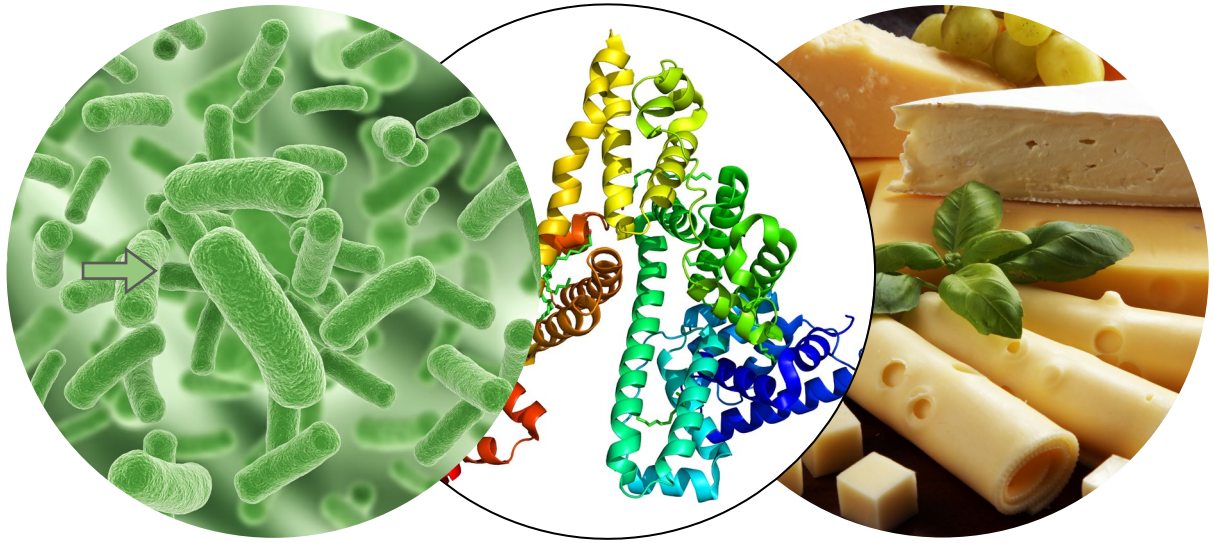
"I would like to eat vegan but
I cannot go without cheese"

Engineering artificial casein micelles for future food

Laurens Antuma, Remko Boom, Julia Keppler



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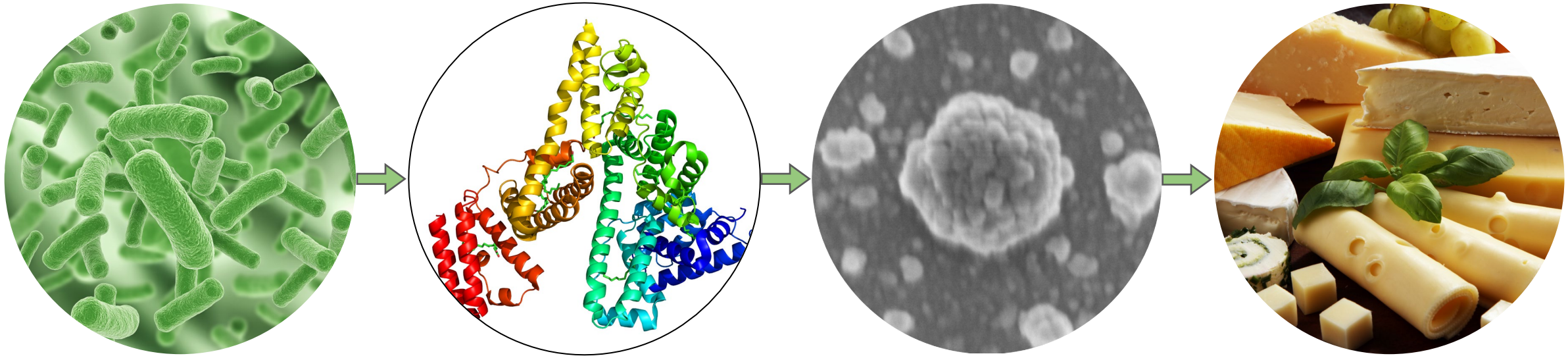
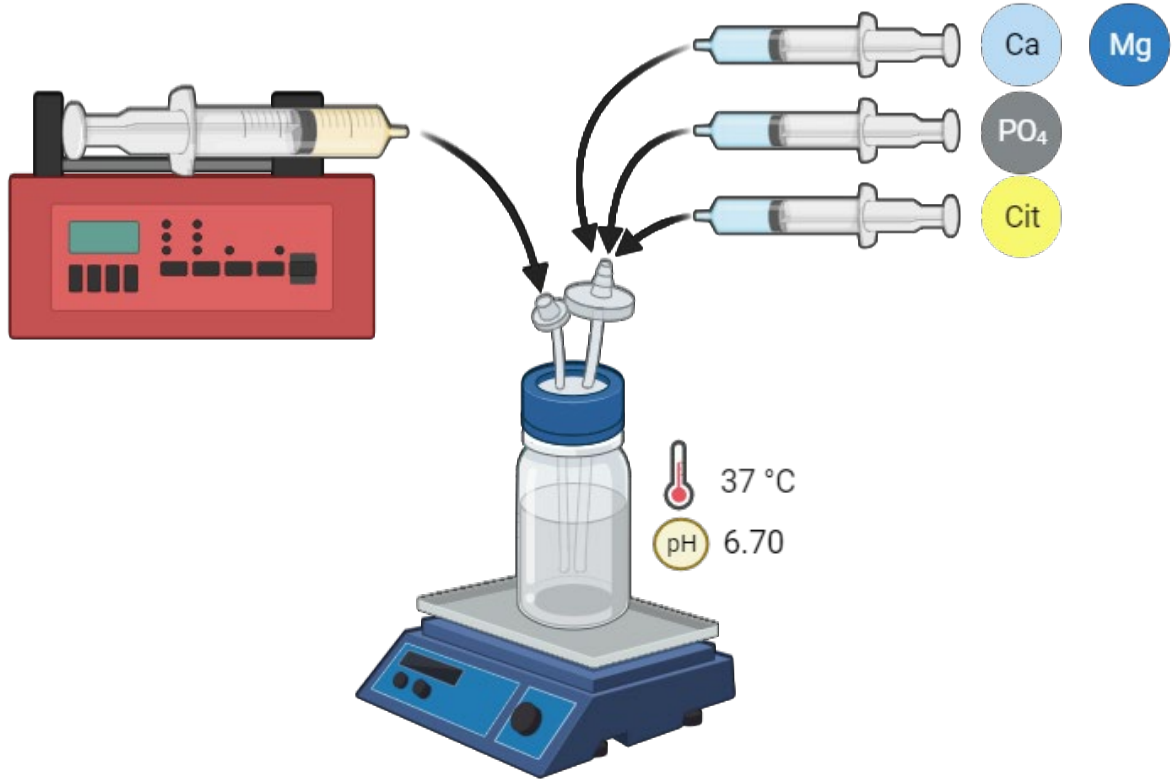


