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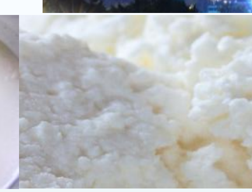
[1] **UR1268 INRA, Biopolymers Interactions Assemblies, Nantes, France**
[2] **UMR1253 INRA, Science and Technology of Milk and Egg, Rennes, France**
[3] **UMR8207 UMET, Interface Processes and Hygiene of Materials, Lille, France**

* E-mail address: **marie.hennetier@purpan.fr**

Milk Valley PROFIL



INRA SCIENCE & IMPACT

bia Biopolymères Interactions Assemblage Nantes

STLO

UMET Unité Matériaux Et Transformations

Context

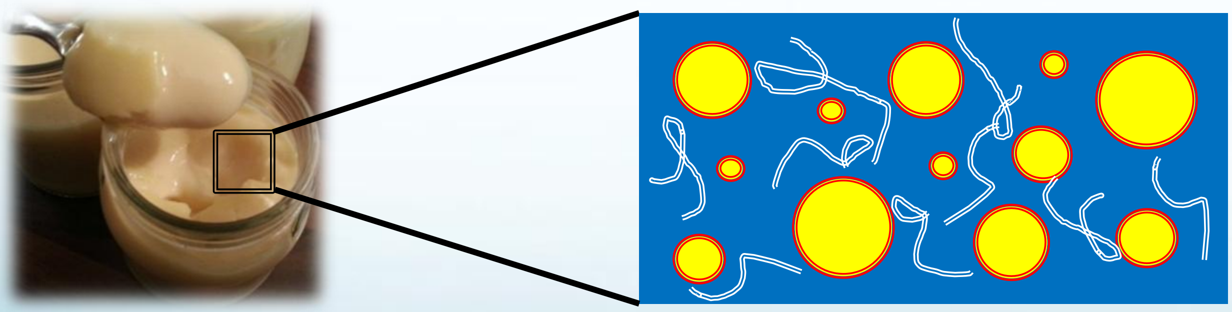
Natural product
please!!!

CLEAN LABEL
CLEAN LABEL

Introduction | Methods | Results | Conclusion and perspectives

Context

Natural product please!!!

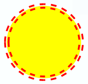


Oil/Water emulsion

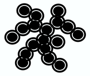
Gelling agents texture the continuous phase and entrap the oil droplets

Whey protein aggregates properties

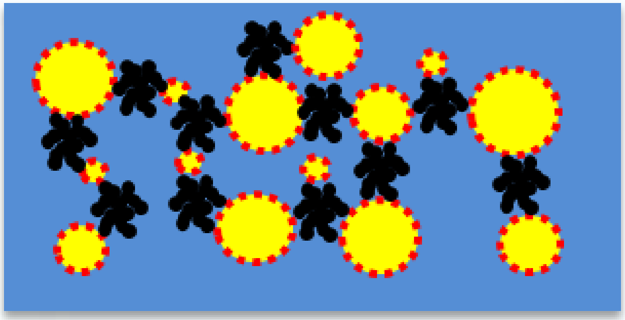
- ✓ Use oil droplets to texture emulsion without gelling agents



- ✓ Connect oil droplets through whey protein aggregates



- ✓ Heating can form aggregates with different shapes and sizes (Nicolai, 2011)



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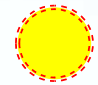

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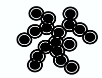

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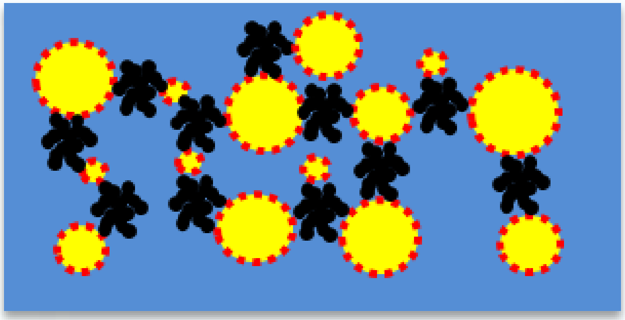

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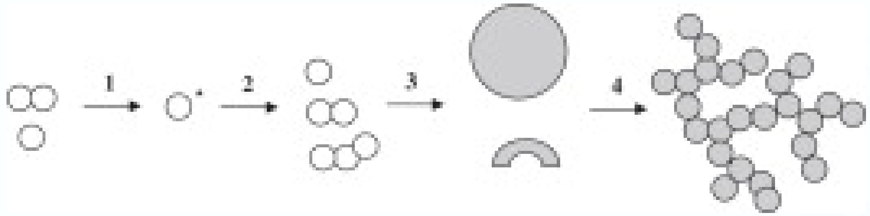
Applications	**Replace food additives like thickening or gelling agents in neutral dairy products**
	Texture emulsions even at low fat content

Fractal aggregates production from Whey Protein Isolate (WPI) at semi-industrial scale

Fractal aggregate formation



(Nicolai, 2011)

1: Dimer/trimer of whey proteins (β-lactoglobulin) are heated at 80°C

→ Denaturation, unfolding and thiol/hydrophobic group exposition

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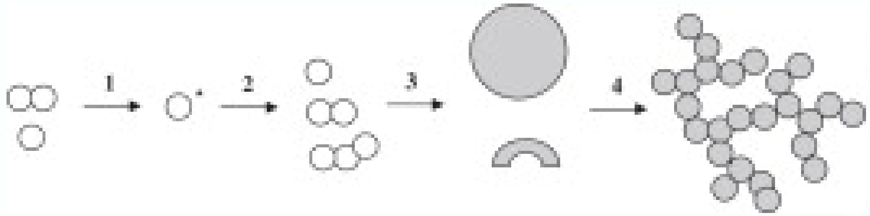

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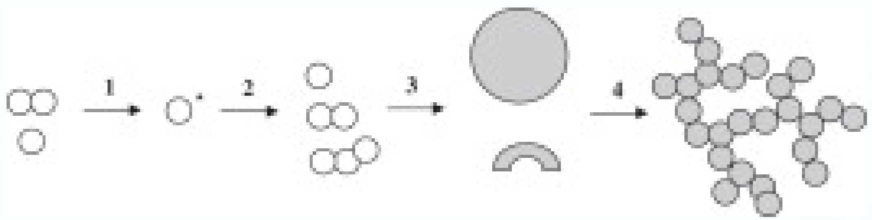

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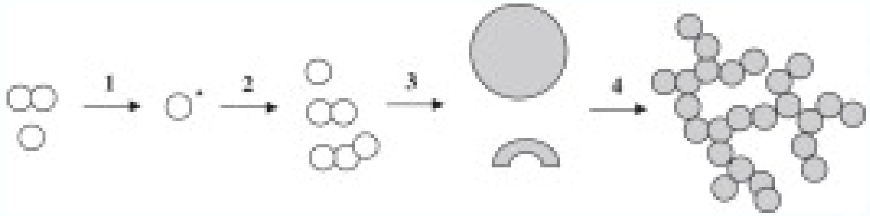

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3: Critical concentration in oligomer : Primary aggregates : curved if pH=7

4: When Salt concentration is sufficient: Branched aggregate formation (disulfides bonds, hydrogen and hydrophobic interactions)

Objective:
Characterization of aggregates produced at semi-industrial scale

Dynamic light scattering (DLS) in batch

- Large objects are exacerbated
- No differentiation in large particle populations

Size exclusion chromatography (SEC)

- Aggregates obstruct the column

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Size exclusion chromatography (SEC)

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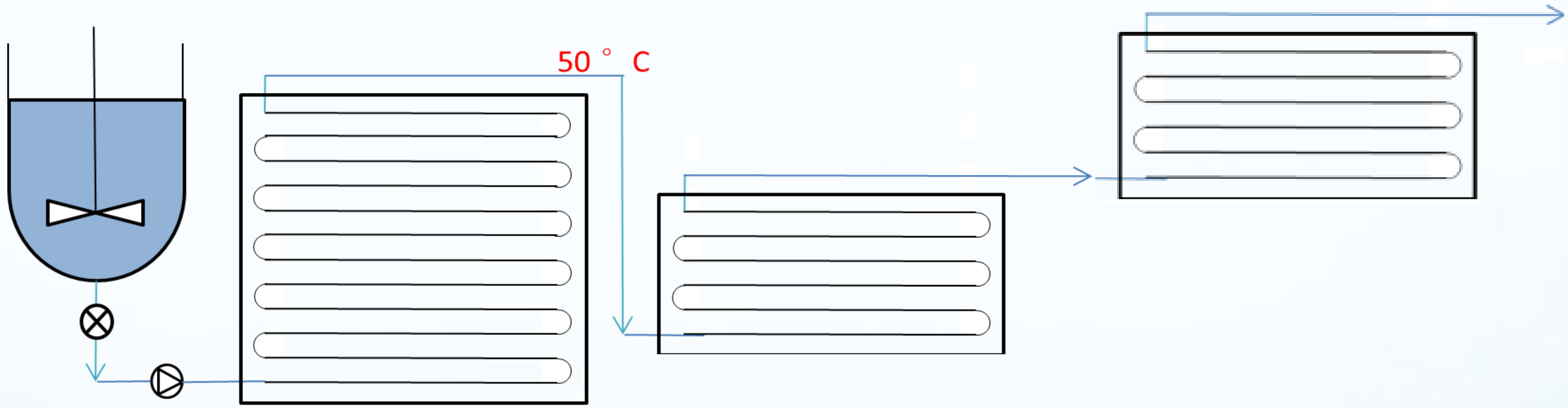
As-FlFFF

- Injection of entire product without filtration
- Fractionation of large particles

Transmission Electronic Microscopy (TEM)

- Correlation between size/form factor (As-FlFFF) and microscopic visual

Aggregates production by semi-industrial way



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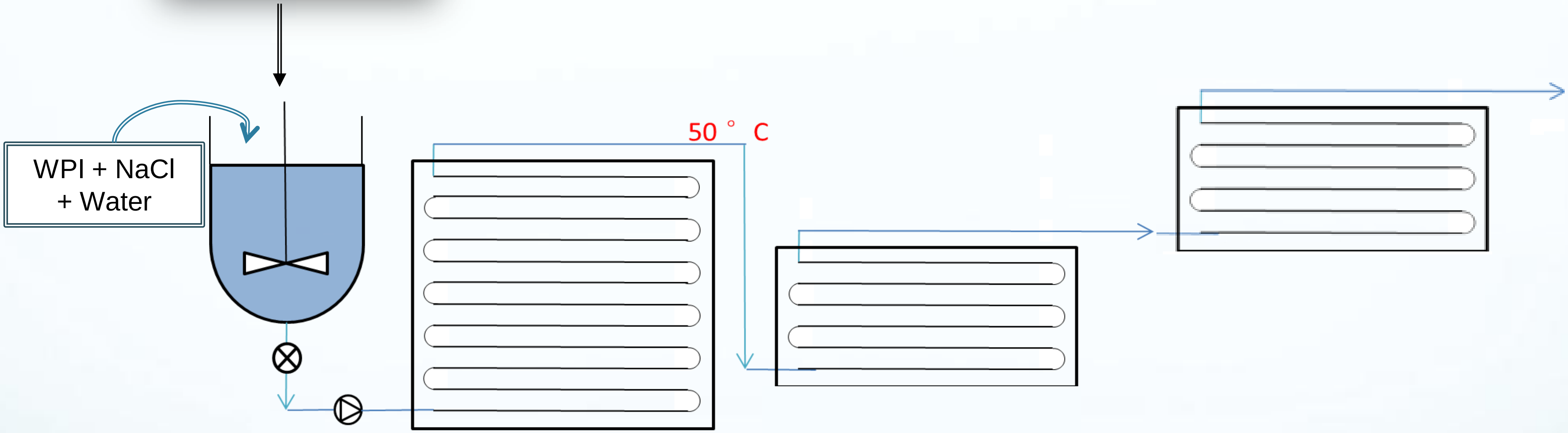

