HOW ENZYME DIFFUSION IN PECTIN AND PECTIN/CELLULOSE COMPOSITE SYSTEMS IS INFLUENCED BY THEIR MECHANICAL PROPERTIES

Catherine Garnier, Camille Jonchère, Bruno Novales, Virginie Hugouvieux

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Camille Jonchère [a], Bruno Novales [a], Virginie Hugouvieux [b], Catherine Garnier [a]

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Diffusion of Enzyme in Biopolymer Systems

Enzymes used in food and non food products to modify their properties :

Protease, amylase, cellulase, xylanase, lipase :

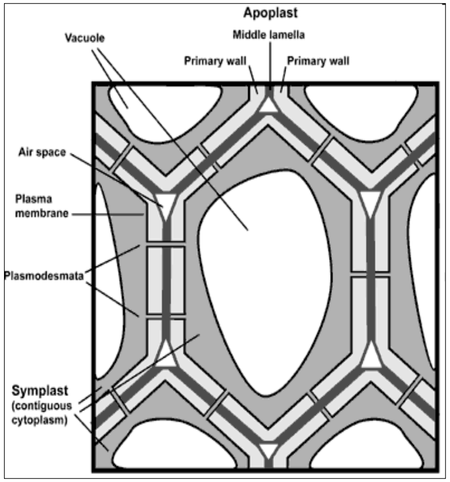
Milk, bread, brewery, fruit industries
Paper and detergent industries

Pepsin in digestion process

Is their diffusion affected by the structure or the texture of the system?

I. Pectins: General points

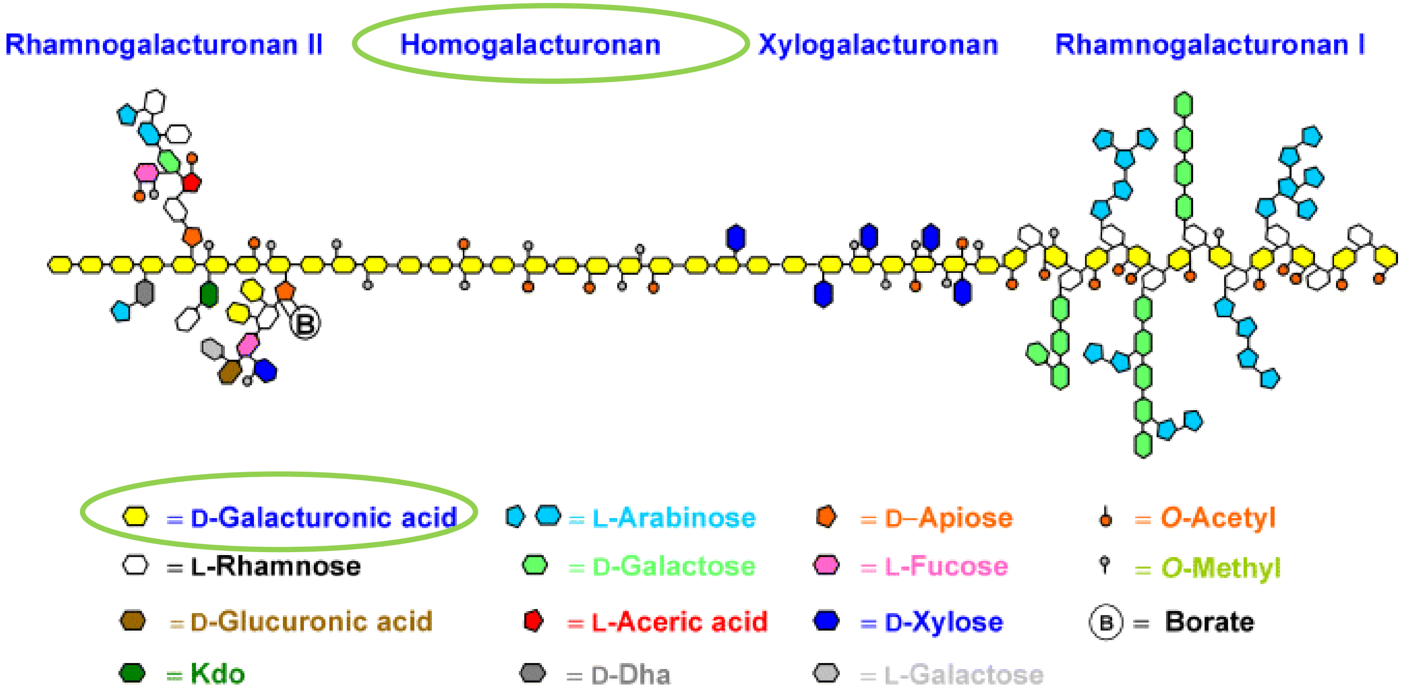
- Plant cell wall polysaccharide:
  - -> Cell shape and mechanical properties
  - -> Cell growth & development
  - -> Defence against phytopathogens



http://www.ccrc.uga.edu/~mao/intro/ouline.htm

- Functionnal ingredients for food and drug industries:
  - -> Gelling agent
  - -> Stabilising agent
  - -> Coating agent

I. Pectins: Structure

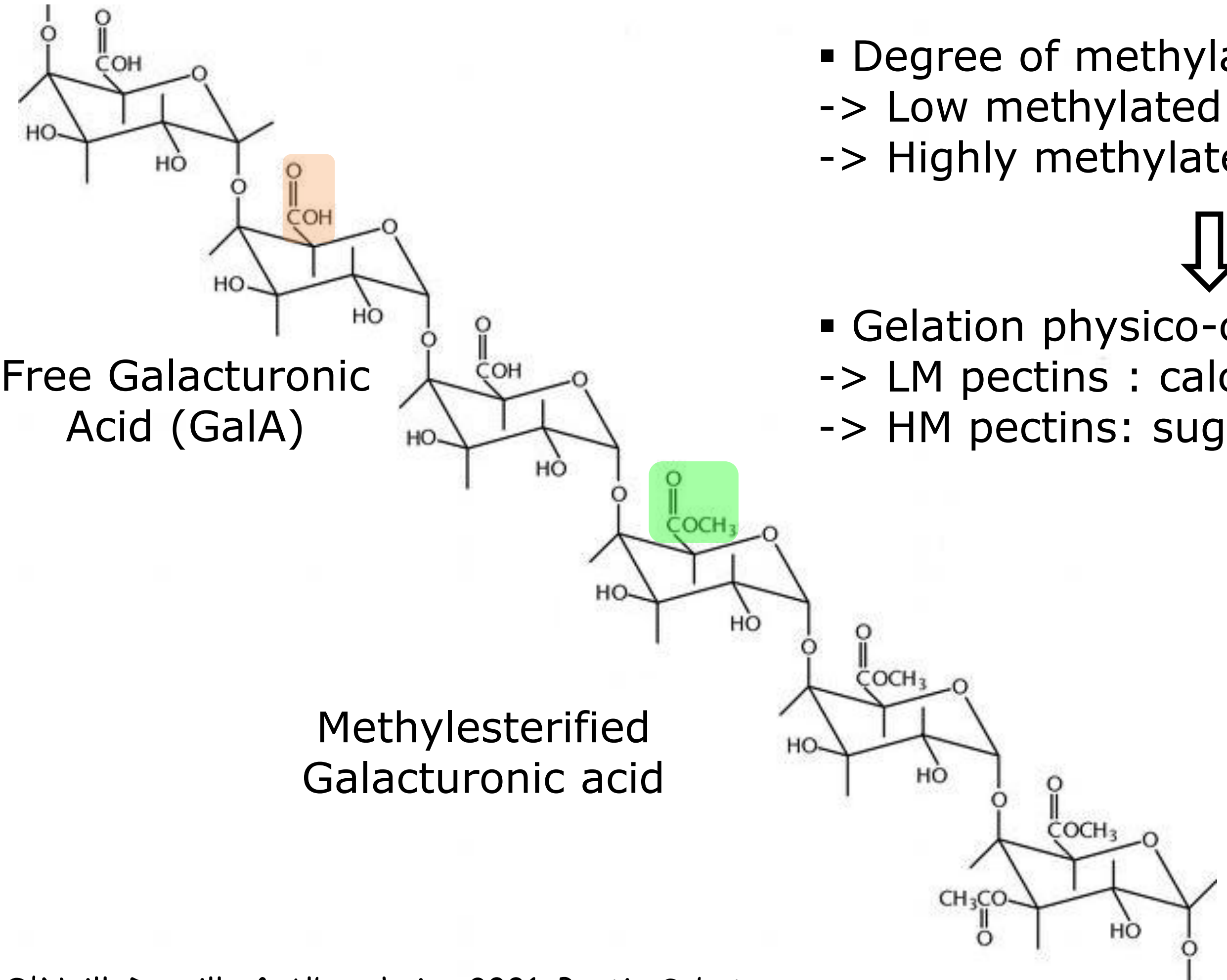


Scheller *et al.*, Physiologia Plantarum, 2007.