Background

Artificial casein micelles

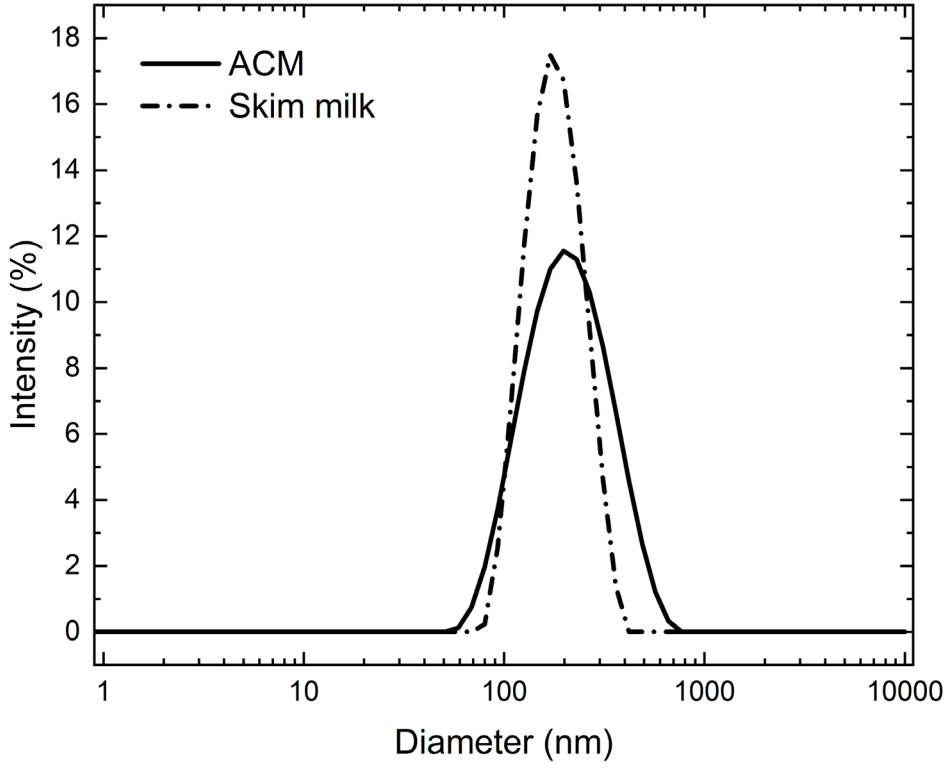
- Controlled mixing of caseins with salts