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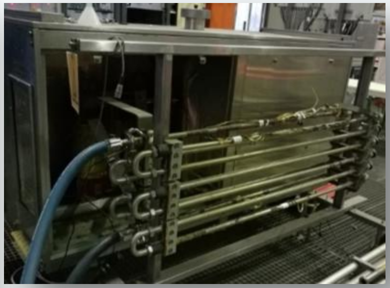
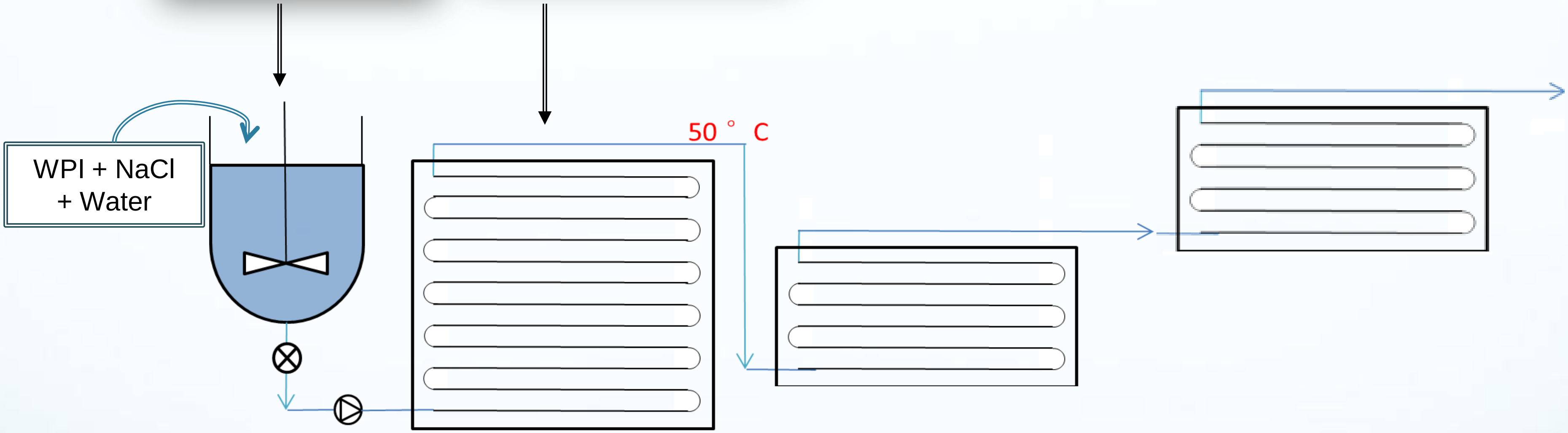
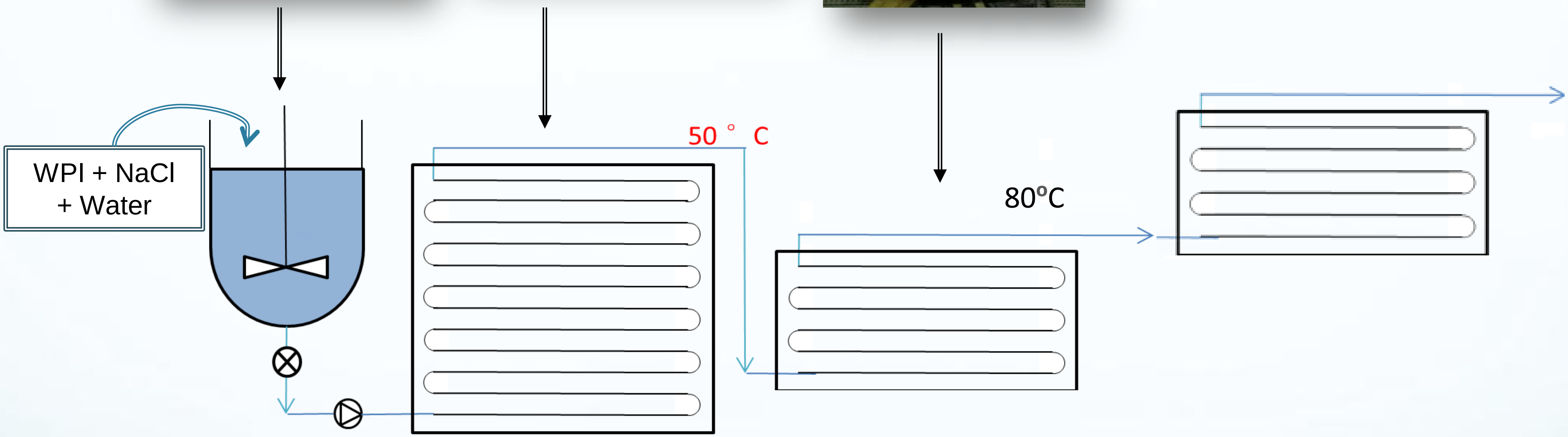




Preheating

20ºC →50ºC

Aggregates production by semi-industrial way



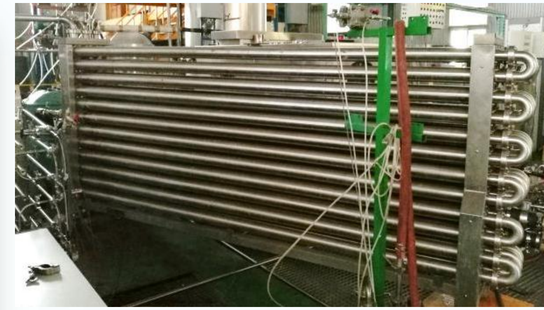
Preheating

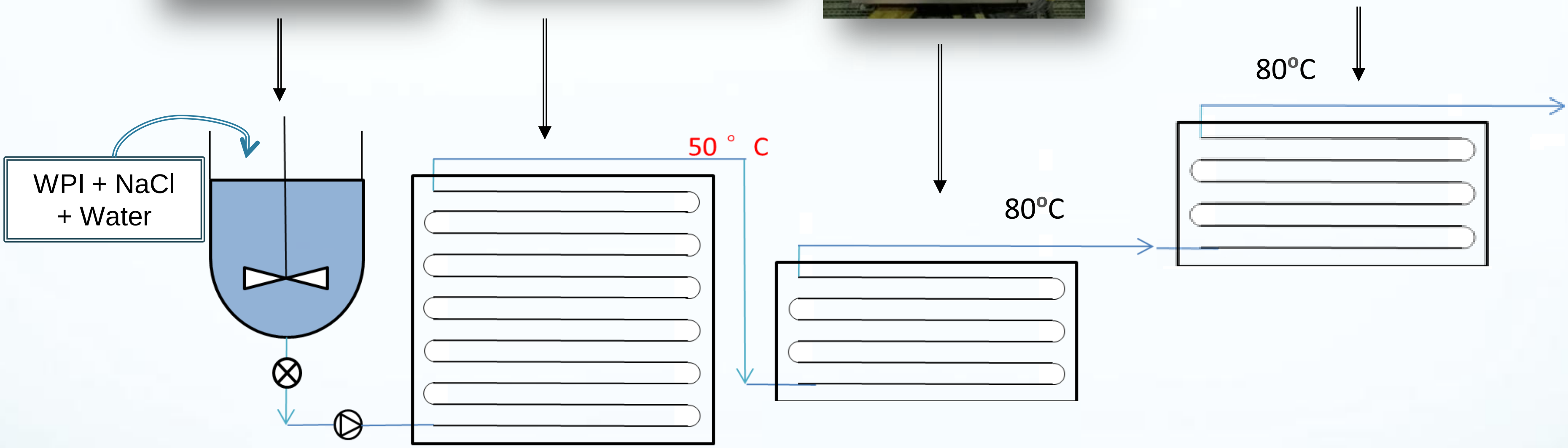
20°C → 50°C

Heating

50°C → 80°C

Aggregates production by semi-industrial way





Preheating

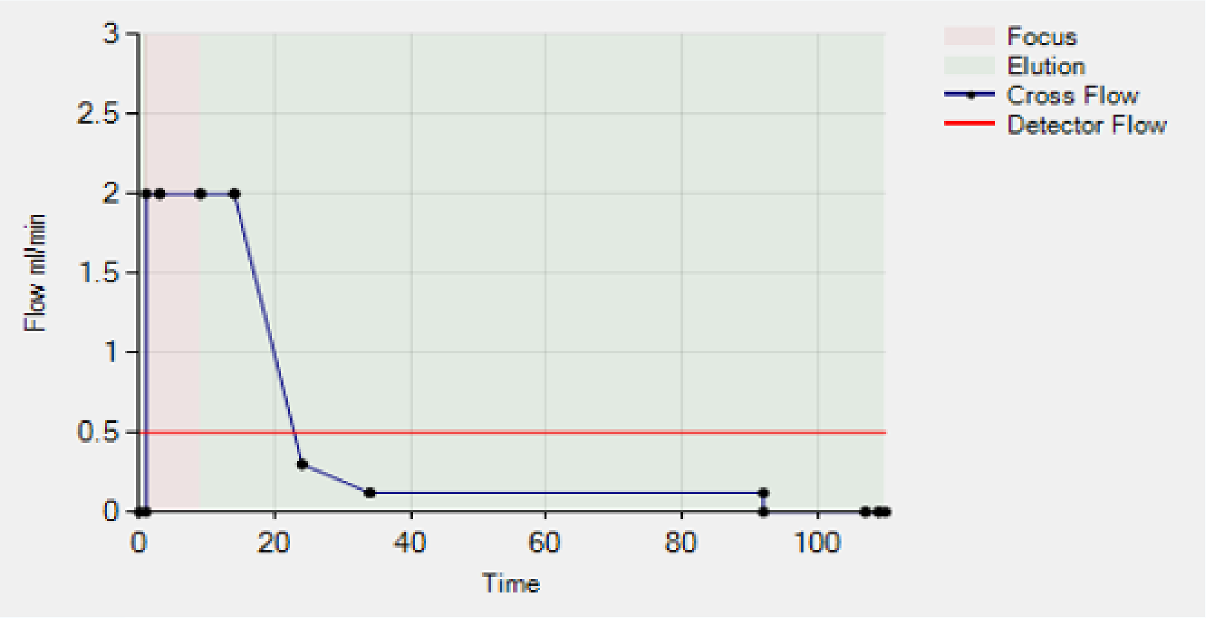
20°C → 50°C

Heating

50°C → 80°C

Static or dynamic holding
80°C ; up to 1h

As-FlFFF method



✓**Wyatt Dualtec System**

✓**MALS**

✓**Differential Refractometer**

Elution : 1 min
Focus : 2 min
Focus + inject : 6 min

Eluent	**Ultrapure water + 0.02% NaN_3 + 45mM NaCl**
Membrane	**Regenerated cellulose, cut-off 10kDa**
Spacer (µm)	**350**
Canal	**Short Canal**
Injected mass (µg)	**20**

Interest parameters

- Radius of gyration (Rg) distribution
- Molar mass (M) distribution

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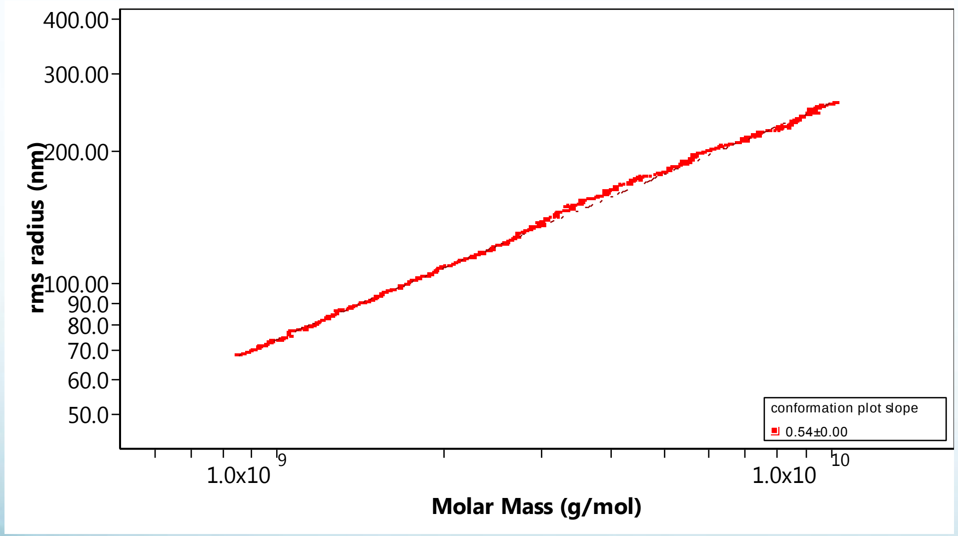
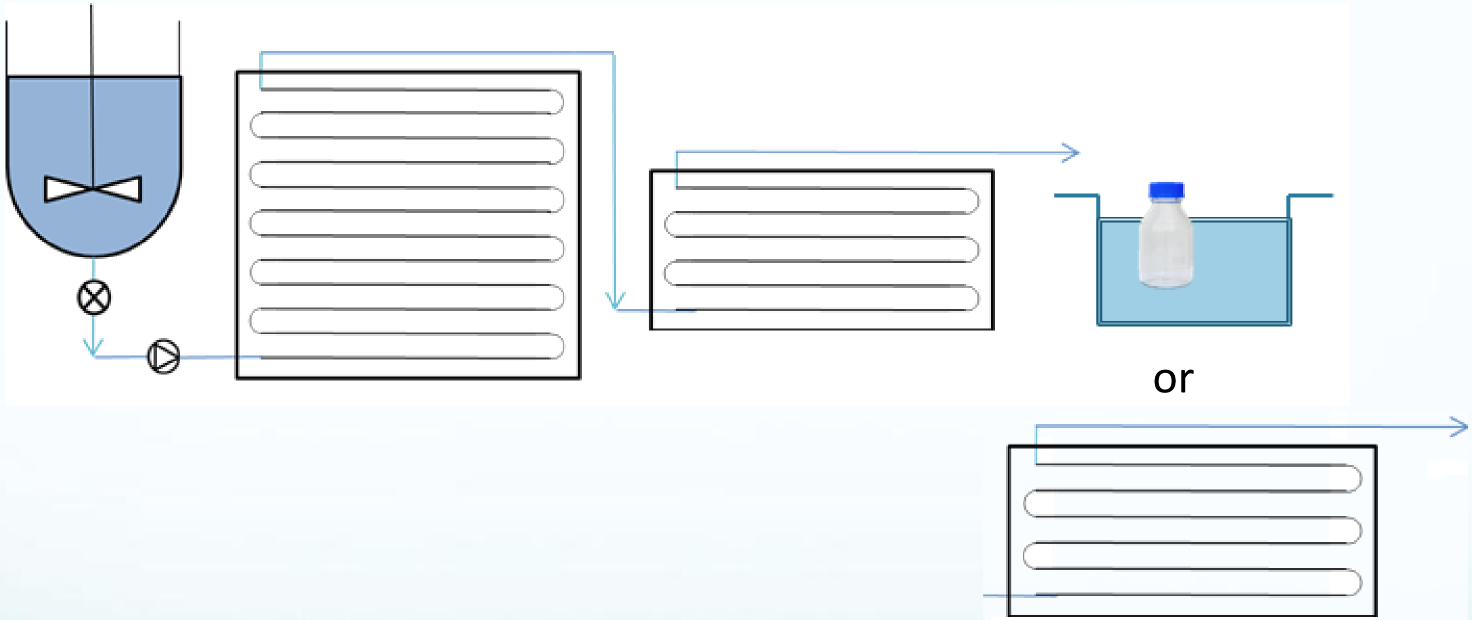
- Radius of gyration (Rg) distribution
- Molar mass (M) distribution
- Normalized concentration: Concentration from RI divided by Calculated Mass (=Mass recovery) from refractometer ; dn/dc=0.1850 (it allows to compare samples)
- Fractal dimension (Df): $\log(\mathrm{Rg}).Df = f(\log(M))$