Very complex structure

Polyelectrolyte character

I. Pectins: Homogalacturonans



Free Galacturonic Acid (GalA)

Methylesterified Galacturonic acid

O'Neill, Darvill, & Albersheim, 2001. Pectic Substances.

- Degree of methylation (DM):

-> Low methylated (LM) pectins : DM < 50%

-> Highly methylated (HM) pectins : DM > 50%

⇩

- Gelation physico-chemical conditions:

-> LM pectins : calcium

-> HM pectins: sugar + acidic pH

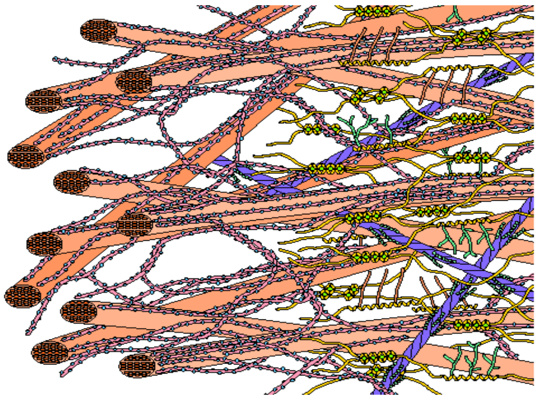
II. Cellulose

Cellulose microfibril

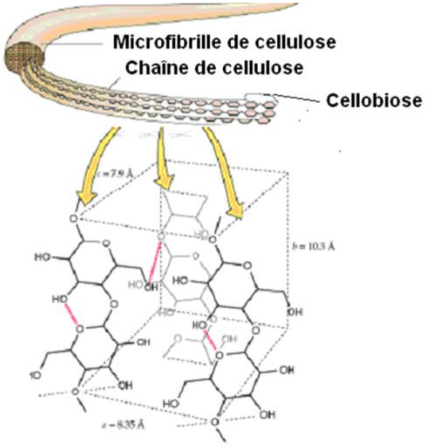
Pectin

Hemicellulose

Proteins



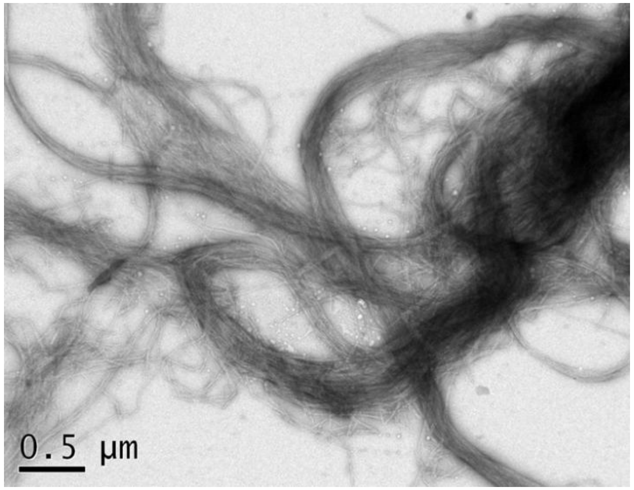
Carpita & Gibeaut The Plant Journal (1993)



Very abondant polymer

β-(1→ 4) linked glucose units

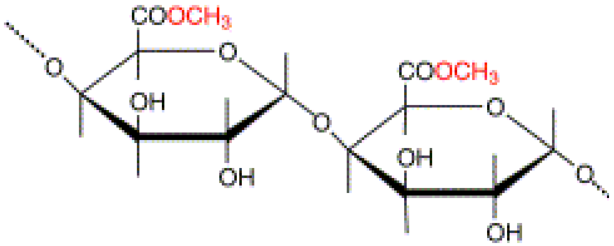
Insoluble



III. Pectin methylesterases (PME)

- Plant or Fungal enzymes
- Decrease of DM

HM pectin



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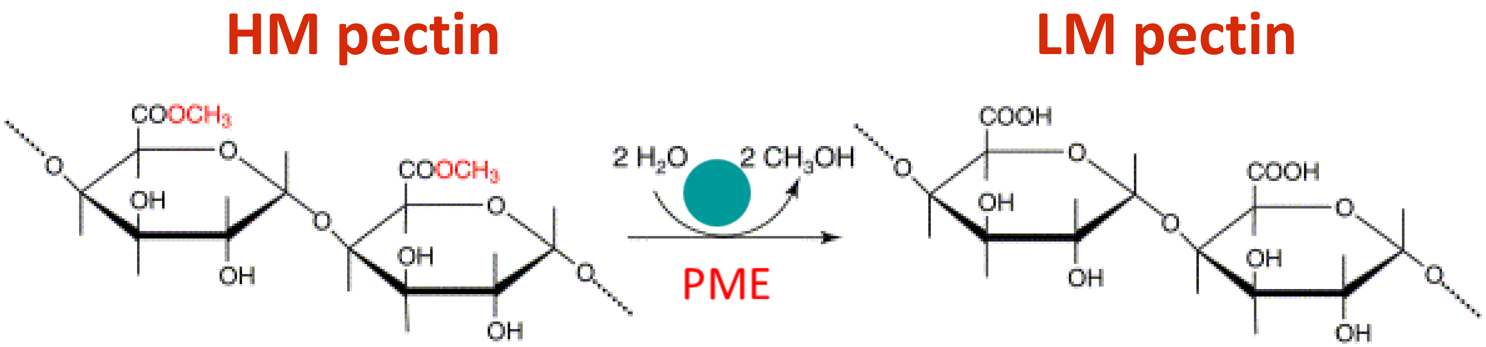
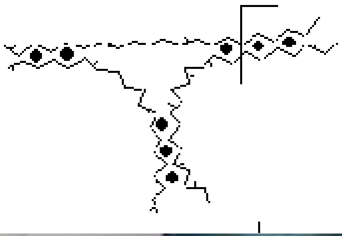


Micheli, Trends in Plant Science, 2001.