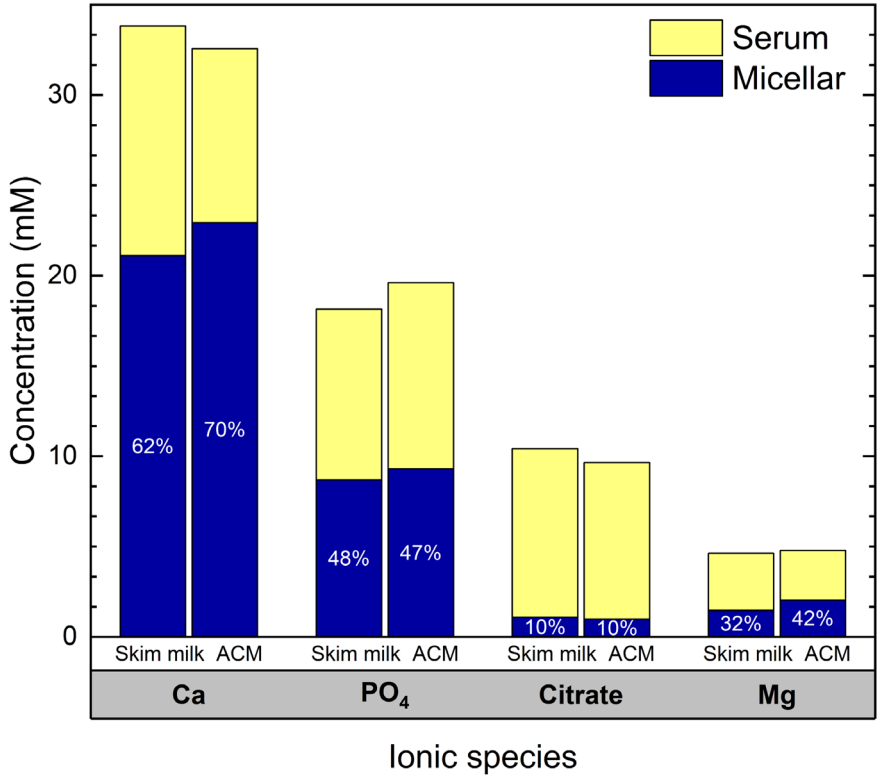


Background

Artificial casein micelles

- Similar properties and functionality as natural casein micelles

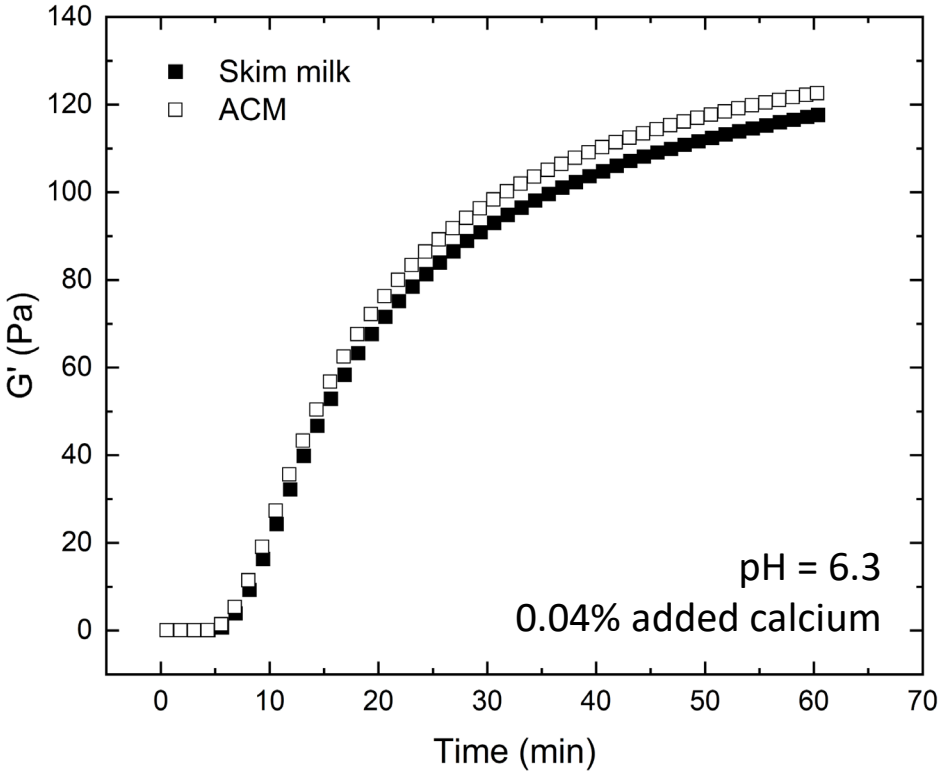




Background

Artificial casein micelles

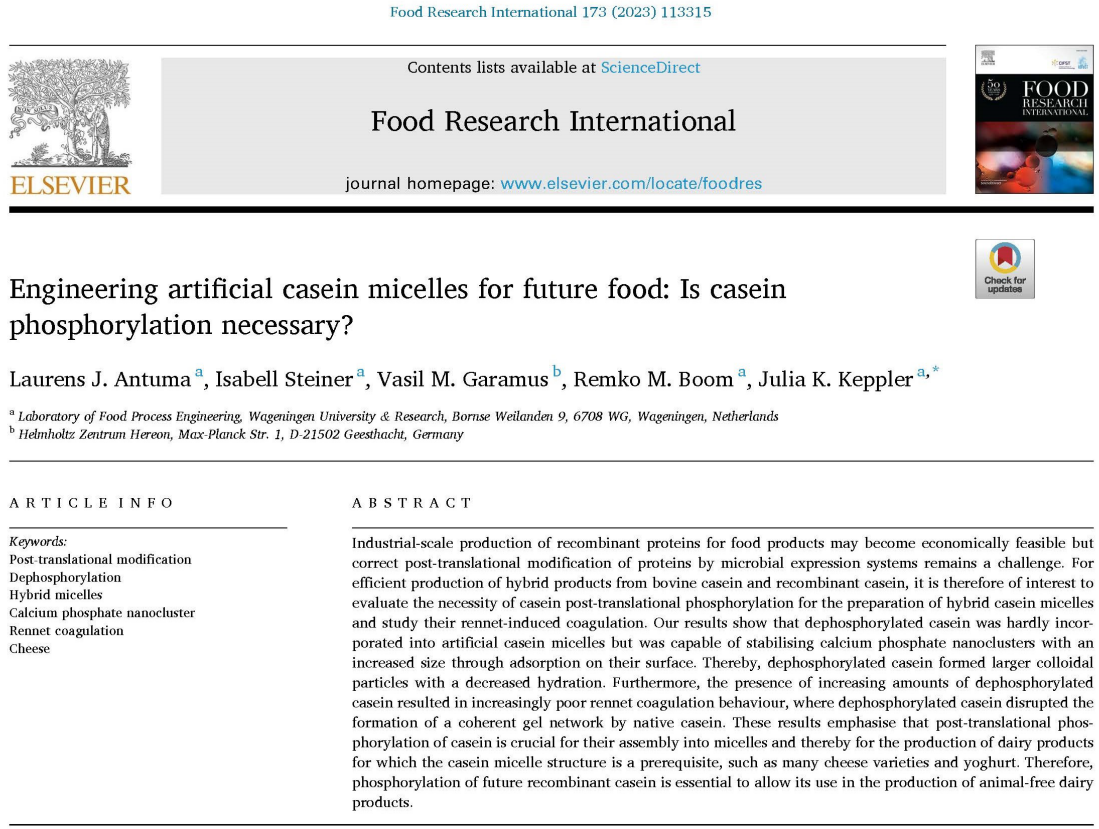
- Similar properties and functionality as natural casein micelles



Background

Challenges

- Production of caseins
  - Yield
  - Downstream processing
  - Posttranslational modifications
- Artificial casein micelles
- Other ingredients
- Upscaling



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Engineering artificial casein micelles for future food: Is casein phosphorylation necessary?

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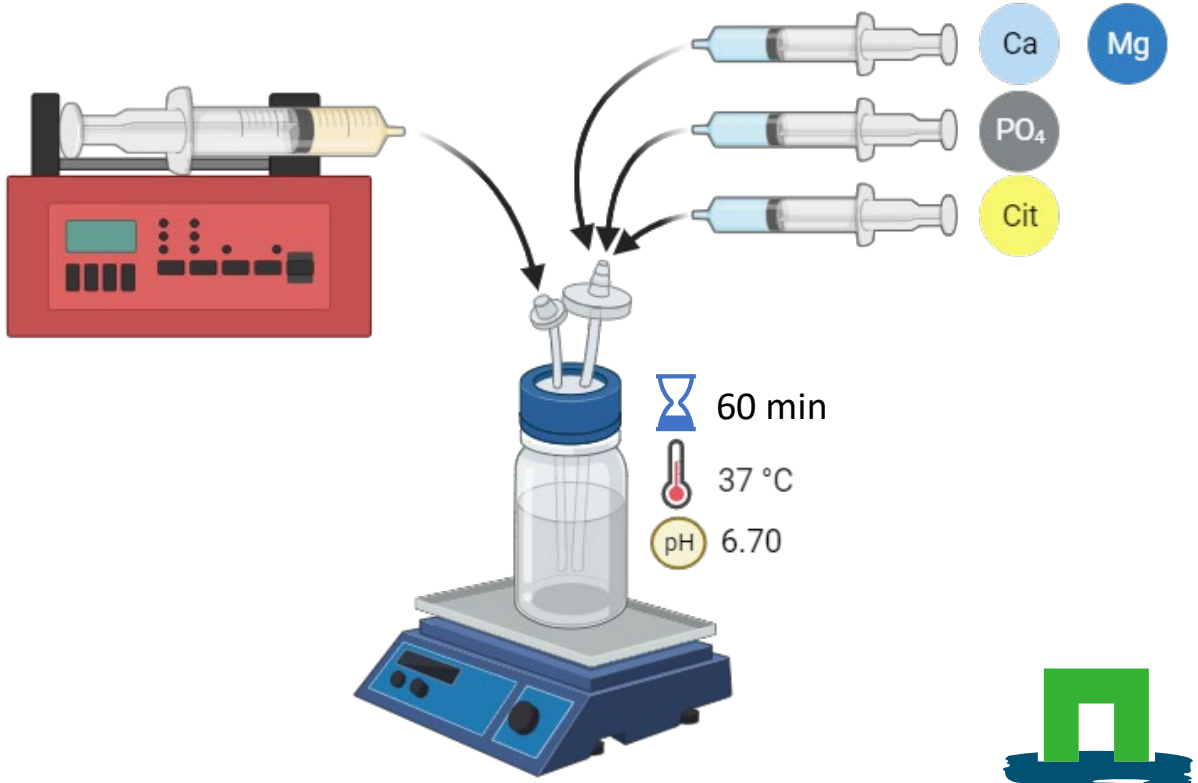
ABSTRACT

