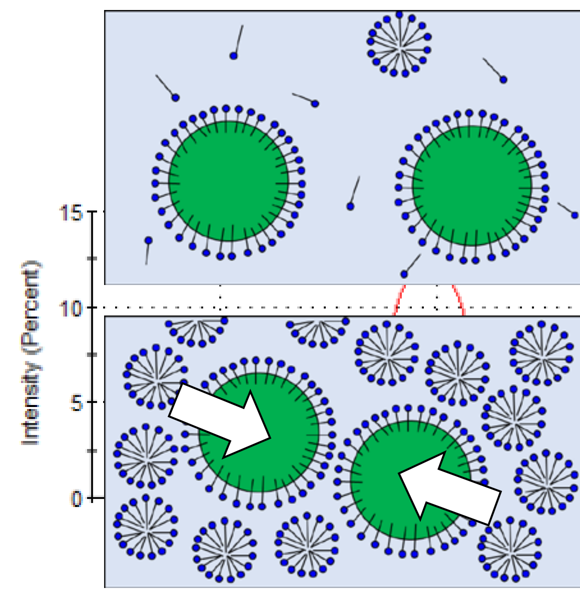
$$A_{tot} = \frac{6 \times m}{d \times \rho}$$

$$A_{tot} = N_{part} \times A_{part}$$

$$A_{part} = \pi \times d^2$$

$$N_{part} = \frac{V_{tot}}{V_{part}} = \frac{6 \times m}{\pi \times d^3 \times \rho}$$

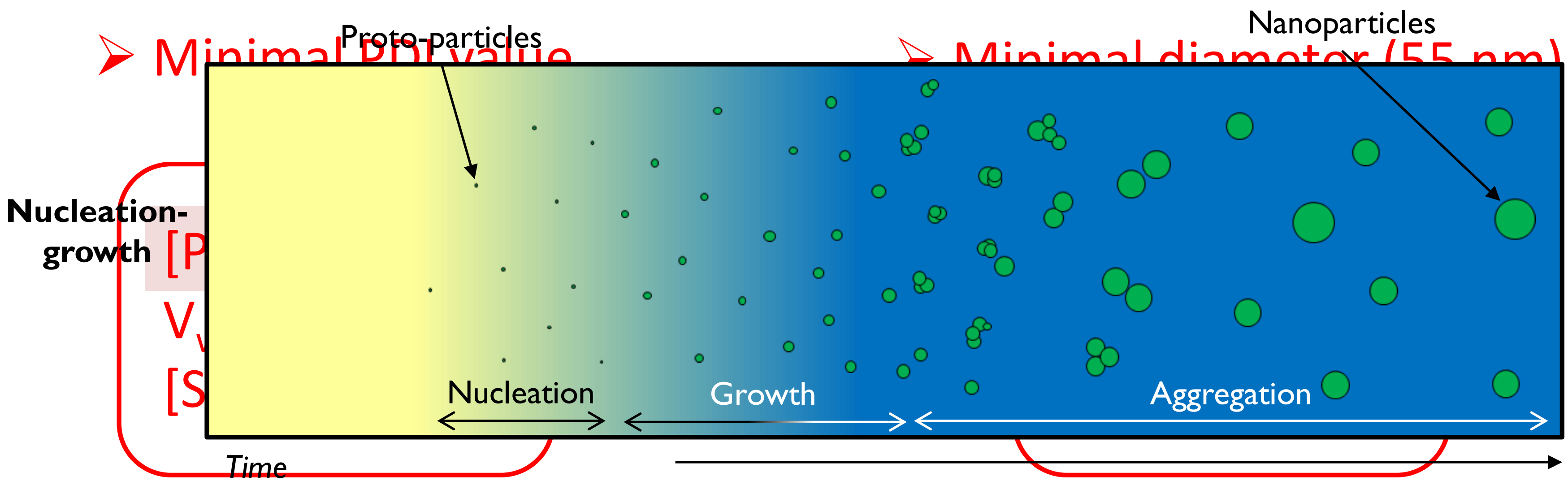




PHU Concentration

➢ Minimal PDI value

➢ Minimal diameter (55 nm)



Numerous PHU nanoparticles

↓Viscosity of the organic phase

↓Destabilizing effect of micelles

↓Protoparticle growth time

↓PDI

↓diameter

C.E. Mora-Huertas, H. Fessi, A. Elaissari, Adv. Colloid Interface Sci., 163 (2011) 90-122
P. Legrand, S. Lesieur, A. Bochot, R. Gref, W. Raatjes, G. Barratt, C. Vauthier, Int. J. Pharm., 344 (2007) 33-43

Conclusion

- ➢ poly(hydroxy)urethane nanoparticle by nanoprecipitation

✓ Optimized

- ➢ Most influent parameters ✓ identified

- ➢ Experimental conditions for Minimal diameter and PDI values

✓ identified

[PHU] = 5g/l V_w = 50 ml [SDS] = 25 mM	[PHU] = 1g/l V_w = 50 ml [SDS] = 25 mM

✓ Good predictive performance for $\hat{Y}_2$ model

- ➢ Presence of SDS mandatory but micelles destabilizing effect

THANK YOU FOR YOUR ATTENTION