Molecular conformation model :

Df = 1 : Rod shape
Df ~ 2 : Coil
Df = 3 : Spherical shape

Size/shape aggregate control : process parameter variations



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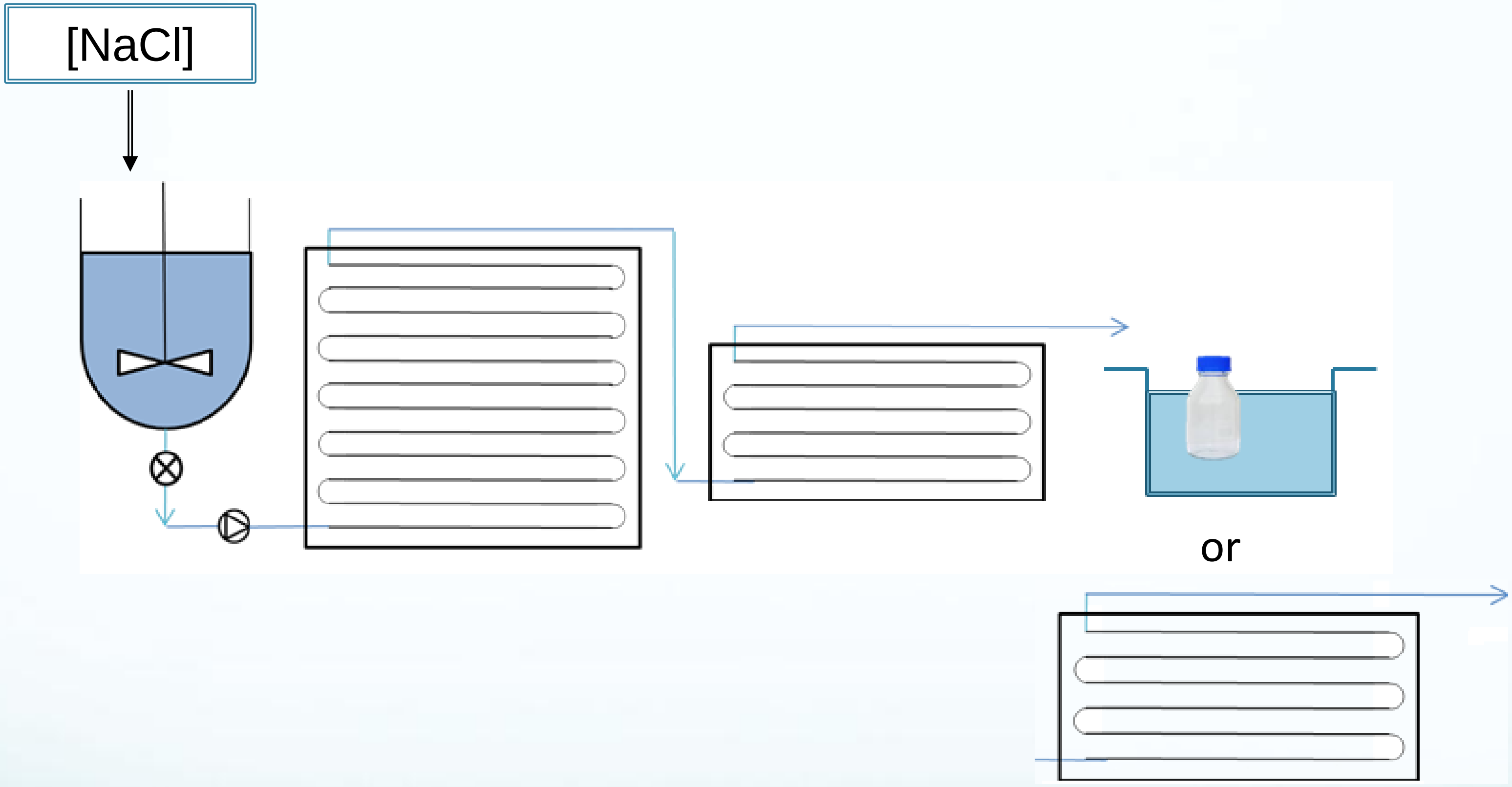

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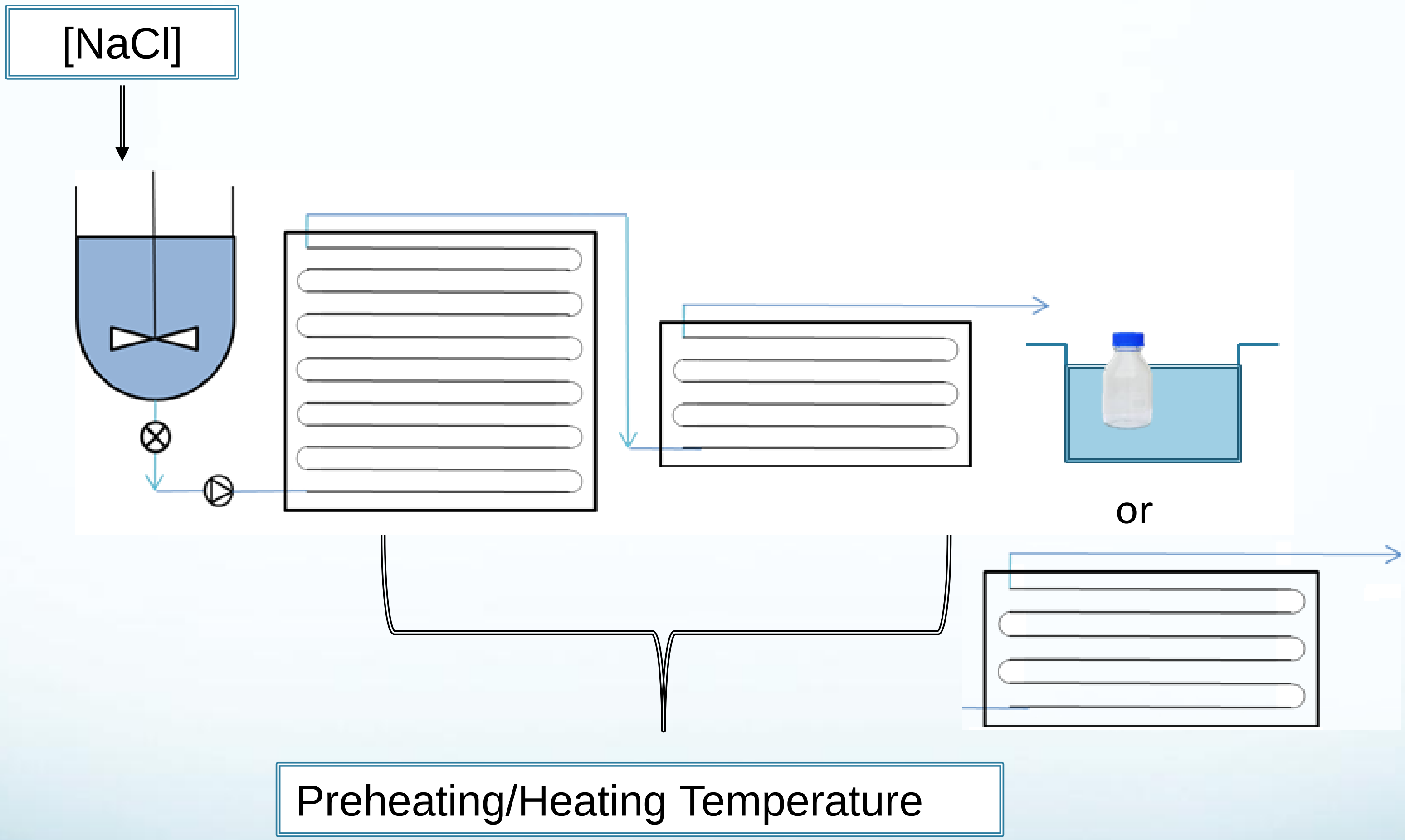

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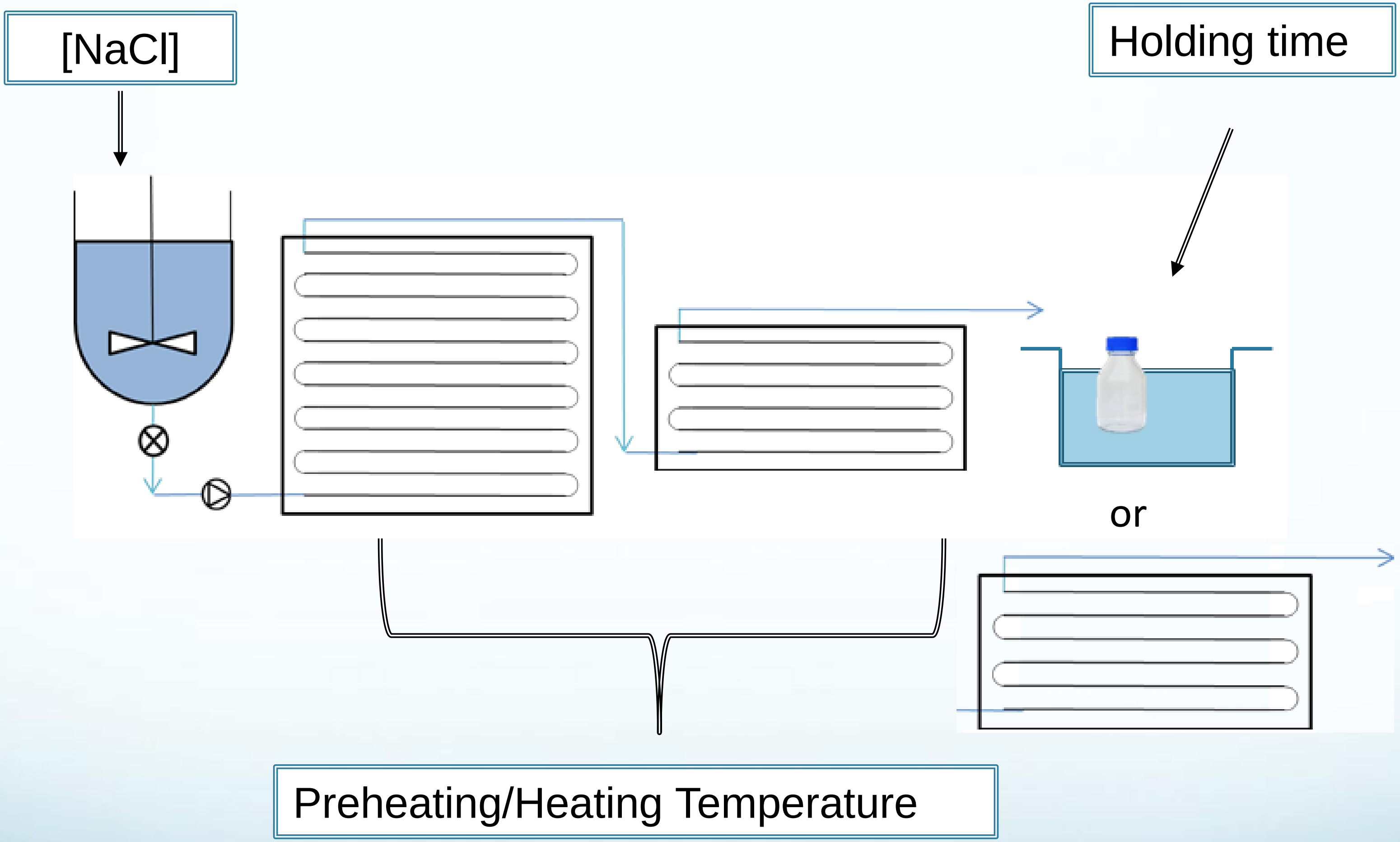