Industrial-scale production of recombinant proteins for food products may become economically feasible but correct post-translational modification of proteins by microbial expression systems remains a challenge. For efficient production of hybrid products from bovine casein and recombinant casein, it is therefore of interest to evaluate the necessity of casein post-translational phosphorylation for the preparation of hybrid casein micelles and study their rennet-induced coagulation. Our results show that dephosphorylated casein was hardly incorporated into artificial casein micelles but was capable of stabilising calcium phosphate nanoclusters with an increased size through adsorption on their surface. Thereby, dephosphorylated casein formed larger colloidal particles with a decreased hydration. Furthermore, the presence of increasing amounts of dephosphorylated casein resulted in increasingly poor rennet coagulation behaviour, where dephosphorylated casein disrupted the formation of a coherent gel network by native casein. These results emphasise that post-translational phosphorylation of casein is crucial for their assembly into micelles and thereby for the production of dairy products for which the casein micelle structure is a prerequisite, such as many cheese varieties and yoghurt. Therefore, phosphorylation of future recombinant casein is essential to allow its use in the production of animal-free dairy products.

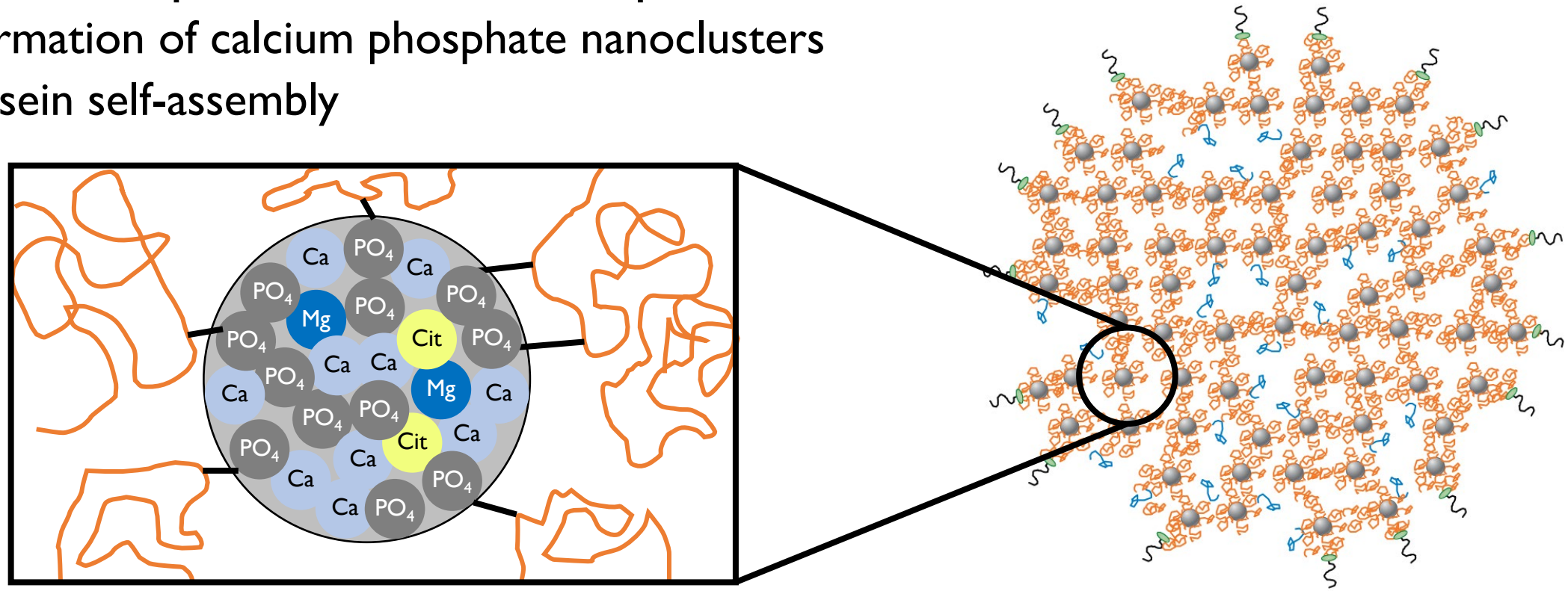
Background
Challenges

- 20th century: Elucidating structure of casein micelles
- 21st century: Practical relevance
- Disadvantages current process
  - Slow
  - Low output
  - Batch process



Hypothesis

- Casein micelle formation occurs upon calcium phosphate supersaturation in the presence of casein
  - Formation of calcium phosphate nanoclusters
  - Casein self-assembly