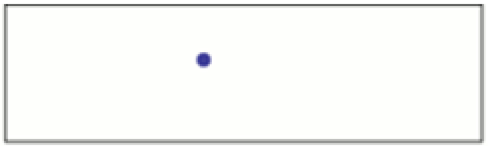
⇨ Modification of gelation properties **with calcium**

Solution

Gel

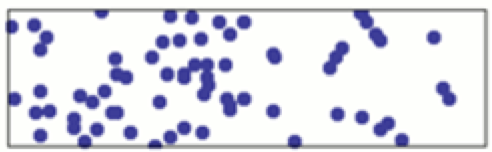


III. PME & Gelation Properties



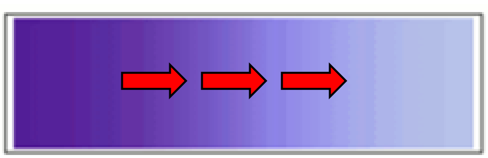
Local scale or
Microscopic scale
(FCS)

→ No influence of the environment
No influence of de esterification type
Diffusion coefficient ~ 100 µm²/s



Mesoscopic scale
(FRAP)

→ Influence of the environment
Influence of de esterification type
Diffusion coefficient ~ 5 µm²/s



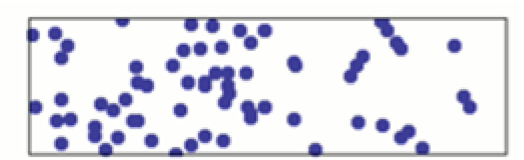
Global Scale or
Macroscopic scale

→ Influence of de esterification type
Diffusion coefficient ~ 100 µm²/s

Videcoq et al., Soft Matter, 2013

IV. Strategy

- Characterisation of PME diffusion in pectin solution, pectin gel in formation, composite systems at different concentrations



Mesoscopic scale → Fluorescent Recovery After Photobleaching (FRAP)

- Characterisation of the rheological properties

- Compare results of experiments with those obtained by modelling

V. Material

- Pectin : Orange, DM 46%, Cargill Texturizing Solutions, 0.25-1.5 wt %

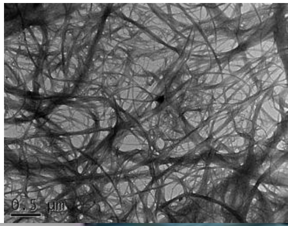
- PME (0,68 mg/ml, covalently labelled by FITC):

  - Aa-PME *Aspergillus aculeatus*, Novozyme

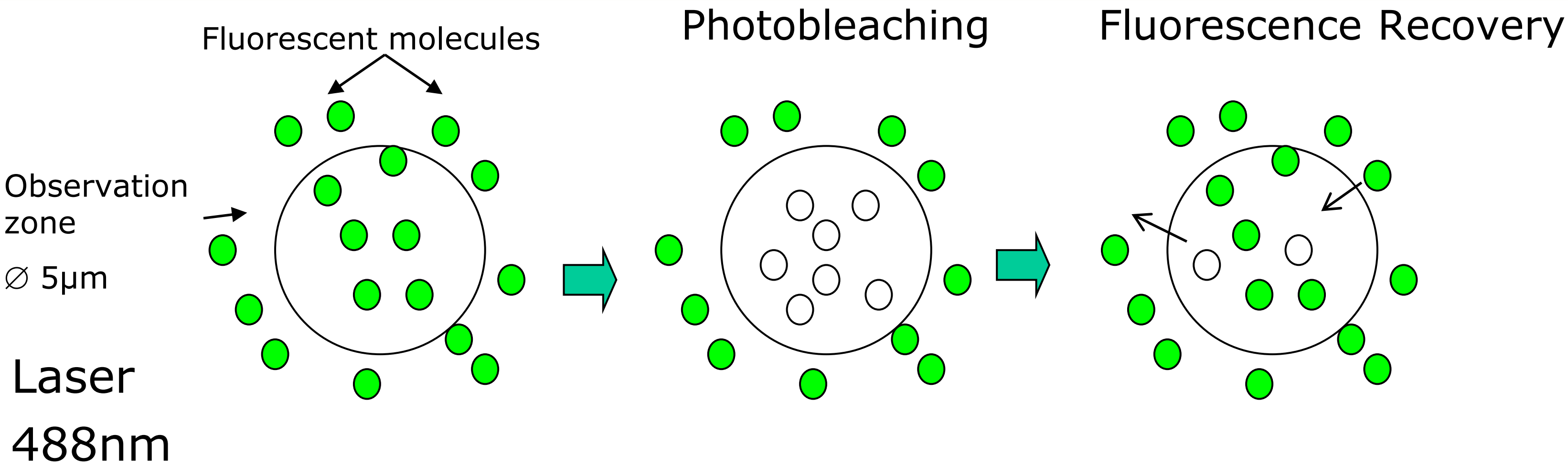
- Pectic environment:

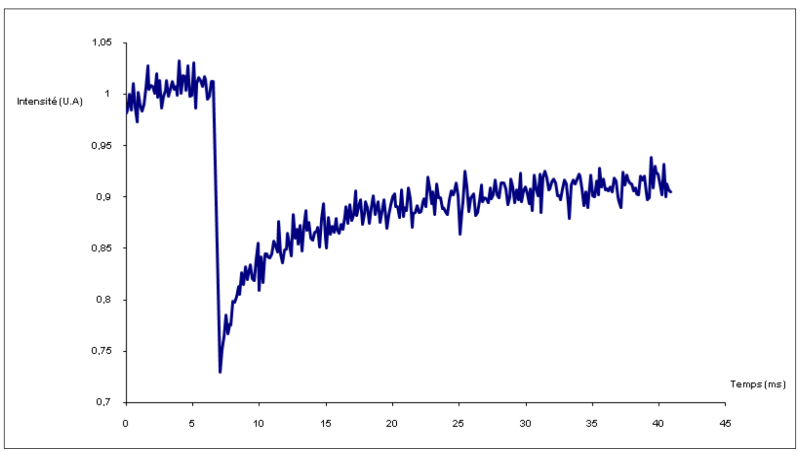
  - Pectin solution : pectin / MES (50 mM, pH 6)/ 30°C/Aa-PME
  - Pectin gel during formation: Pectin/ Calcium 5 mM /30°C /Aa-PME

- Cellulose: Micro-fibrillated, from sugar beet *(Agoda-Tandjawa, 2009)*



VI. Results – Mesoscopic scale





$$\Longrightarrow \quad C(x,y,t) = \left(\frac{C_0 w^2}{8Dt + w^2}\right).e^{\left[-\left(\frac{2(x^2+y^2)}{8Dt+w^2}\right)\right]}$$

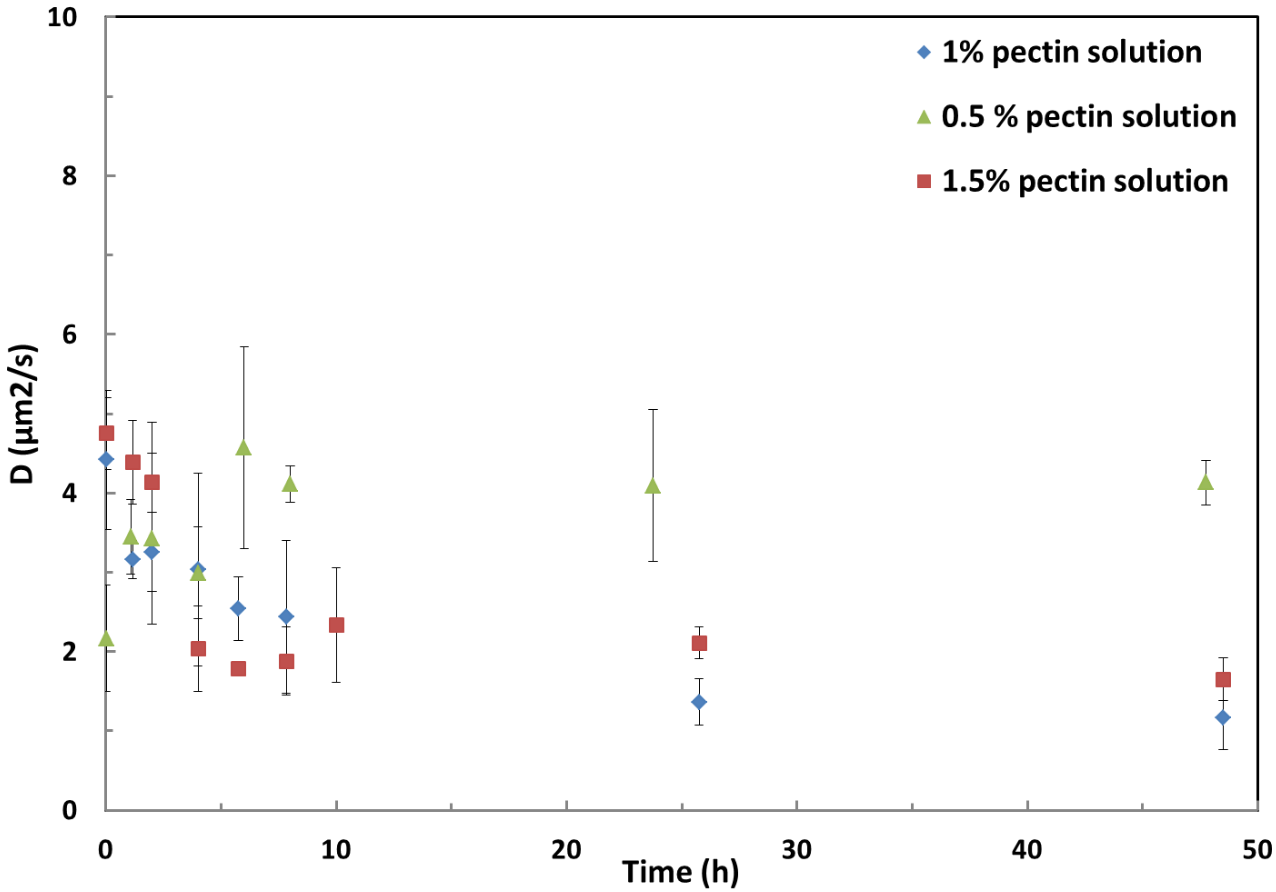
Waharte *et al.*, Journal of Applied Microbiology, 2010