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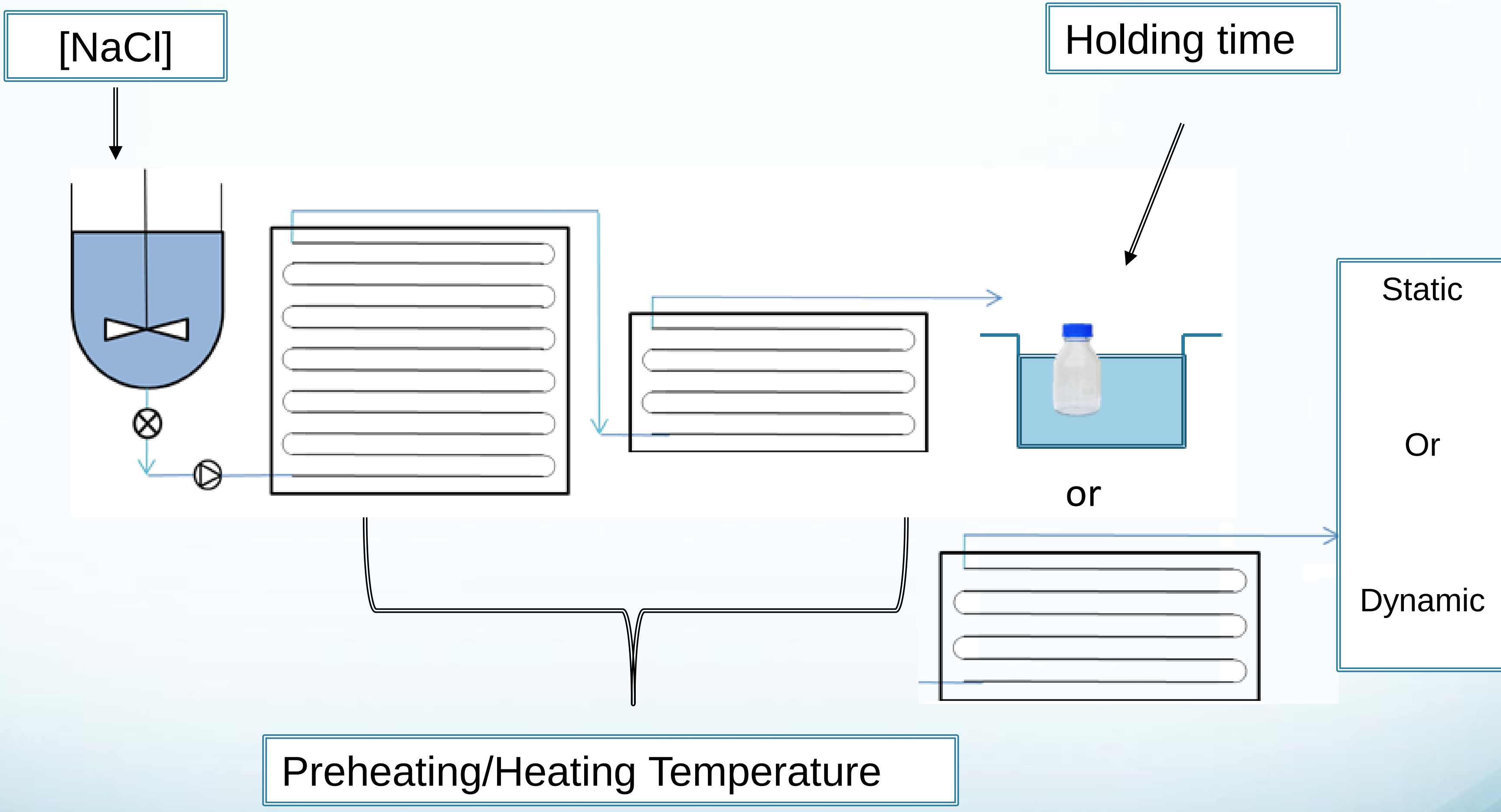

First production results

5% proteins ; **10mM** NaCl ; **Static** holding ; **15min** of holding time ; **50/80˚C** Preheating/Heating

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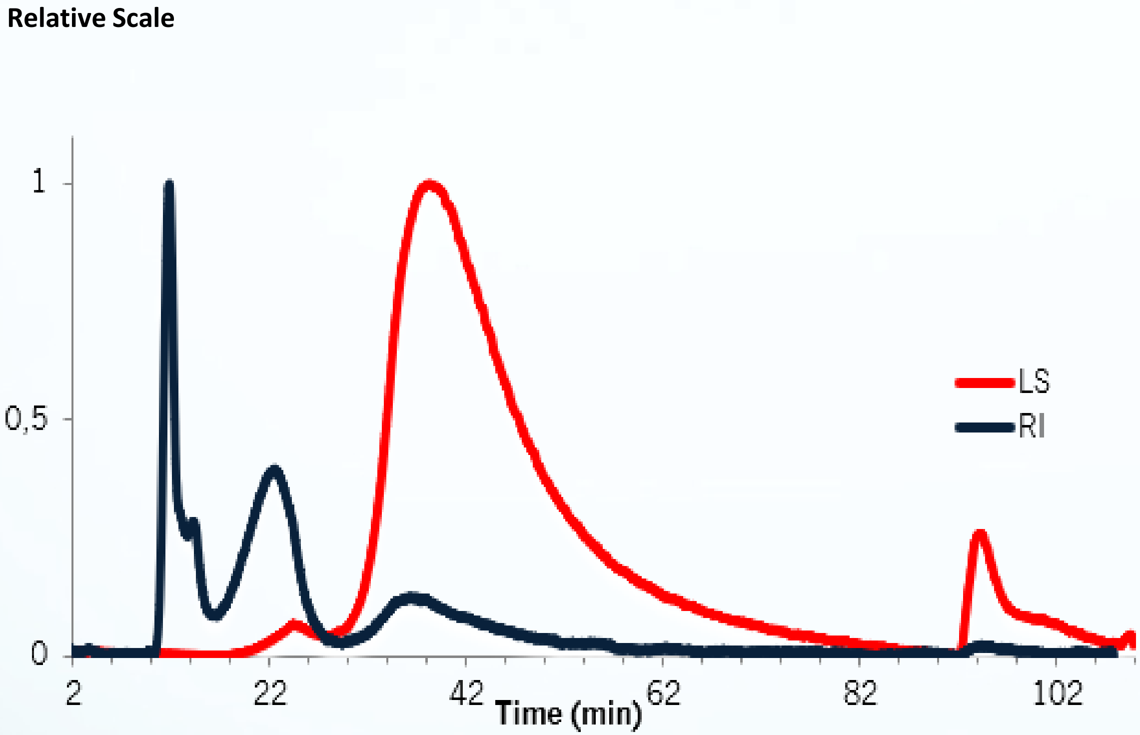

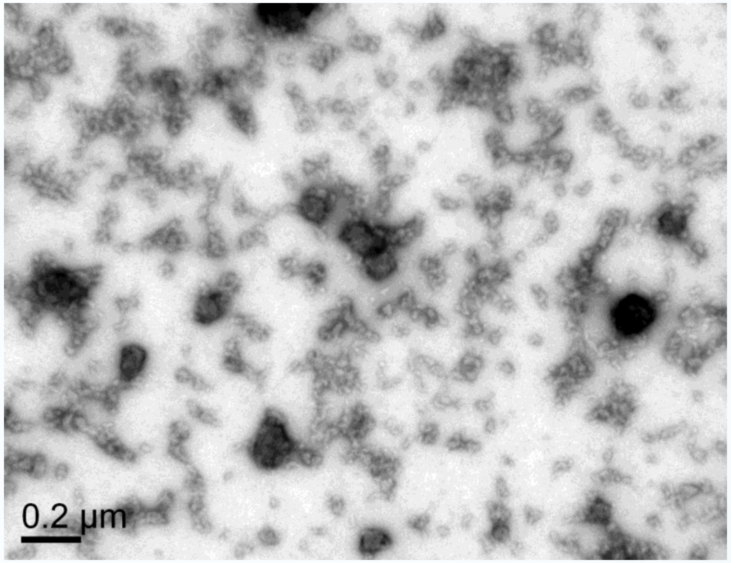

Transmission Electronic microscopy (negative coloration)

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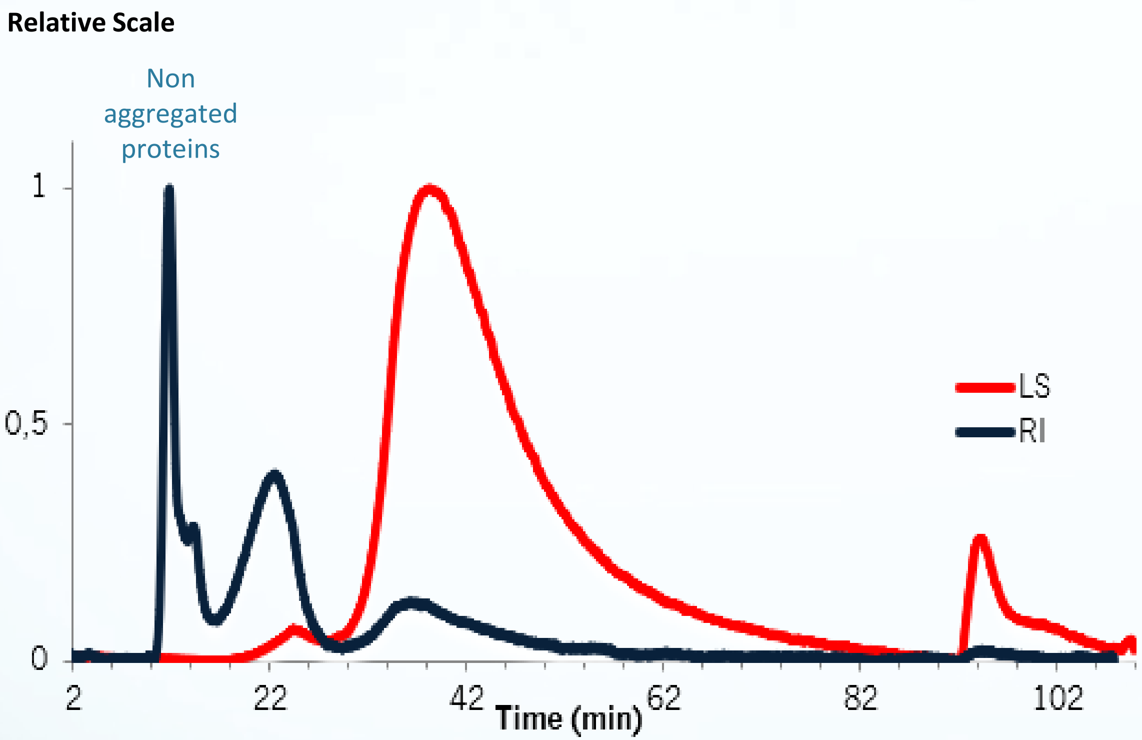

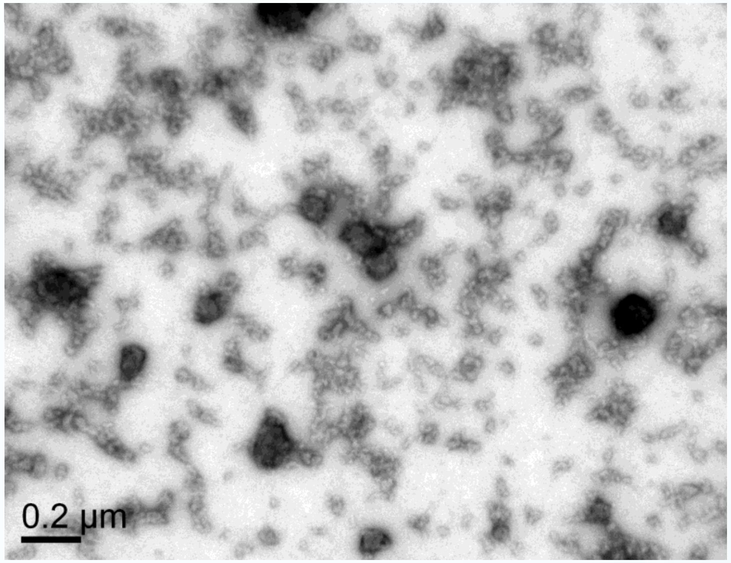

Transmission Electronic microscopy (negative coloration)