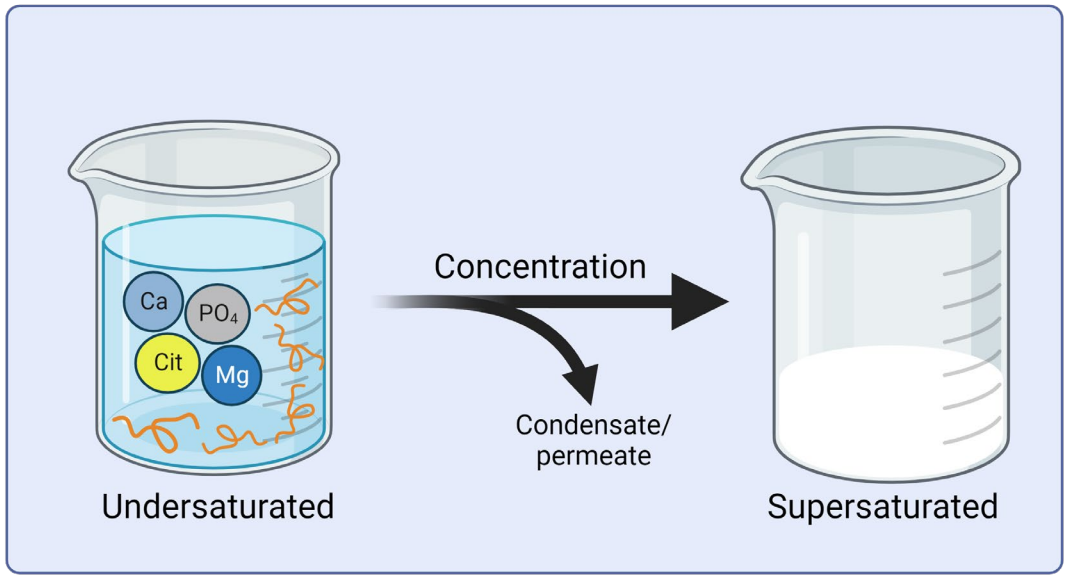
Background

Calcium phosphate supersaturation

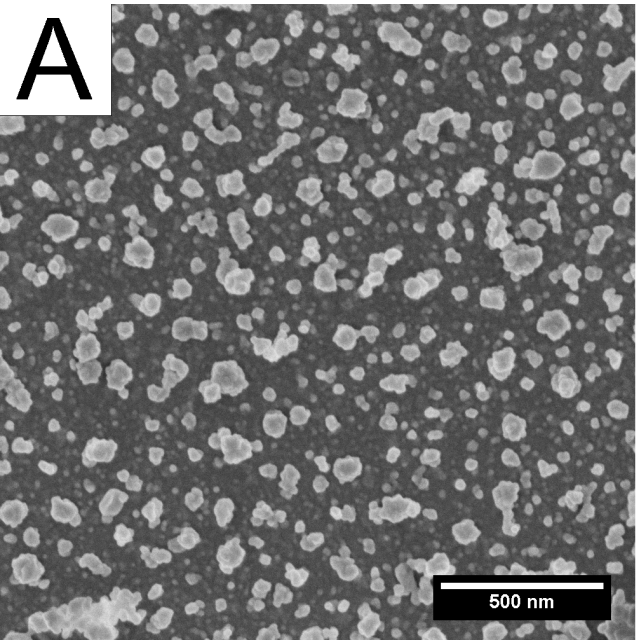
- Multiple ways to achieve supersaturation
  - Increasing pH
  - Increasing temperature
  - Increasing concentration
    - Adding calcium and phosphate ions (Schmidt method)
    - Concentrating the solution

Approach

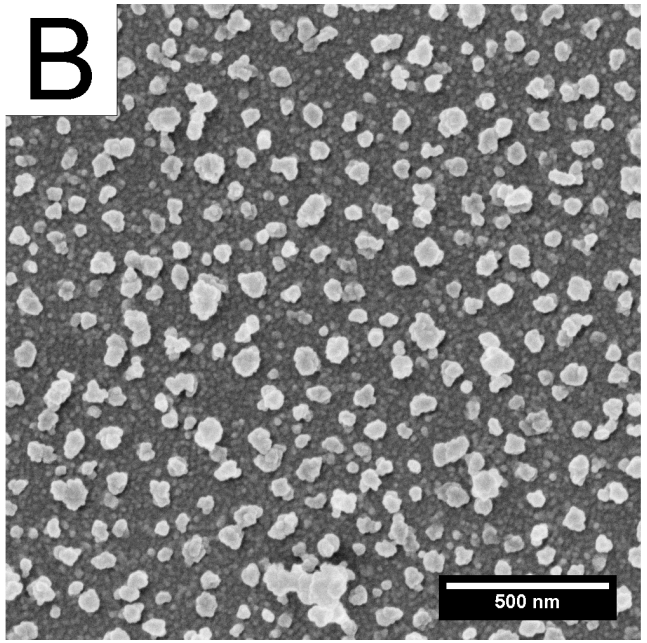
- Induce micelle formation through calcium phosphate supersaturation in the presence of casein by means of concentration
  - Vacuum evaporation (VE)
  - Forward osmosis (FO)
  - Reverse osmosis (RO)
- Varied preparation rate