$\Downarrow$

Self diffusion coefficient D

Nikon A1 CLSM

VI. Results – In macromolecular solutions



Stokes-Einstein

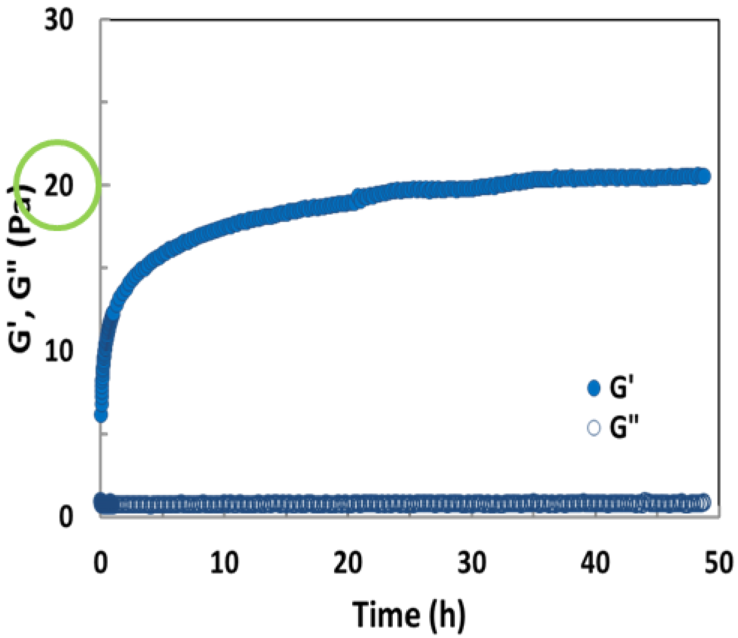
$$D = \frac{kT}{6\pi\eta R_h}$$

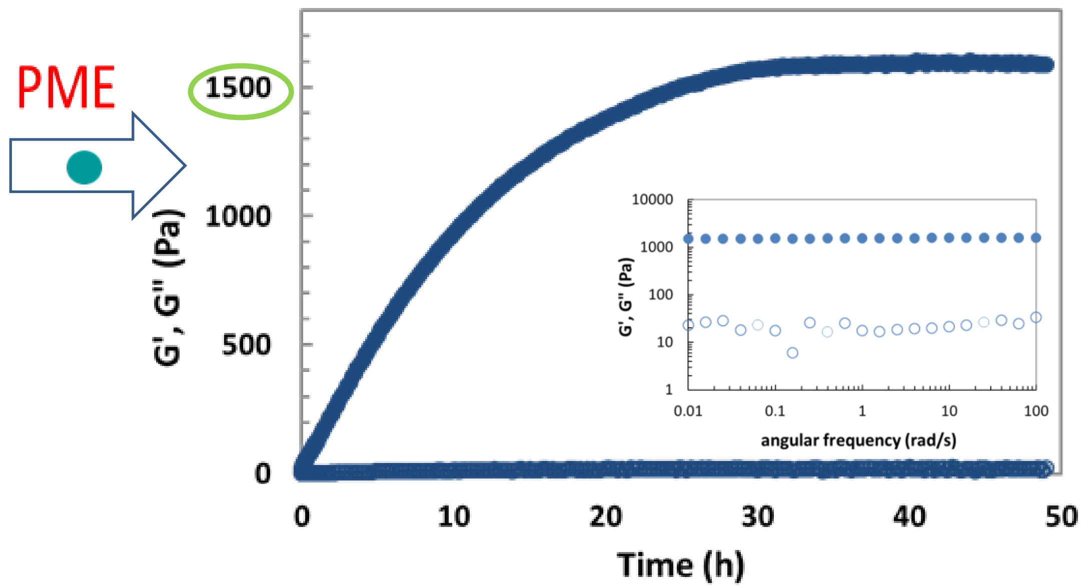
	D calc (µm²/s)
0.5% Pectin solution	6.7
1% Pectin solution	0.4
1.5 % Pectin solution	0.1

Slight decrease of D along time, without changes in the physical state
Decrease of D with increasing pectin concentrations