- 1rst population (10-15min): non aggregated whey proteins

Introduction | Methods | **Results** | Conclusion and perspectives

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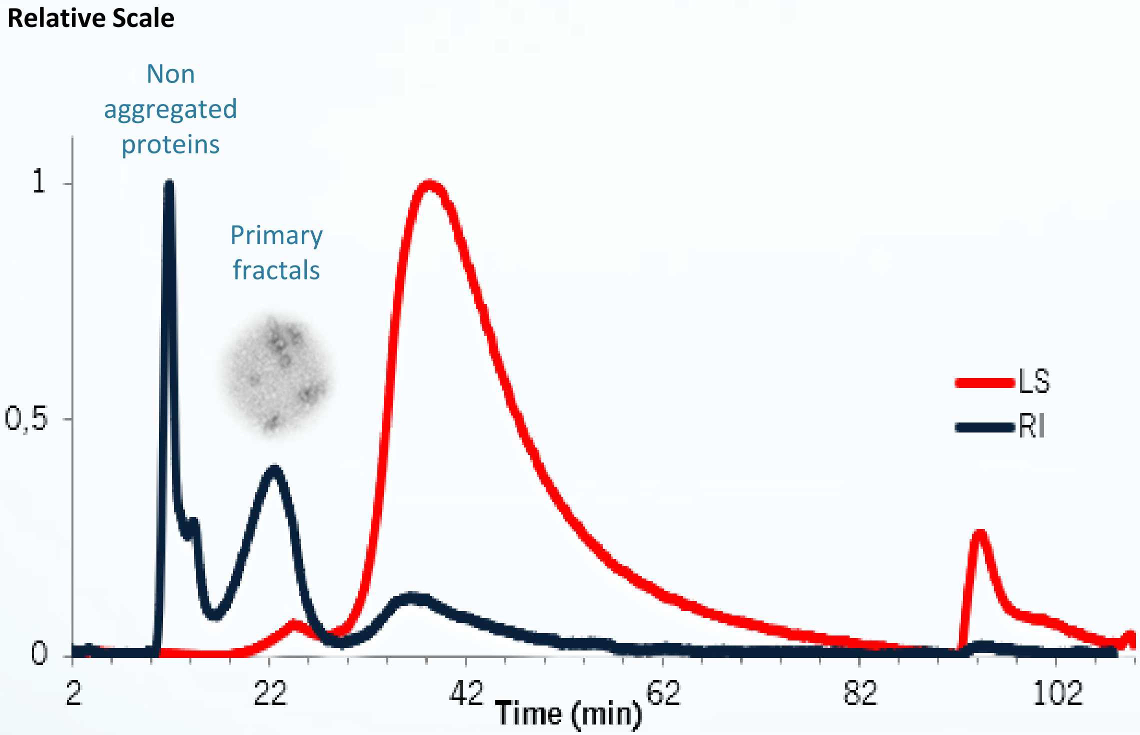

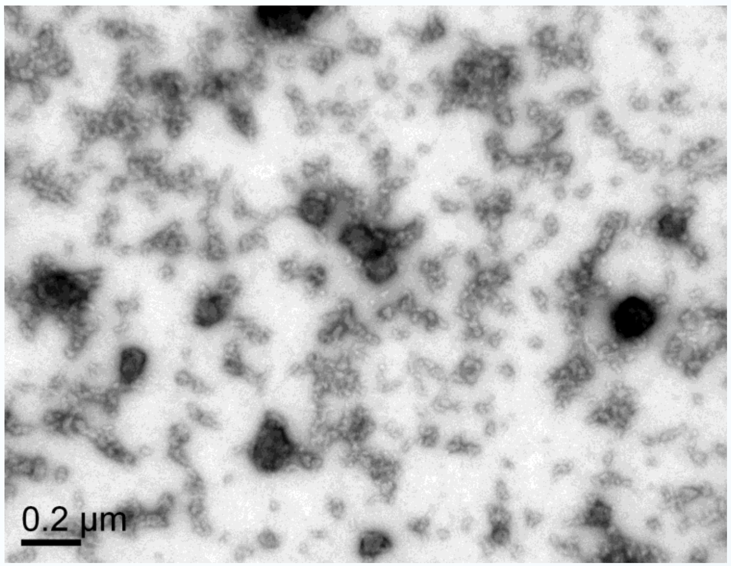

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- 1rst population (10-15min): non aggregated whey proteins
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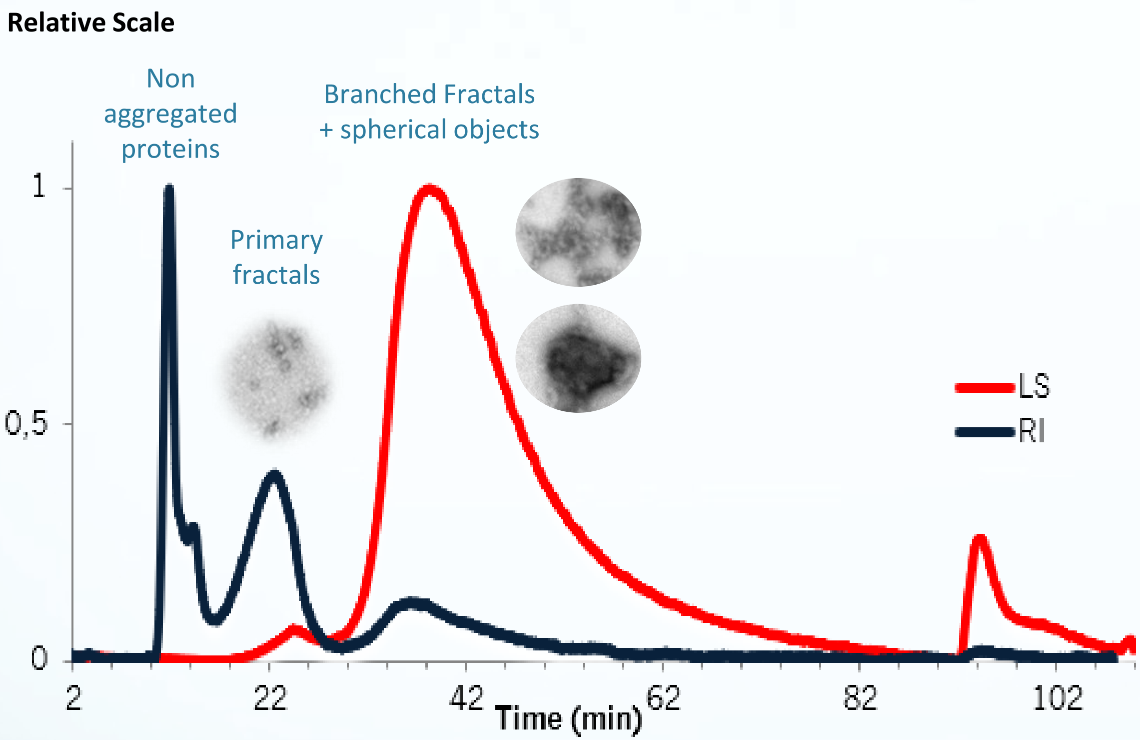
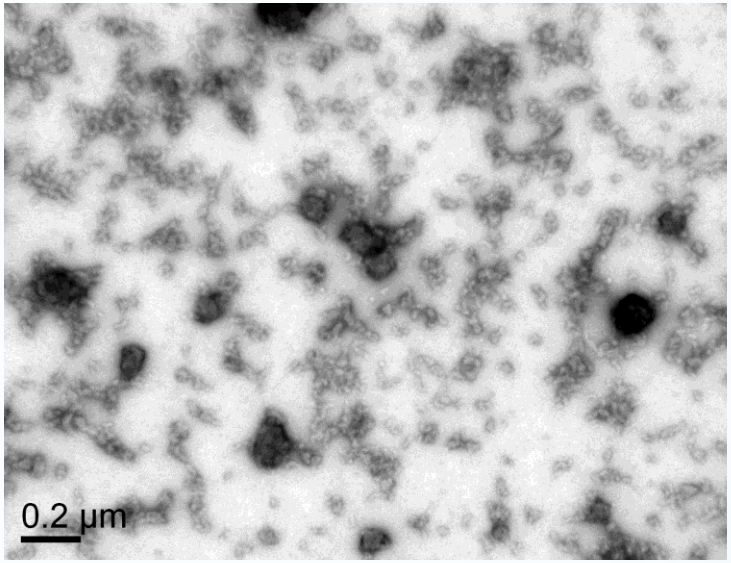
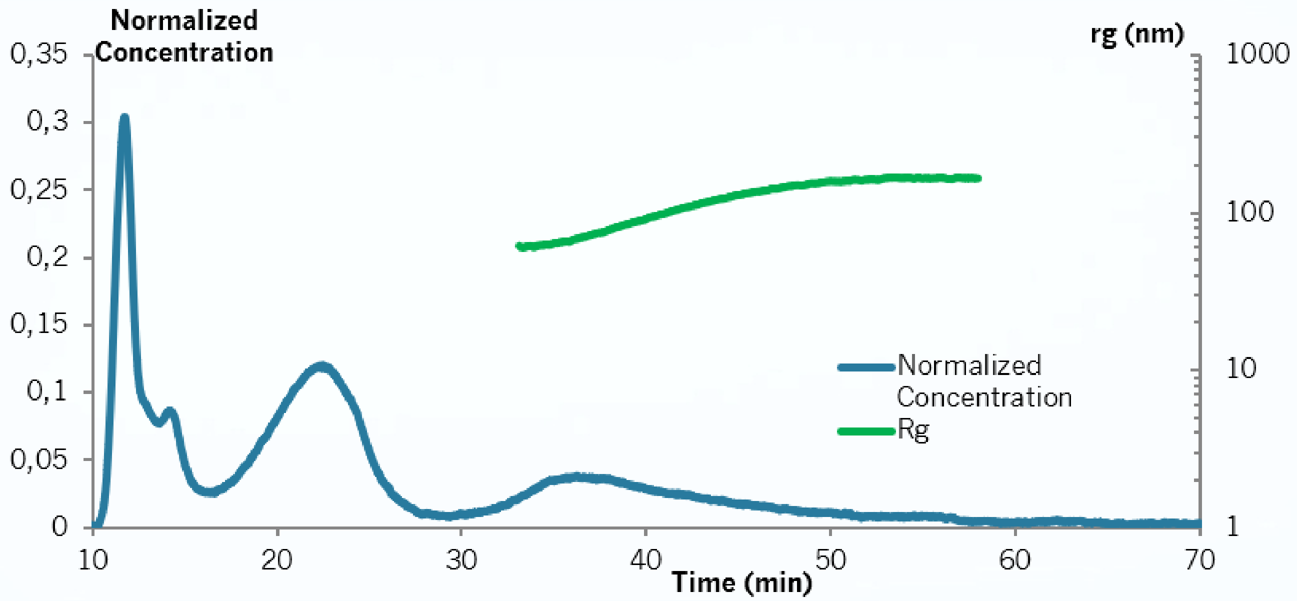




Transmission Electronic microscopy (negative coloration)

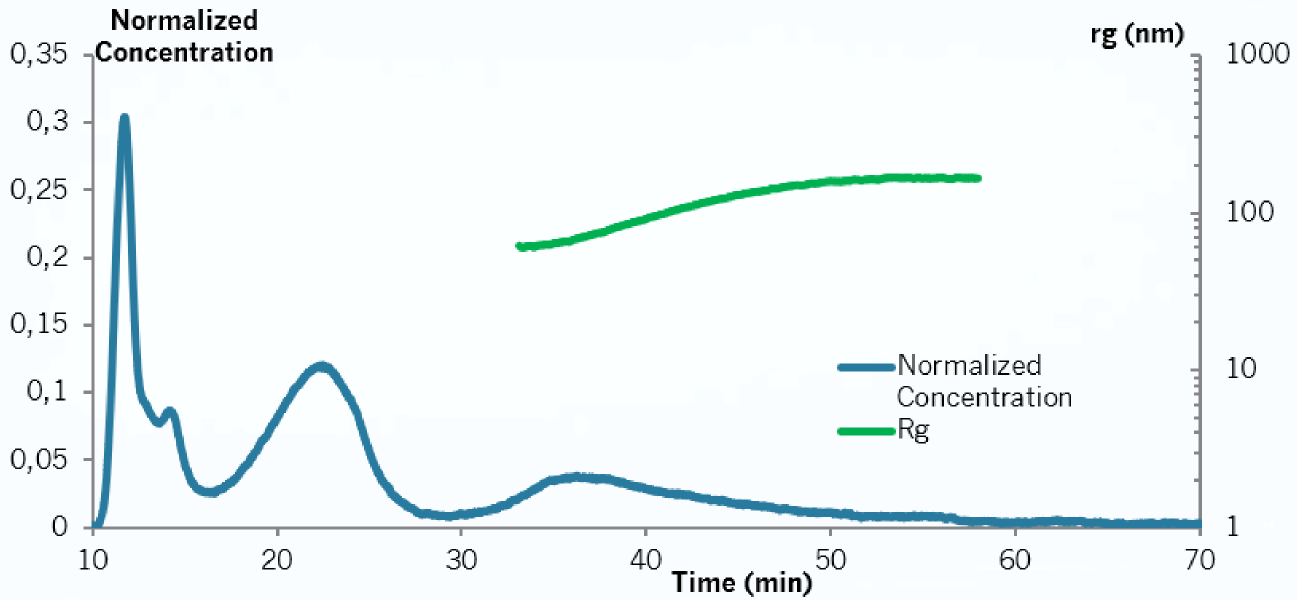
- 1rst population (10-15min): non aggregated whey proteins
- 2nd population (15-30min): primary fractals
- 3rd population (after 30min): mix between large fractals and spherical objects