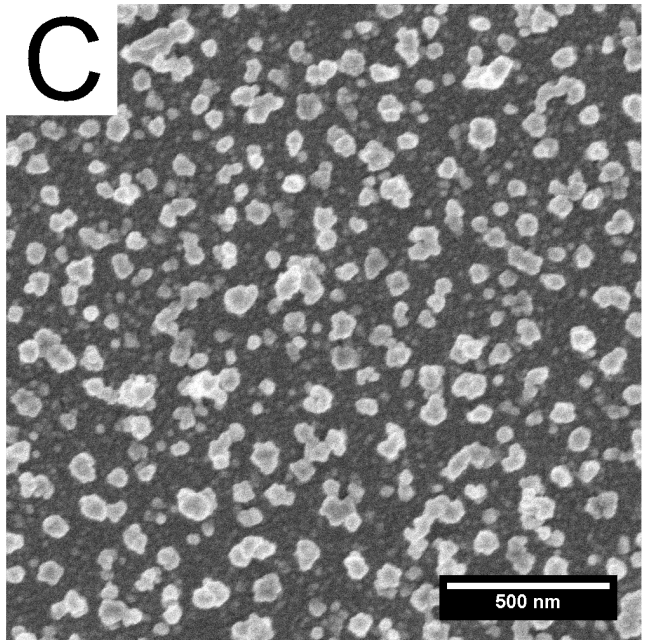
Results



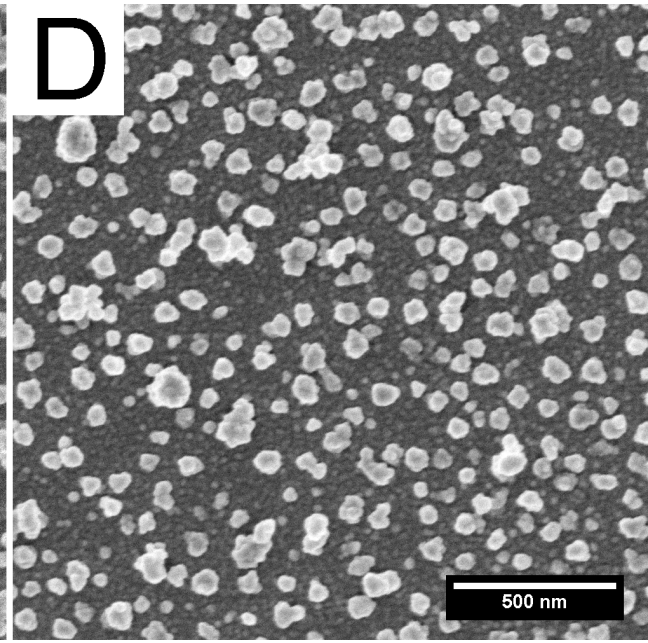
S-ACM



VE-ACM

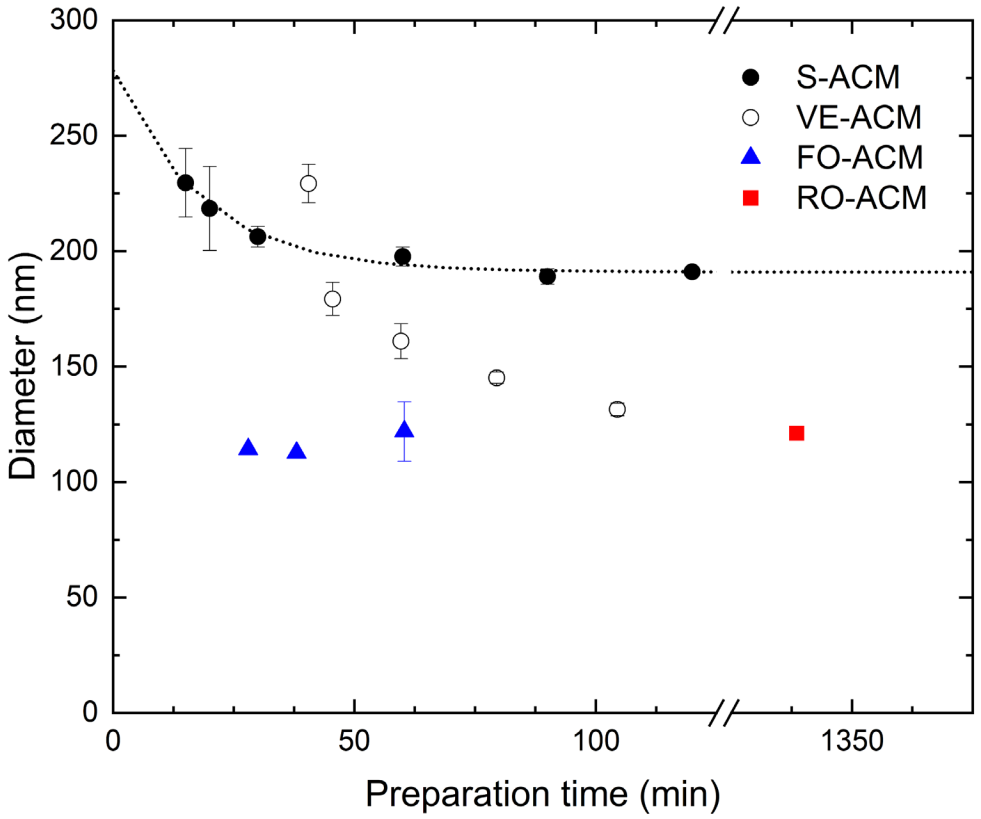


FO-ACM

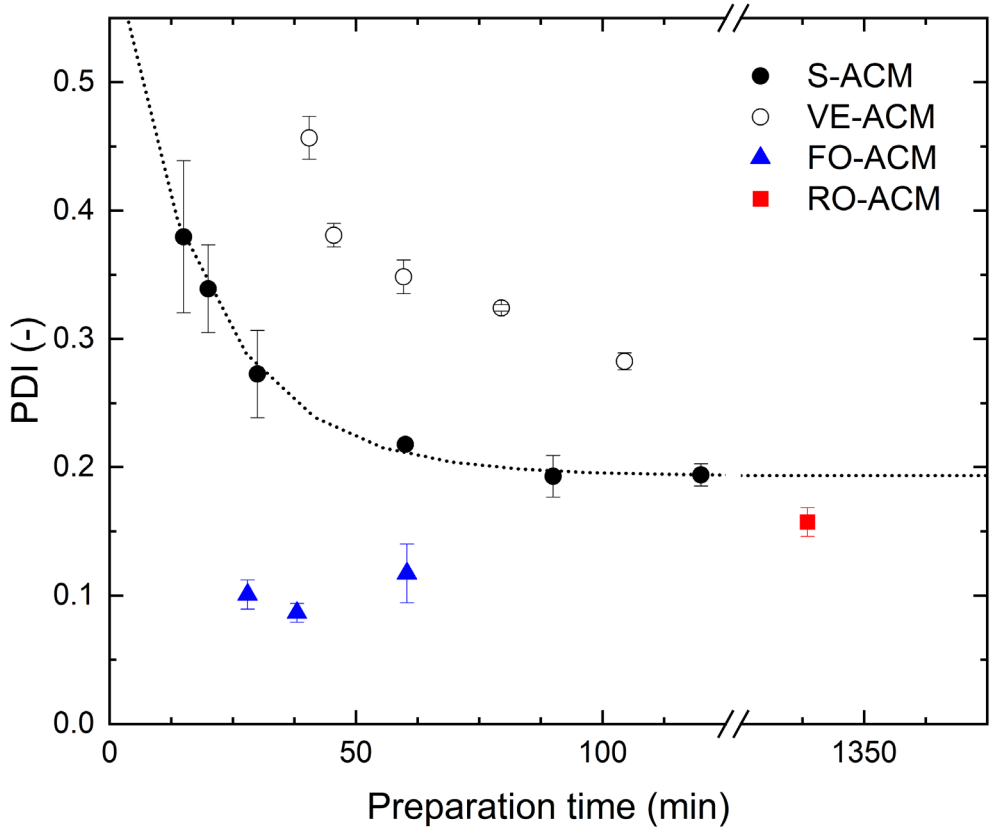


RO-ACM

Results



$$d(t) = d_\infty + (d_0 - d_\infty) * e^{-\frac{t}{\tau}}$$



$$PDI(t) = PDI_\infty + (PDI_0 - PDI_\infty) * e^{-\frac{t}{\tau}}$$