VI. Results – During pectin gel formation

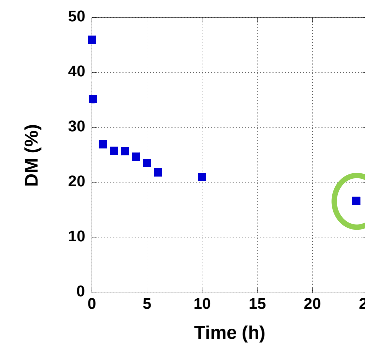
1% Pectin + 5 mM Ca^{2+}





Strong increase of G’ upon demethylation

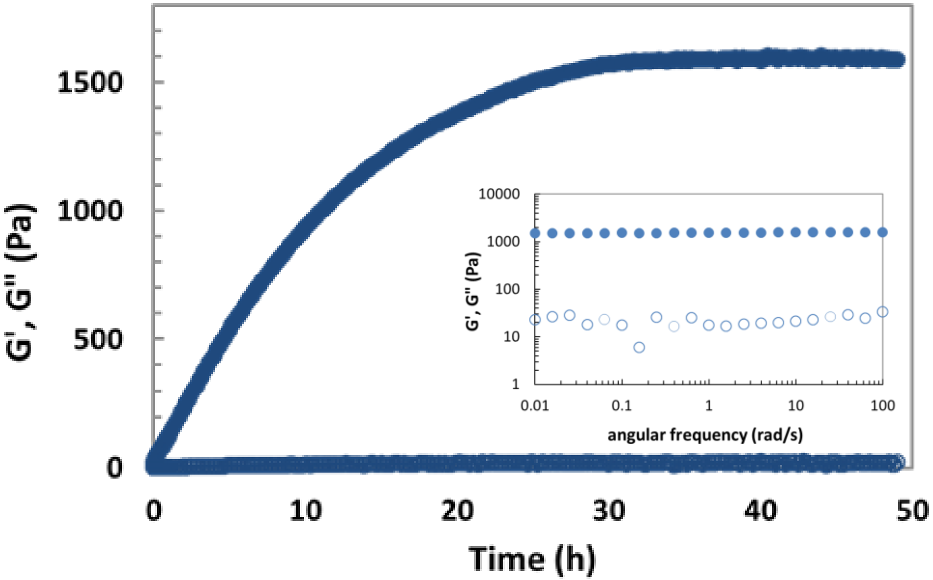
Delayed gelation kinetics

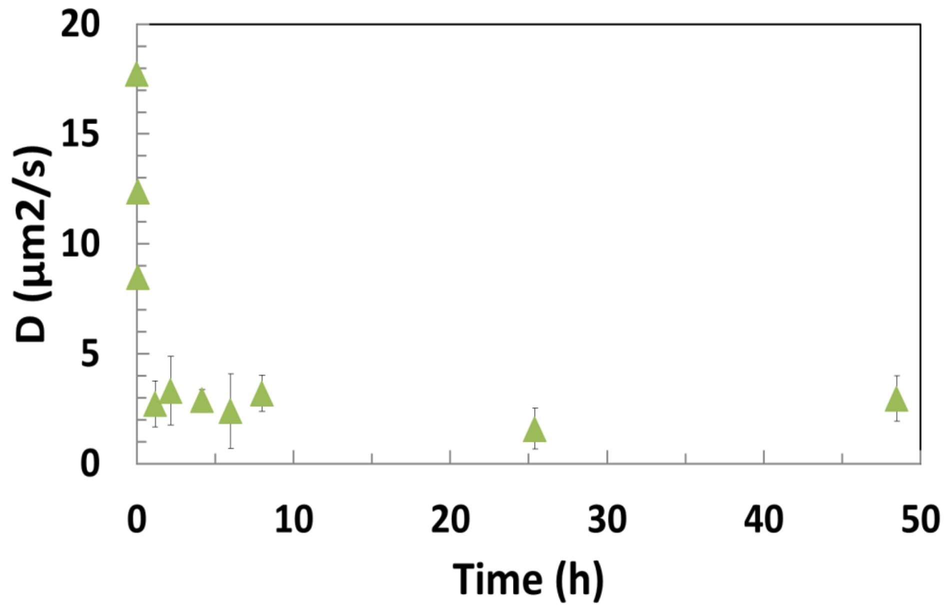


RFSII & AR2000, TA Instruments

VI. Results – During pectin gel formation

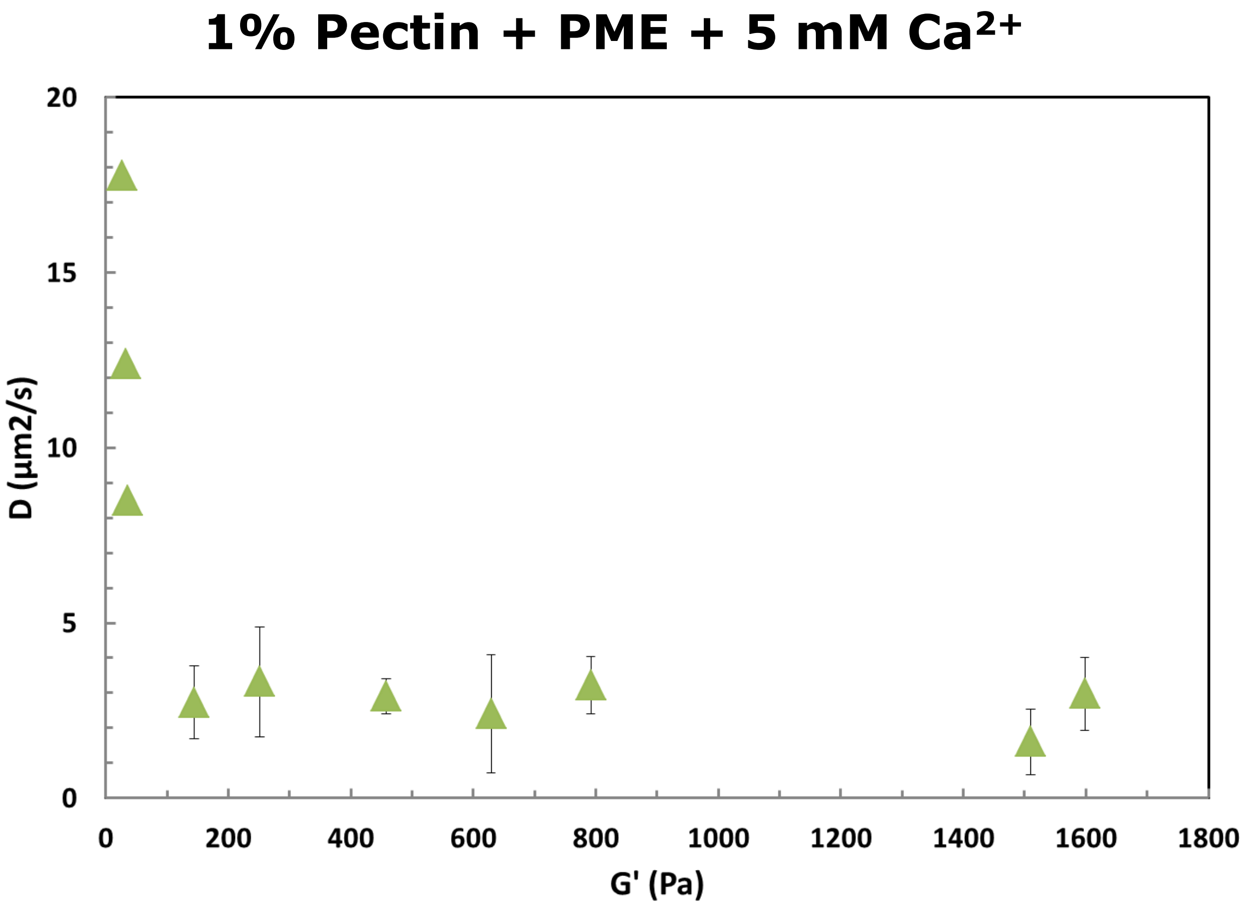
1% Pectin + PME + 5 mM Ca^{2+}





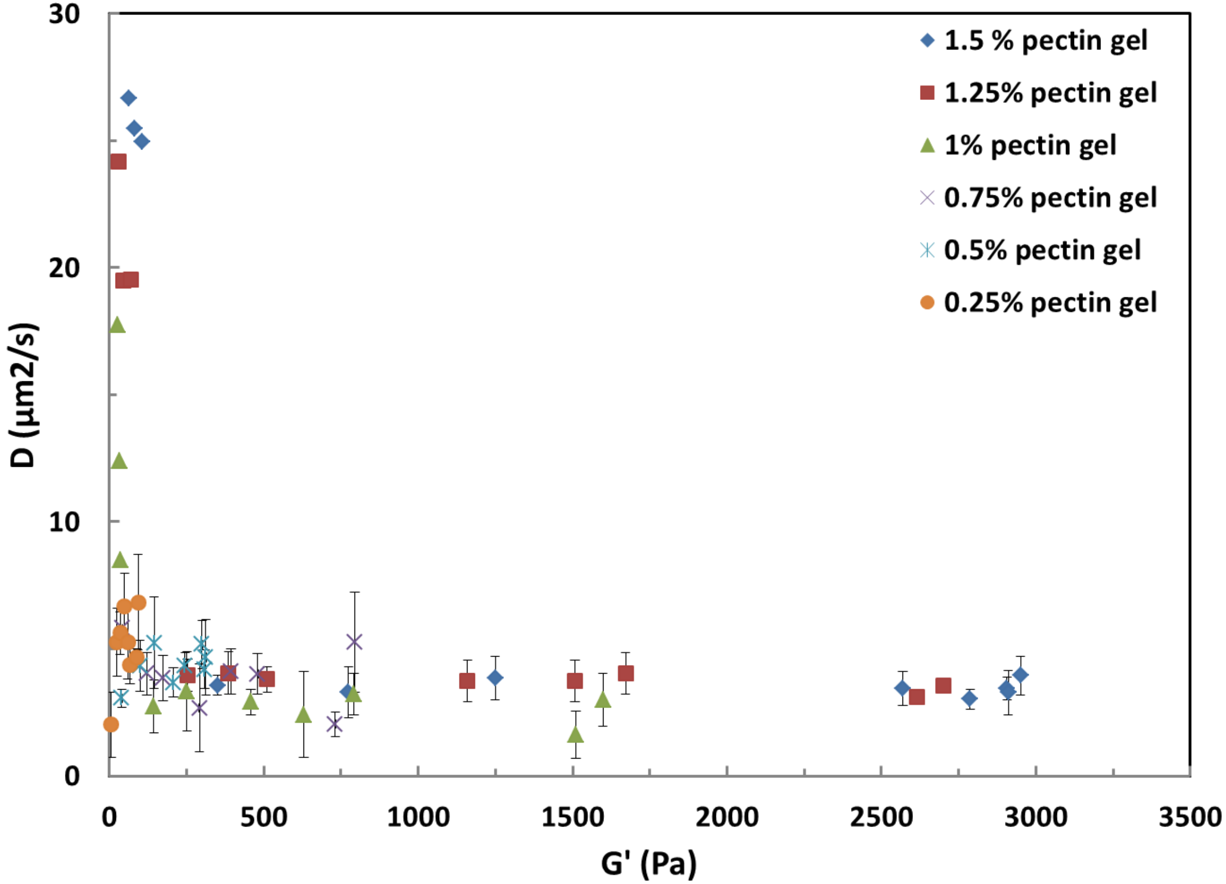
Higher D values obtained during the very first steps of gelation
Higher values than those found in solution
At longer times, D values are in the same range