First production results



Introduction | Methods | **Results** | Conclusion and perspectives

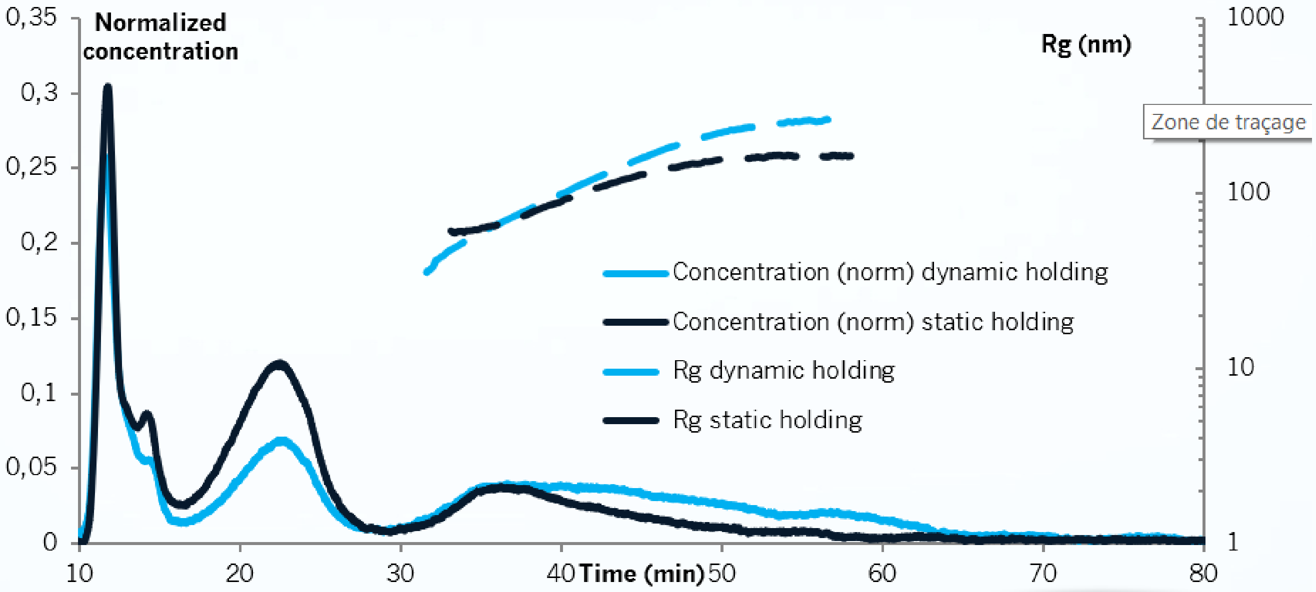
First production results



	$\overline{Mw}$ (g/mol)	$\overline{Rg}$ (nm)	Mass fraction (%)
1st population	/	/	30
2nd population	$8{,}5.10^{6}$ ±14%	/	40
3rd population	$8{,}3.10^{8}$ ±1,3%	79±3%	30

Objective : Vary process parameters to obtain largest aggregates

Dynamic/static holding



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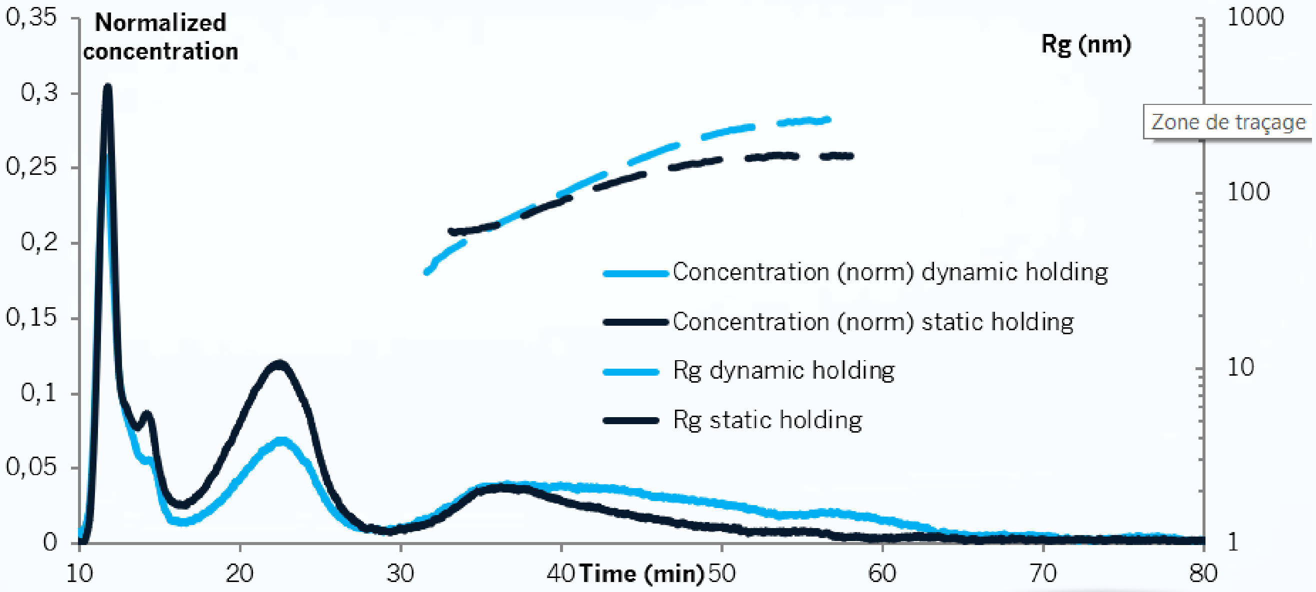

Primary fractals aggregate together to form larger objects

$\overline{Rg}$ =95nm$\pm$3%

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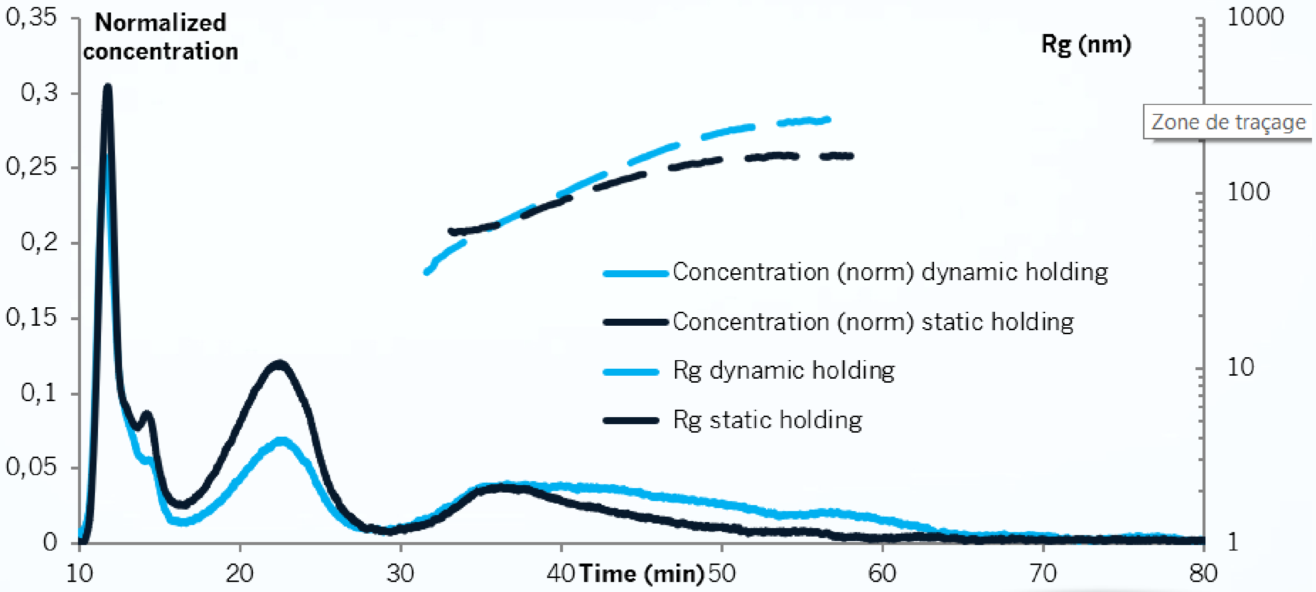
Dynamic/static holding

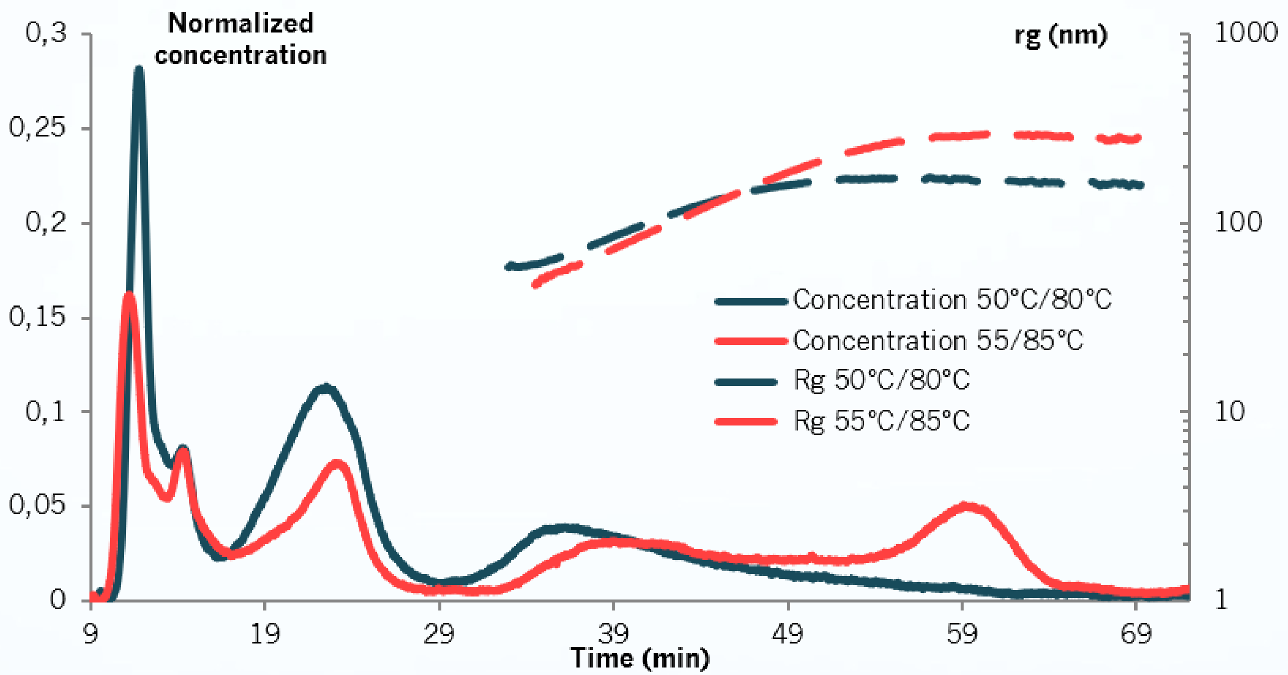


	Static Mass fraction (%)	***Dynamic*** Mass fraction (%)
1st population	30	30
2nd population	40	26
3rd population	30	44

Primary fractals aggregate together to form larger objects
$\overline{Rg}$ =95nm ± 3%

Interaction probability between fractals is more important in dynamic system

Preheating/Heating Temperature Variation
50/80° C ⟶ 55/85° C



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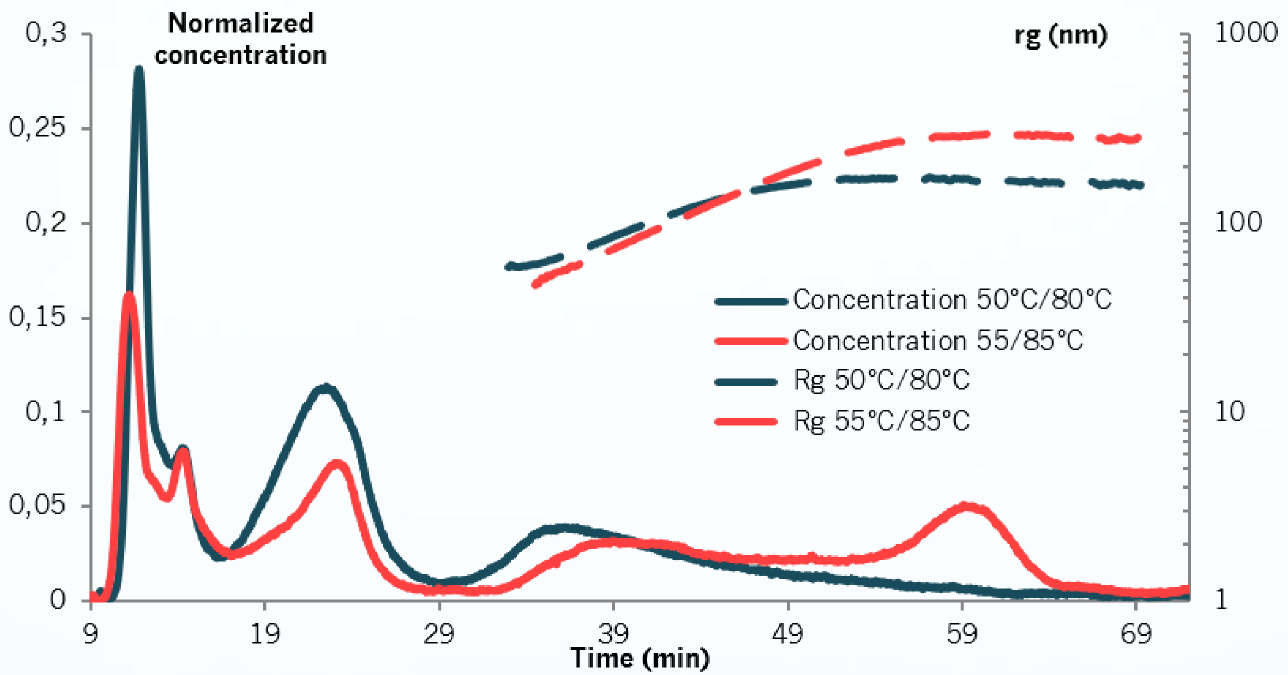