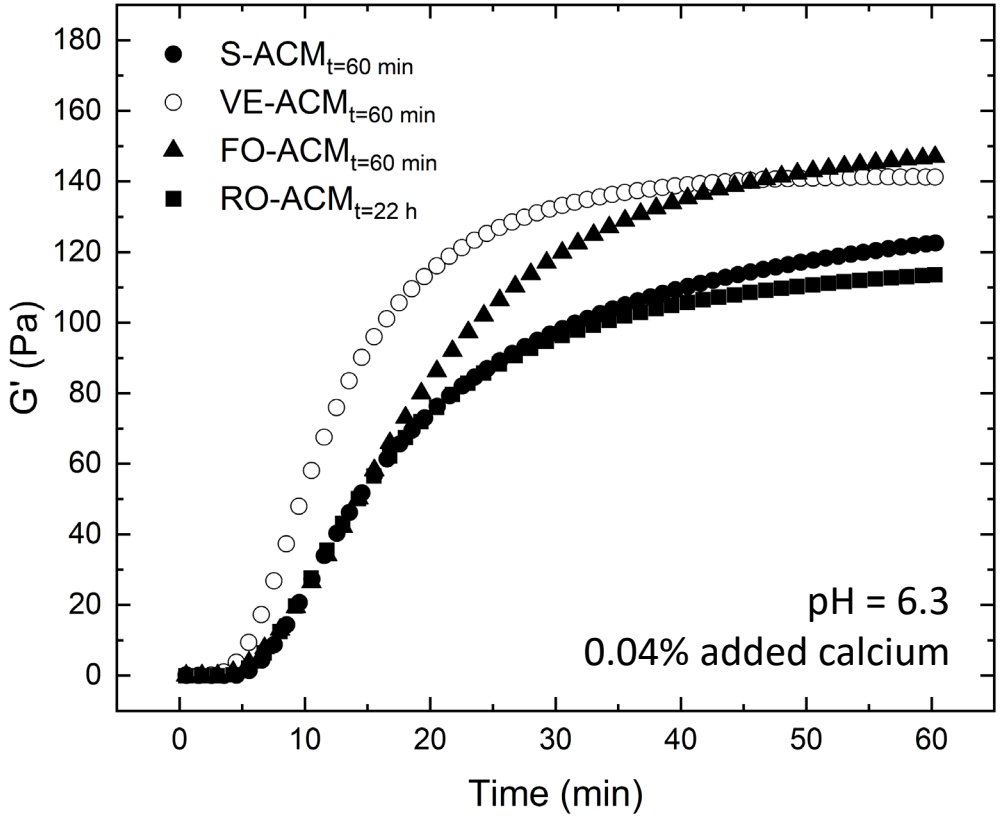
Results

- Micellar casein
- Casein mineralization
- Micelle hydration
- Internal structure (SAXS)

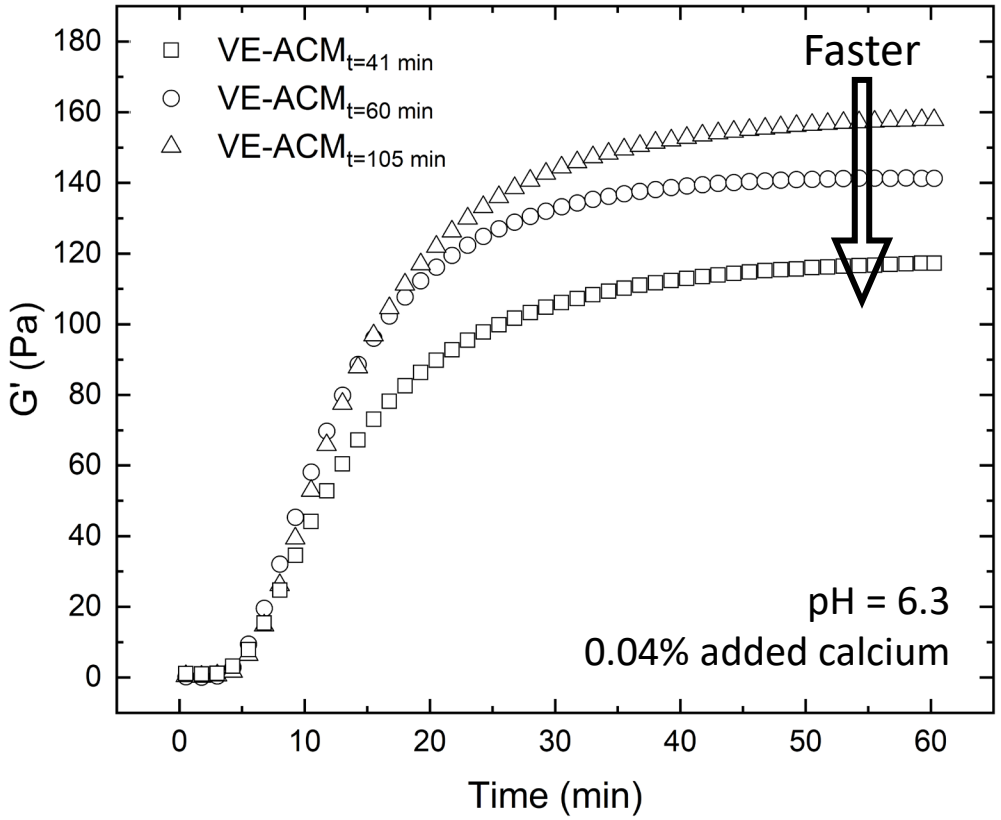
- Little influence of preparation method or preparation time

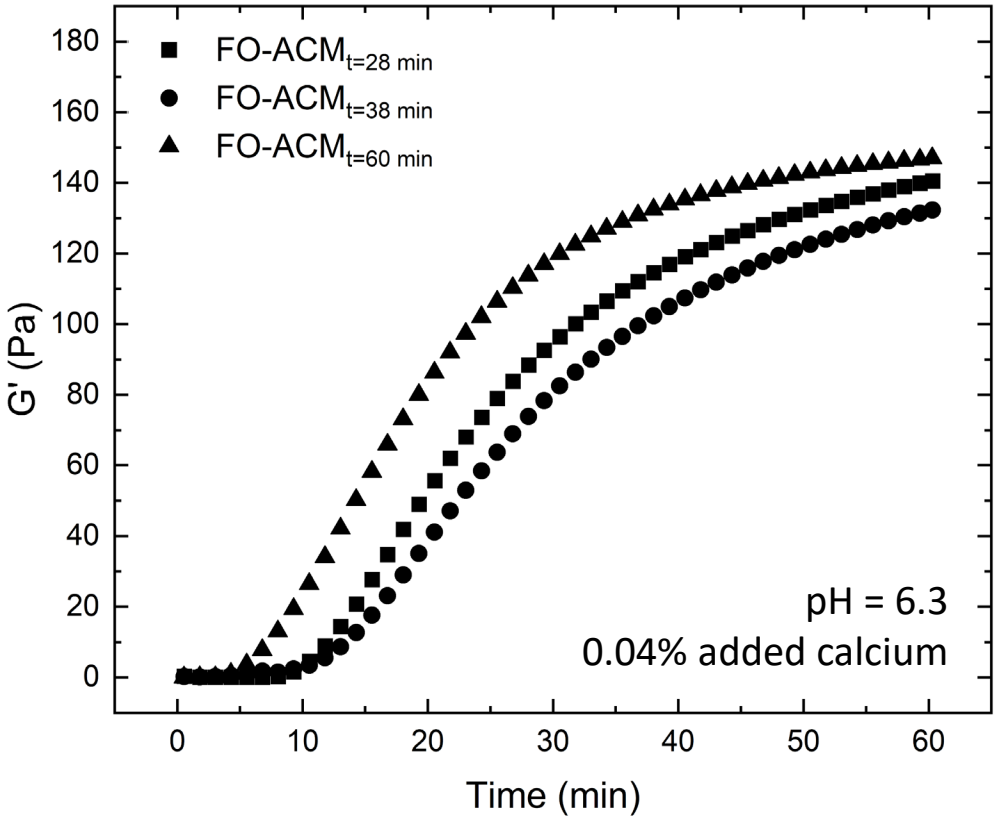
Results

- Improved coagulation behaviour
- Fouling during reverse osmosis
  - Lower concentrations