VI. Results – During pectin gel formation



Higher D values obtained for low storage moduli

VI. Results – During pectin gel formation (1.5-0.25%)

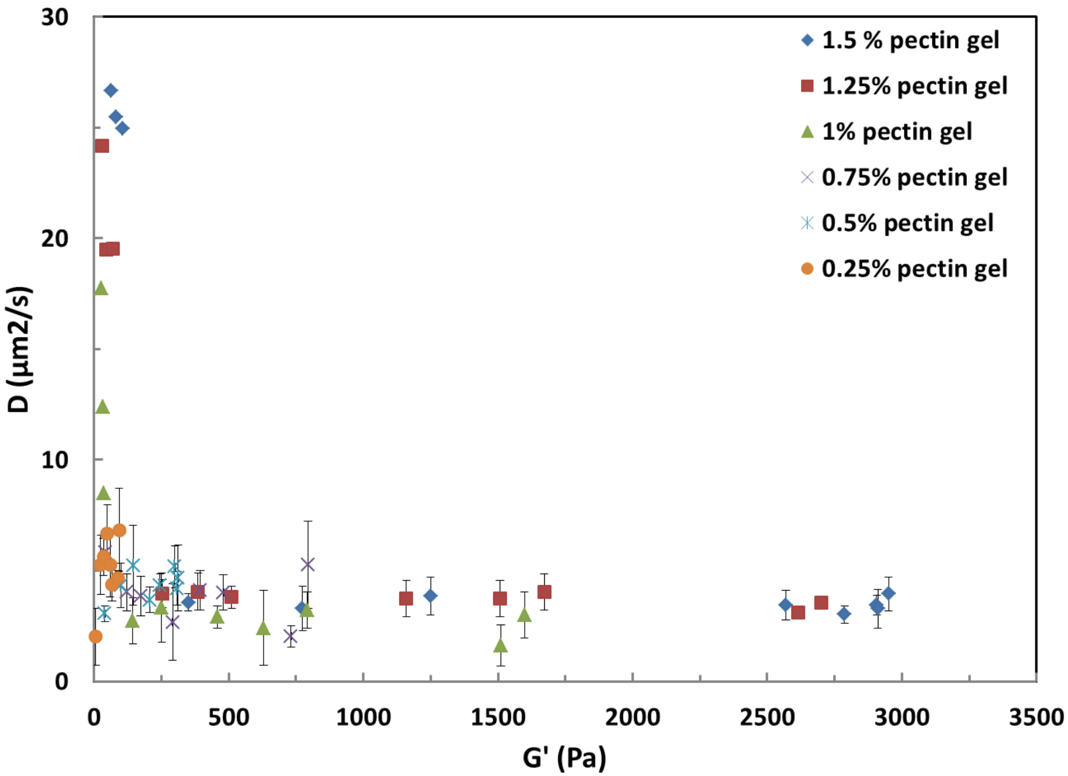


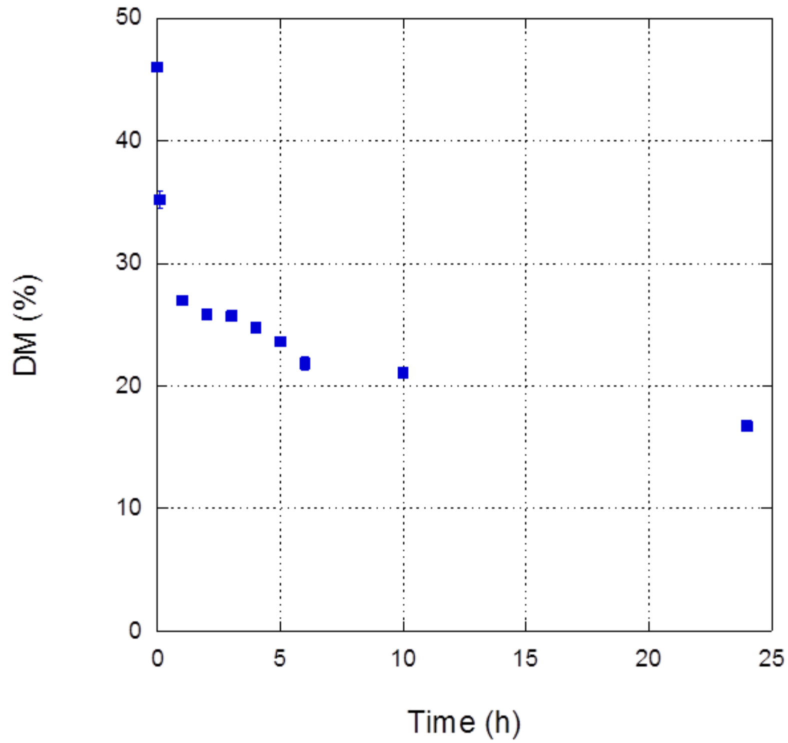
Higher D values obtained during the first steps of gelation