New population formation (55-65min) by increasing preheating/heating temperature

$\overline{Rg}$ =285nm±0,7%

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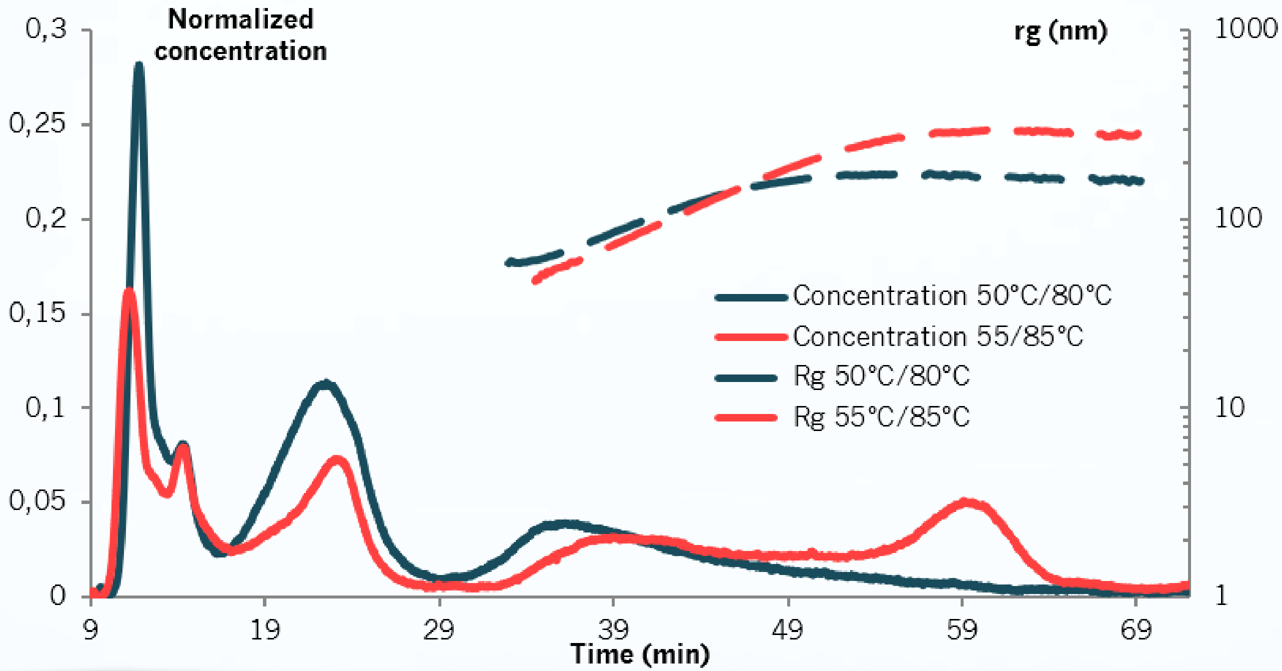
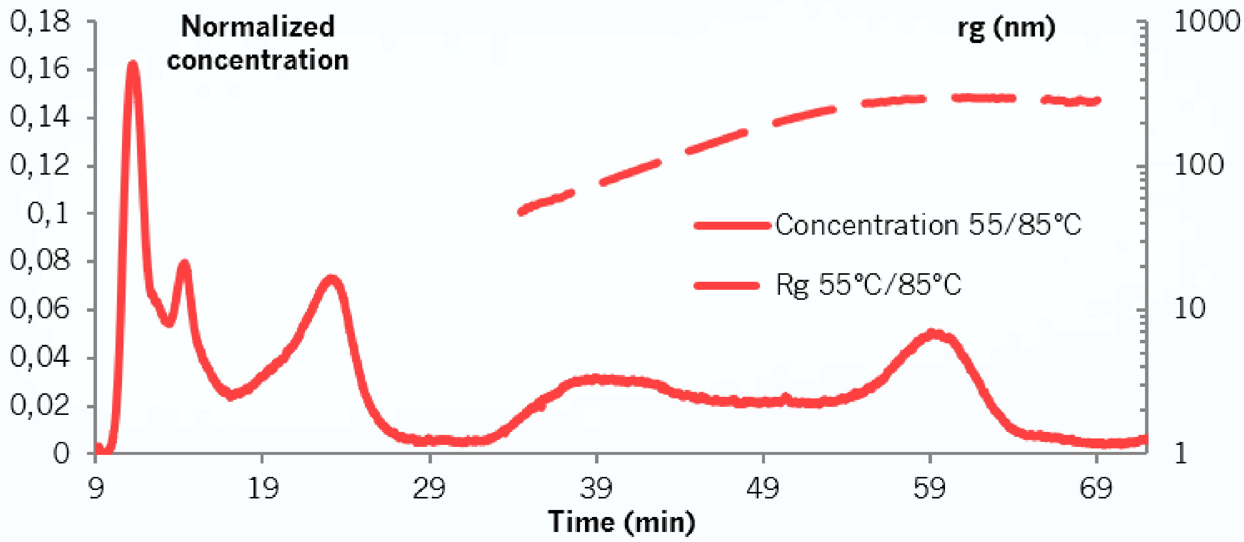


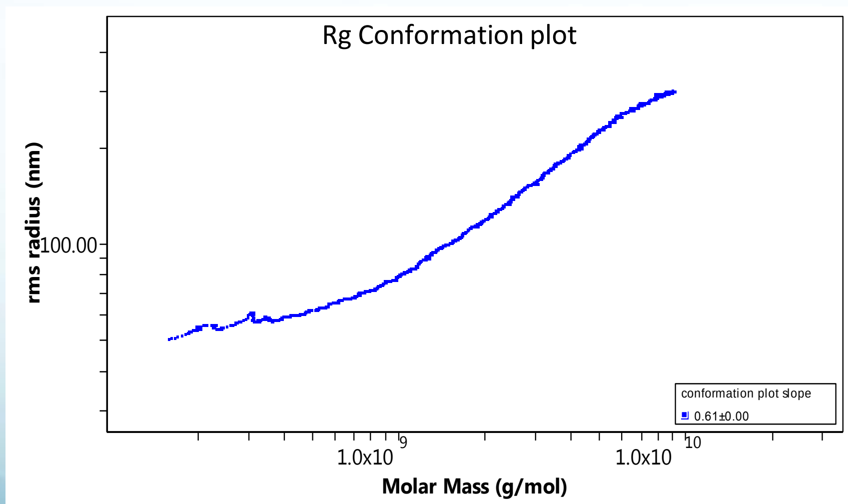
New population formation (55-65min) by increasing preheating/heating temperature

$\overline{Rg}$ =285nm±0,7%

⟶ Whey proteins and primary fractals concentration reduces to form a 4th largest population

Fractal Dimension

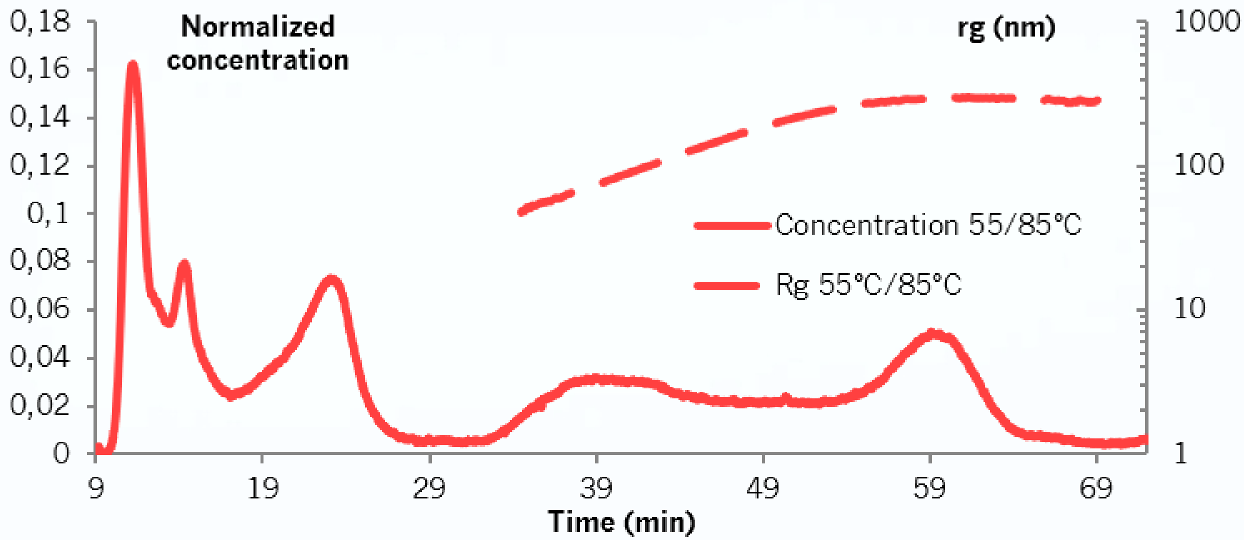


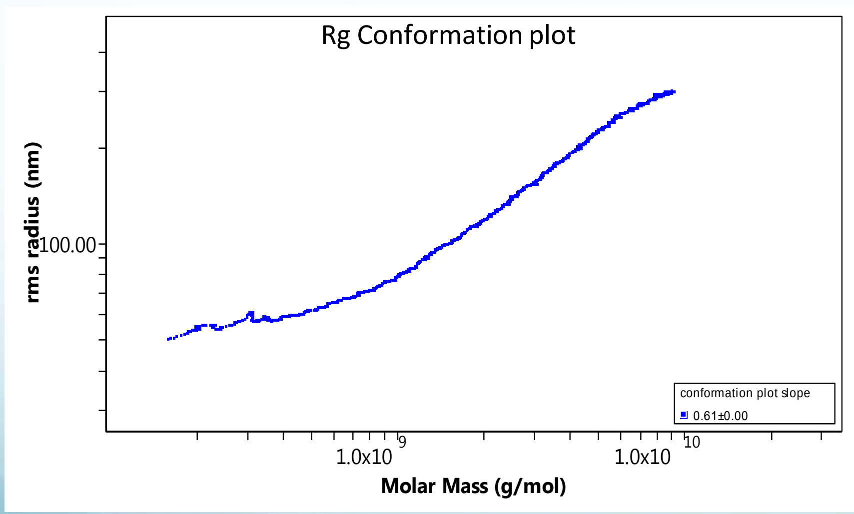


$$\log(\mathrm{Rg}) = \frac{1}{Df} \cdot f(\log(Mw))$$ between 35-65min

Introduction | Methods | **Results** | Conclusion and perspectives

Fractal Dimension





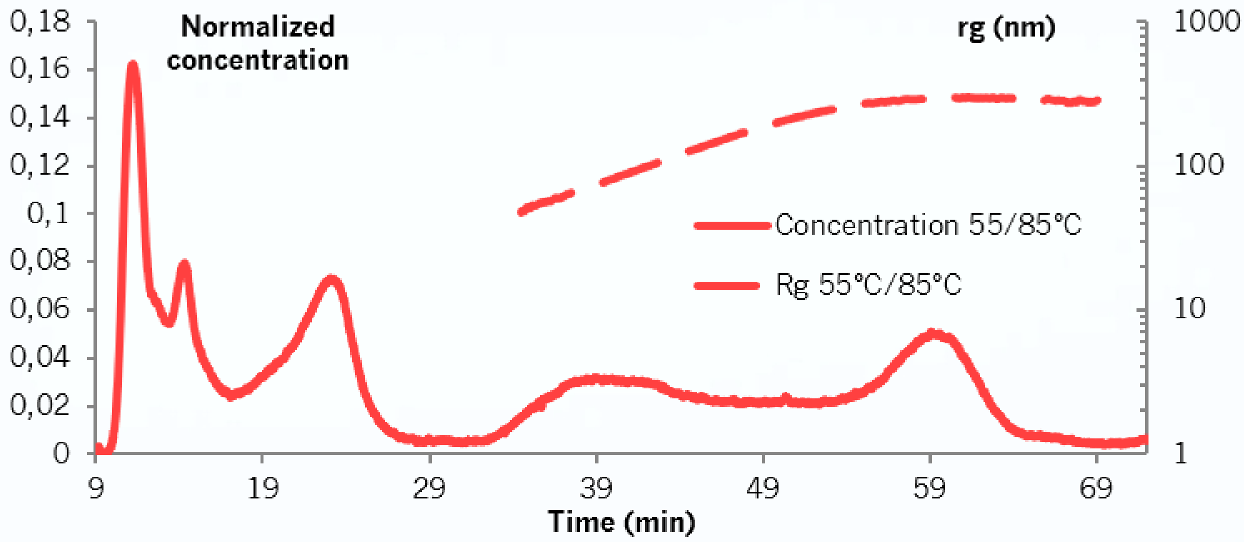
$$\log(Rg) = \frac{1}{Df} \cdot f(\log(Mw))$$ between 35-65min

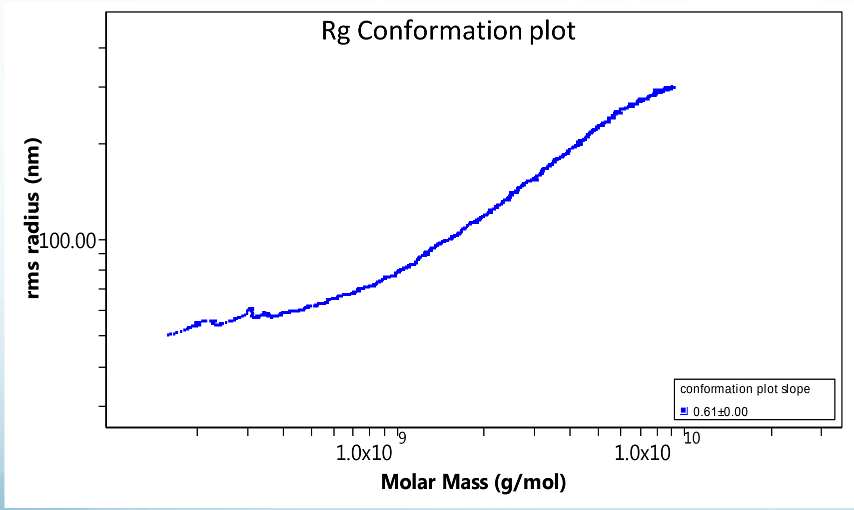
The slope increases over time (Df decreases)

→ 4th population is less dense than 3rd one

Maybe there are less spherical objects?