Results

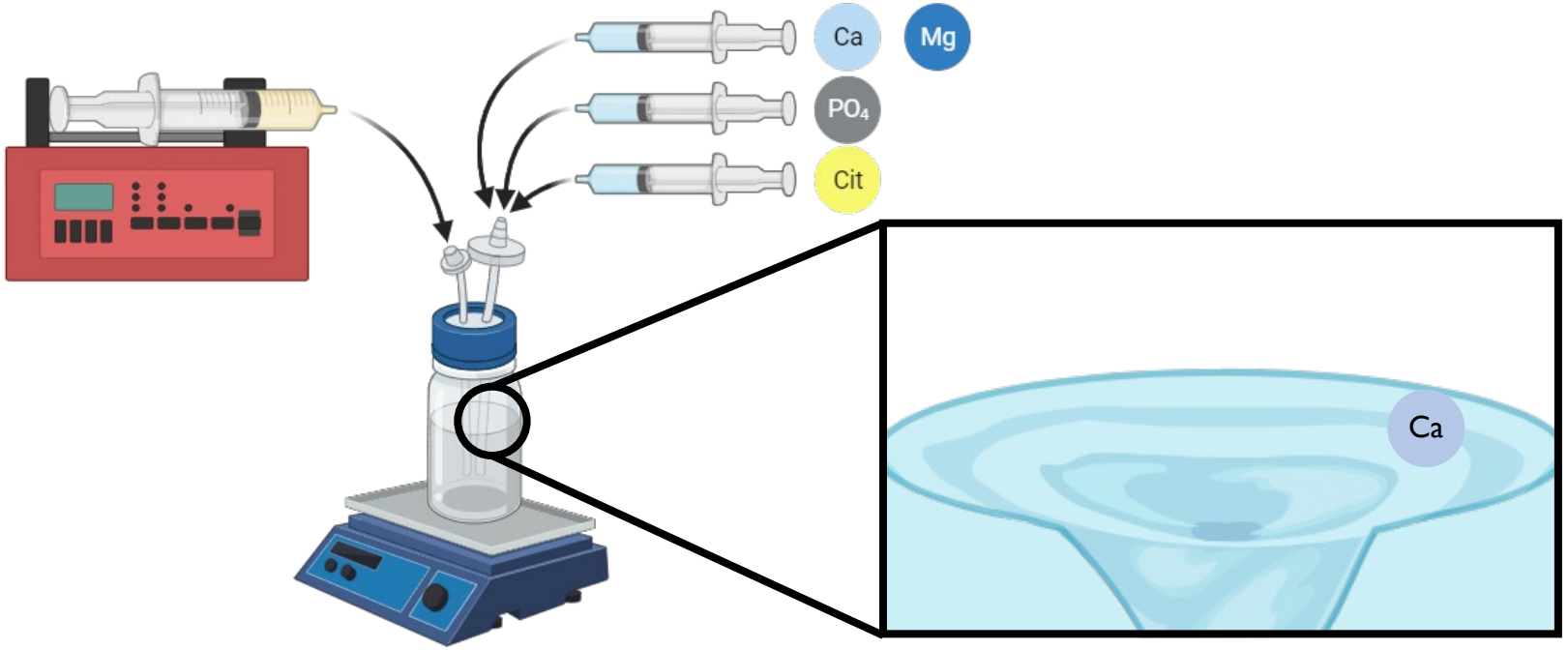




Method: rheometry time sweep

Discussion

- Faster preparation = increased size and PDI, impaired coagulation
  - Except for FO-ACM
- Local differences in the concentration of ions



Discussion

- Faster preparation = increased size and PDI, impaired coagulation
  - Except for FO-ACM
- Local differences in the concentration of ions
- Area of evaporation/permeation
  - Vacuum evaporation dm^2-range
  - Reverse osmosis 0.9 dm^2
  - Forward osmosis 2.3 m^2

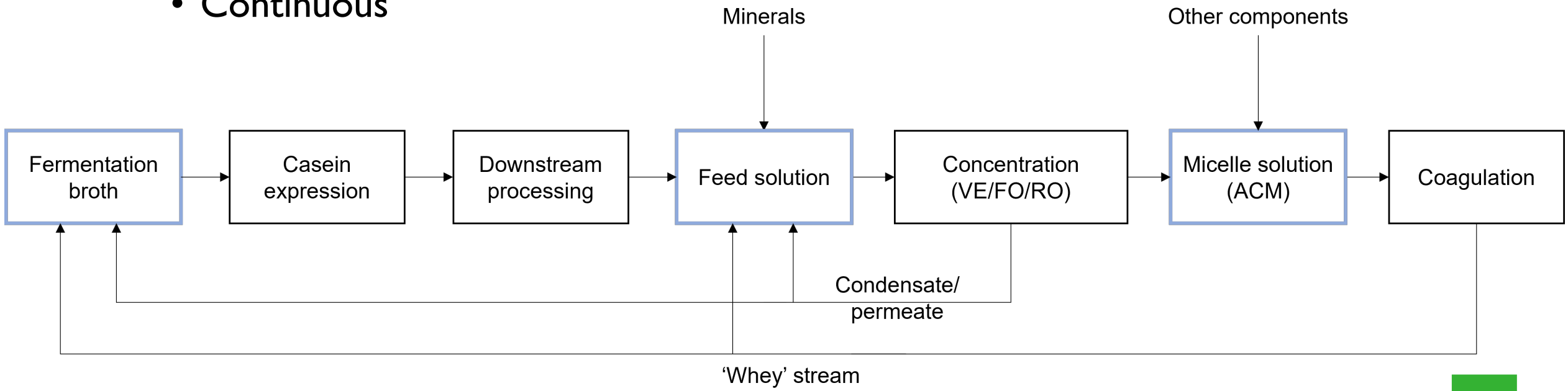
Conclusions

- Casein micelle formation occurs upon calcium phosphate supersaturation in the presence of casein
  - Artificial casein micelles can be produced through vacuum evaporation, forward osmosis and reverse osmosis

- Local concentration differences affect micelle properties and functionality
  - Preparation time
  - Area

Outlook

- Advantages of the proposed preparation methods:
  - Start with dilute feed
  - Continuous



Recommendations

- Fouling (especially reverse osmosis)
- Forward and reverse diffusion during forward osmosis
- Resource efficiency

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Thanks for your attention!

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