Higher D values with increasing concentrations

For all concentrations, the evolution of D with the storage modulus is the same

VI. Results – During pectin gel formation (1.5-0.25%)





Higher D values obtained during the first steps of gelation

In agreement with a rapid decrease of the DM at the beginning of the reaction

VI. Results – Modelling of gel formation

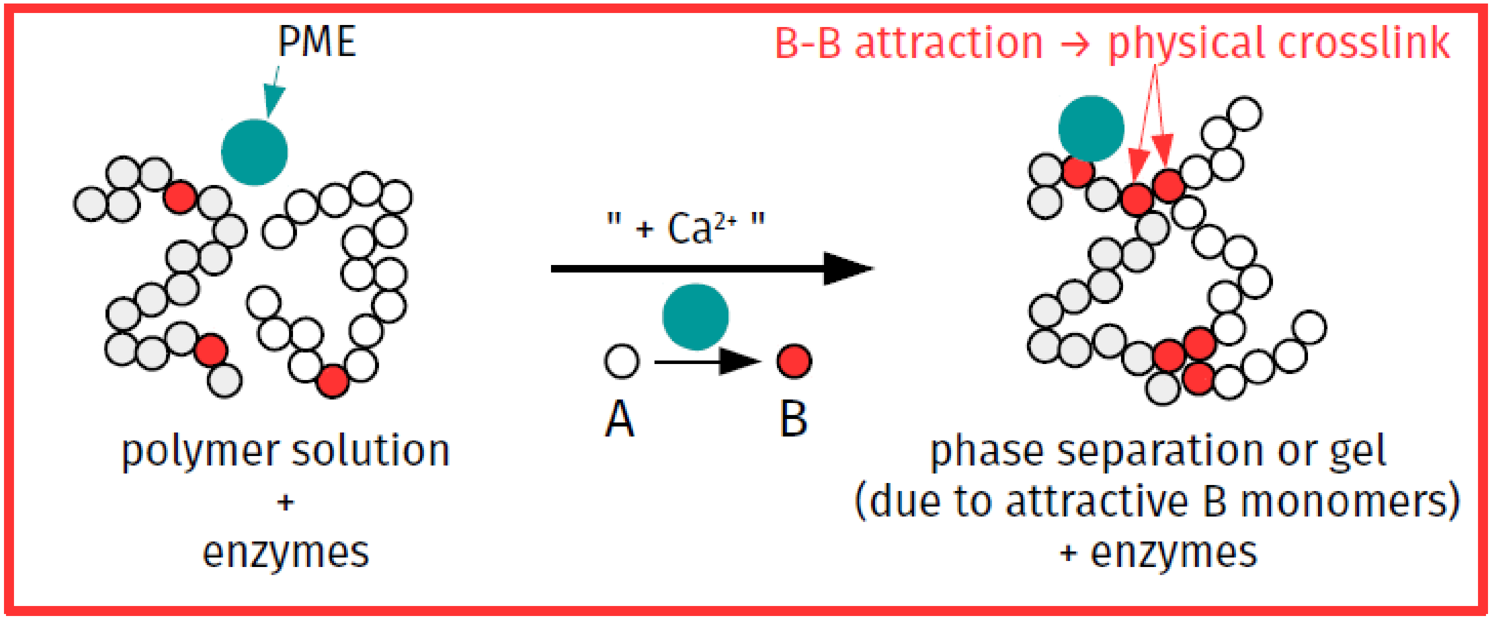
in collaboration with W. Kob, Univ. Montpellier

Coarse-grained generic model

PME

○ methylated monomer : A

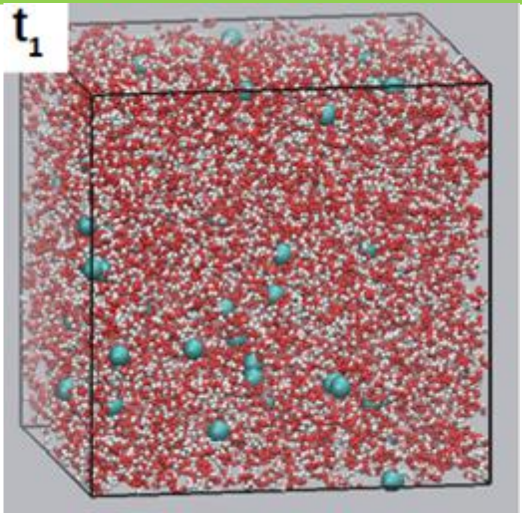
● demethylated monomer : B

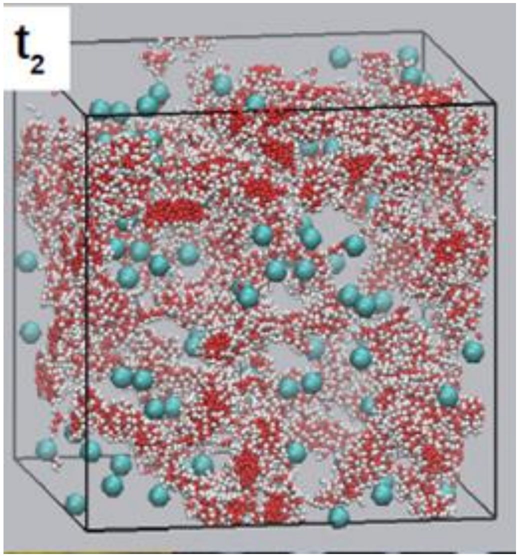


Molecular dynamics simulations

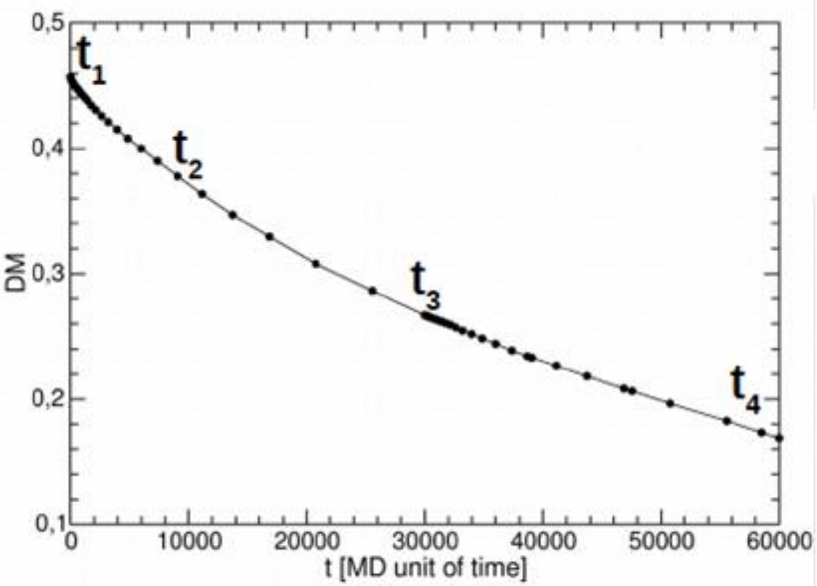
Polymers and enzymes evolve due to forces acting on them → structure and dynamics

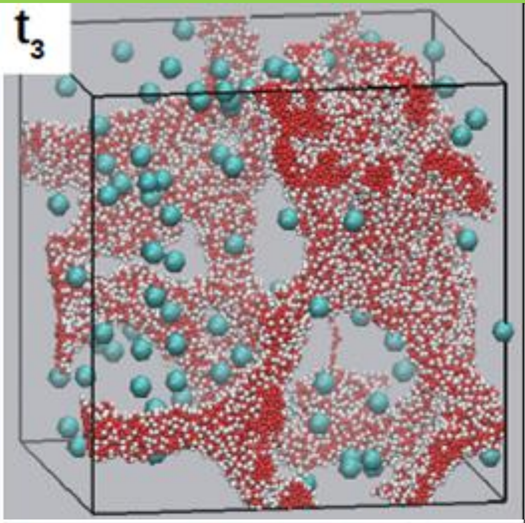
VI. Results – Modelisation of gel formation

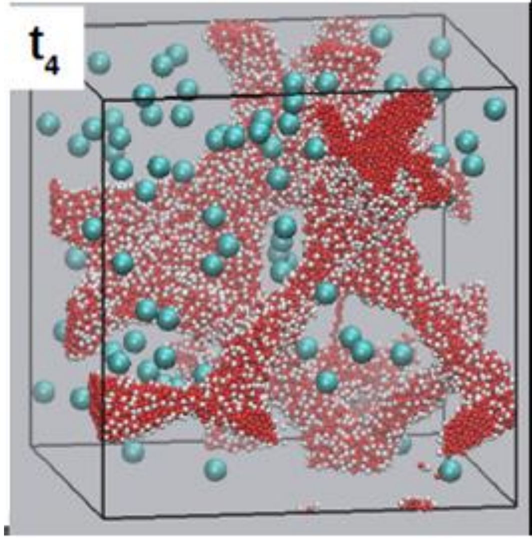


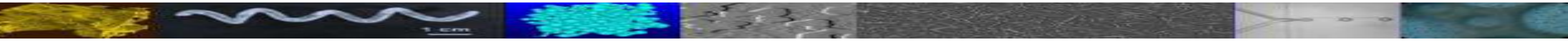


Evolution of the DM and structure with time
→ From a homogeneous polymer solution to a coarsening polymer gel