Fractal Dimension





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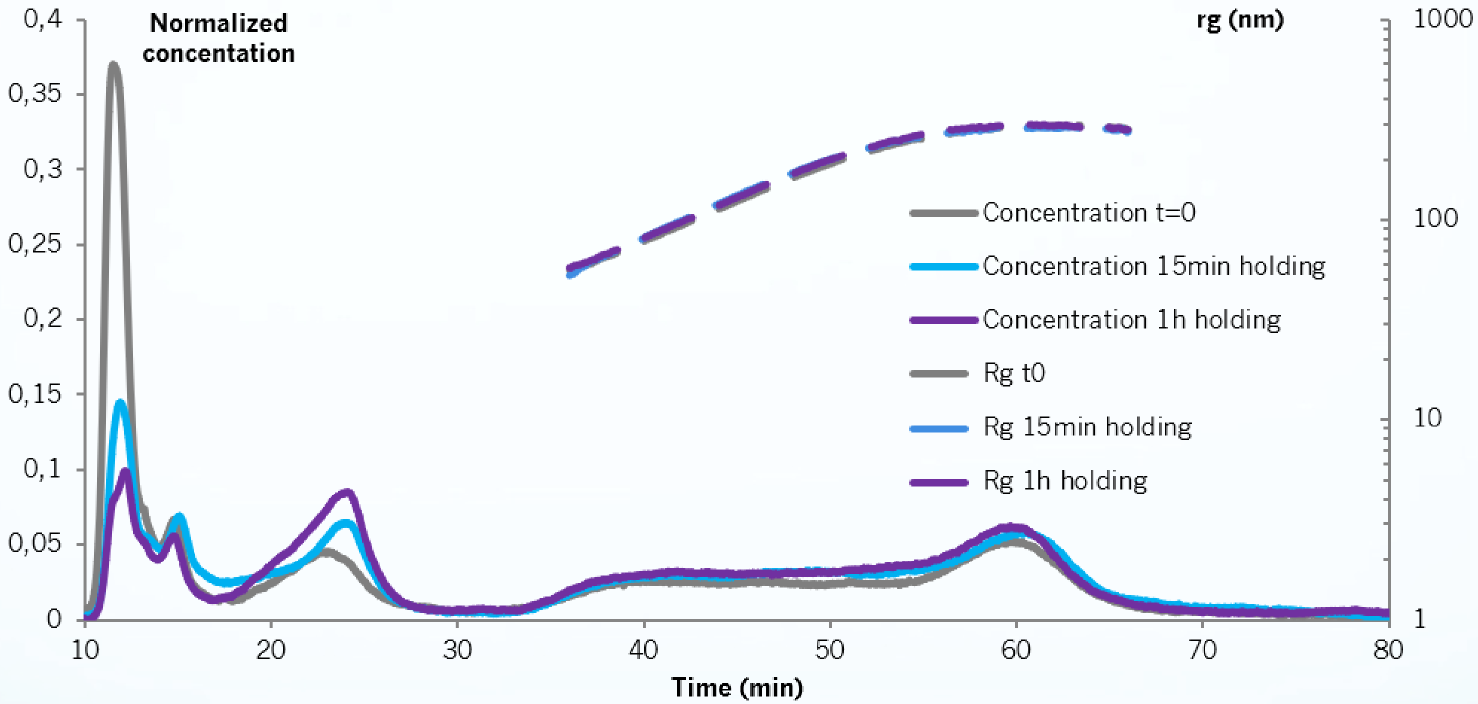
Maybe there are less spherical objects?

→ Fraction collection + TEM

Holding time variation

Holding time : 0/15min/1h at 80° C

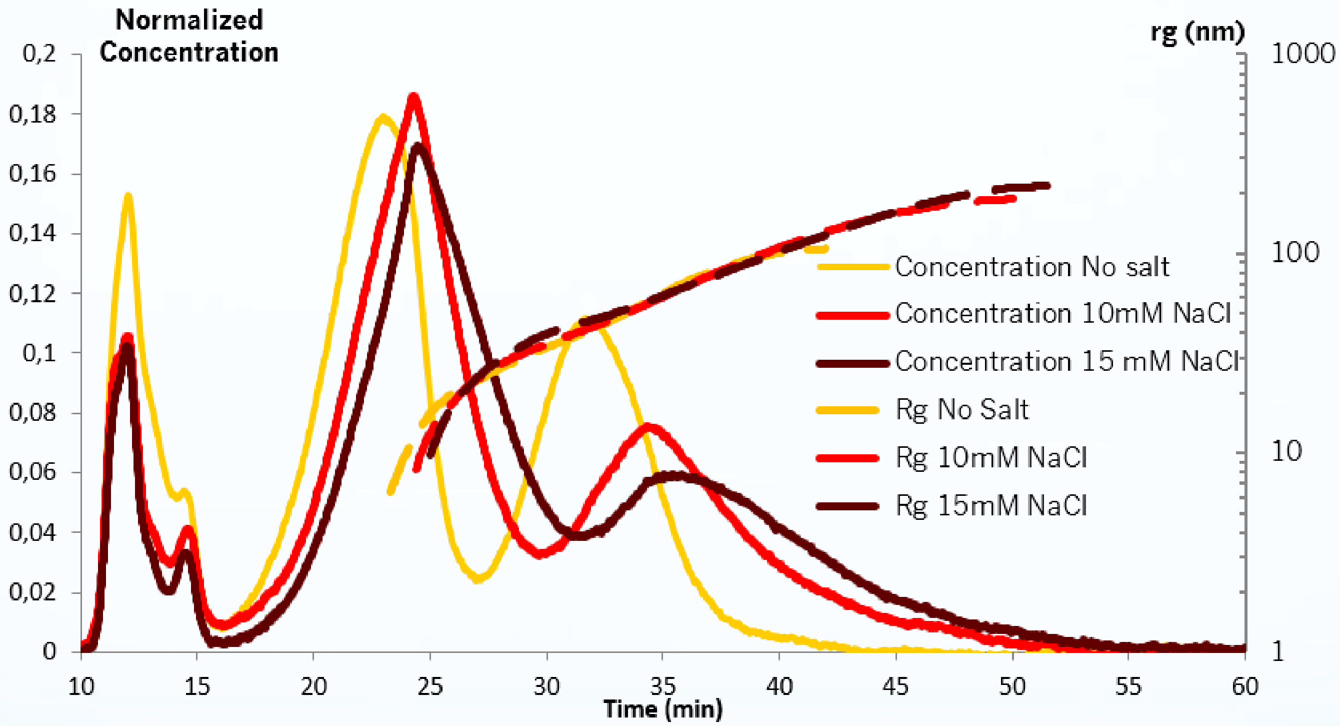


- Last population size does not change with holding time
- Large aggregates are present after heating without holding (t=0)
- During holding, non-aggregated proteins form primary fractals

→ Work on pre-heating and heating parameters

Salt Variations

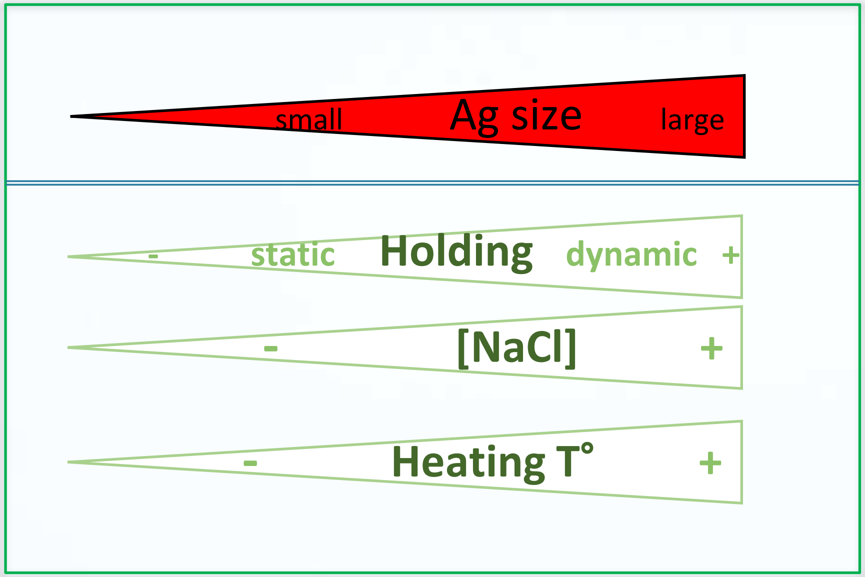
No Salt/10mM/15mM NaCl



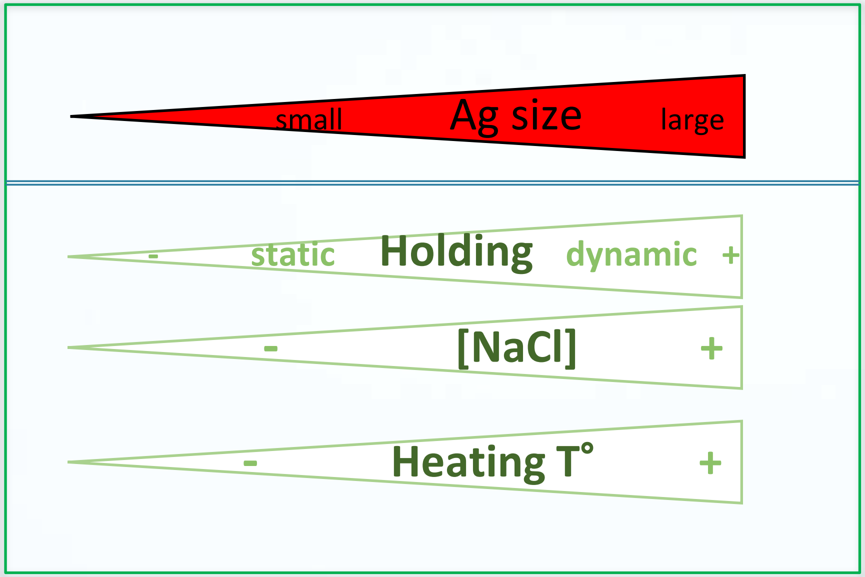
The more salt concentration increases, the larger aggregates are formed

→ Salt decreases interaction hiding charges: aggregates are produced easier

Conclusions and Perspectives



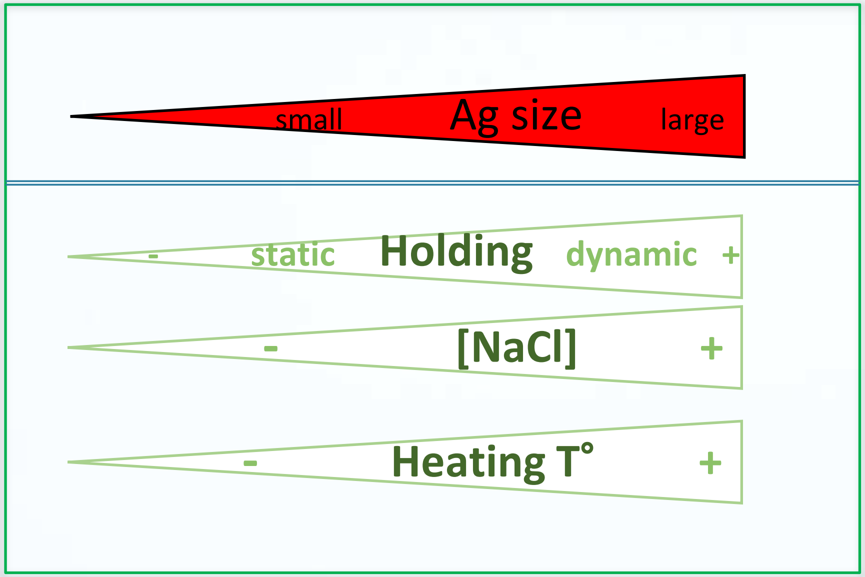
Conclusions and Perspectives



As-FlFFF

- Fractionate whey protein aggregates
- Filtration-free injection
- Informations: Radius of gyration / Molar mass / Conformation
- Tool to analyze and compare semi-industrial productions

Conclusions and Perspectives



As-FlFFF

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<u>Perspectives</u> :

- Flow rate variations
- Tube dimension variations
- Heating rate variations
- Fraction collection + TEM

Thank you for your attention