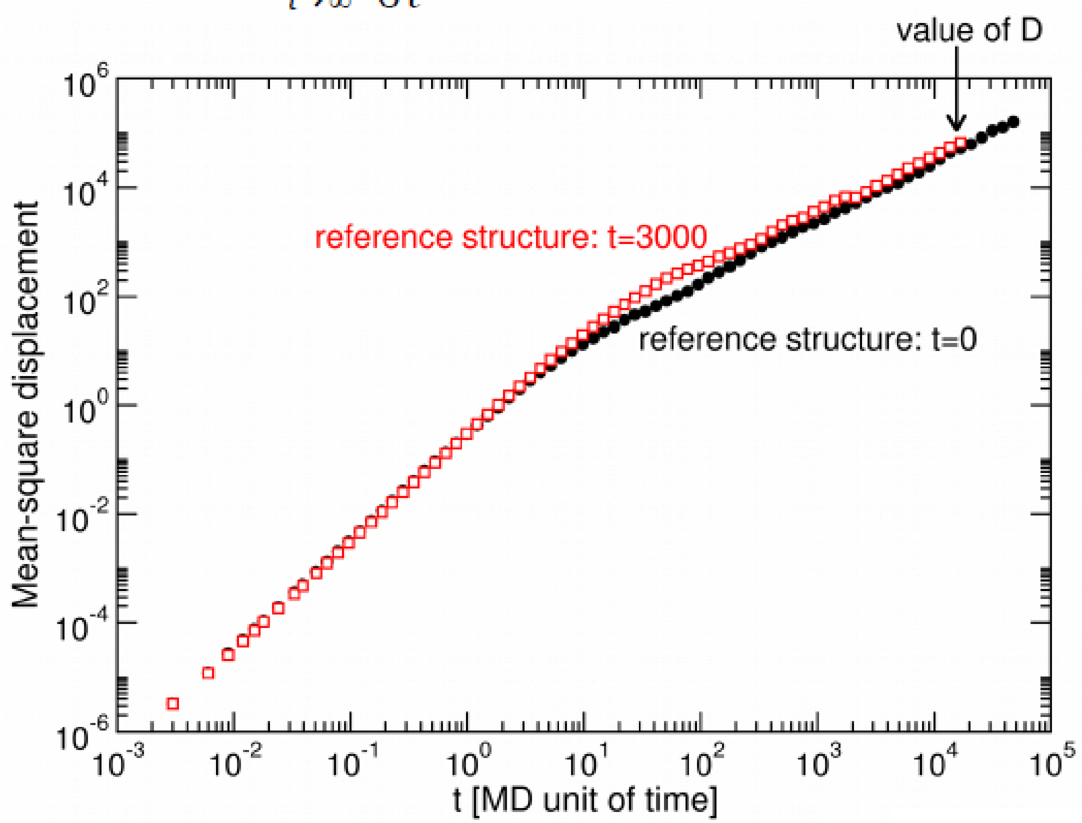




VI. Results – Modelisation of gel formation

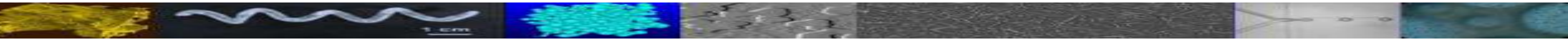
Diffusion coefficient of the enzymes

$$D = \lim_{t \to \infty} \frac{1}{6t} MSD(t) \quad \text{with} \quad MSD(t) = \frac{1}{N} \sum_{i=1}^{N} |r_i(t+t_0) - r_i(t_0)|^2$$

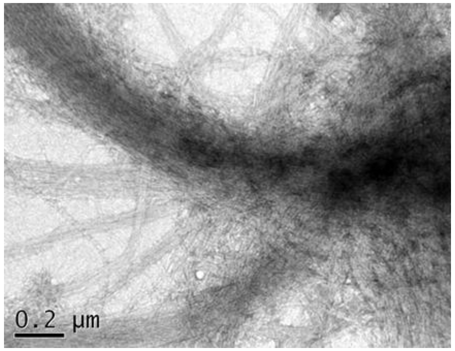


Evolution of the structure in this concentration range
→ No sensible variation of D (except for a slight increase)

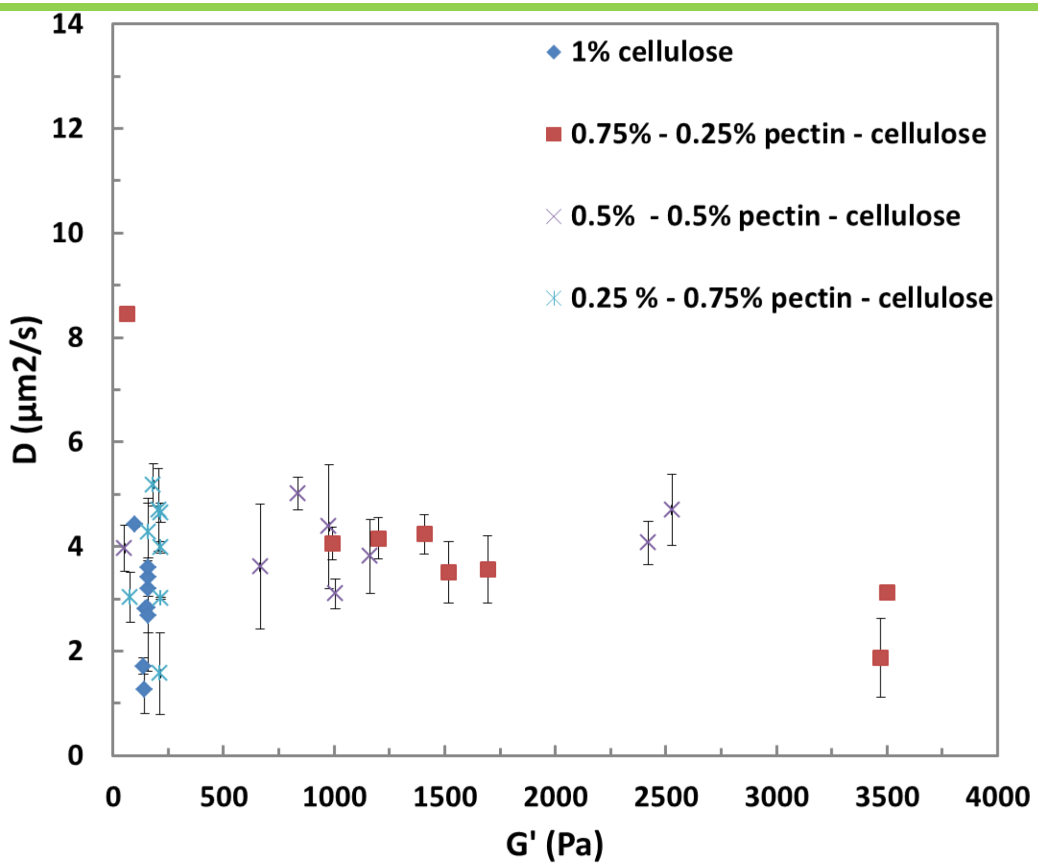
Hugouvieux & Kob, Soft Matter, 2017



VI. Results – Gelation in presence of cellulose



Agoda-Tandjawa, 2009



Slighly higher D values obtained in the first steps of reaction compared to pectic gels alone at the same concentation

Steric effect increases the local pectin concentration leading to increased viscoelastic properties

VII. Conclusions

Strong decrease of D during gelation for all the pectin gels

In the first steps of gelation, D increased with polymer concentration

At longer times (higher G' values): diffusion coefficients are in the same range (< 5 μm2/s)

In agreement with the demethylation profile of PME and modelling

Diffusion and activity are they linked?

Experiments with inactivated PME

Determine the fine morphological parameters of gels
(size of pores, correlation length) and microrheology experiments

Acknowledgements

CEPIA (Research Department) for funding

Estelle Bonnin, Sylvie Clerjon, Juliane Floury, Gabriel Paes
for fruitfull discussions

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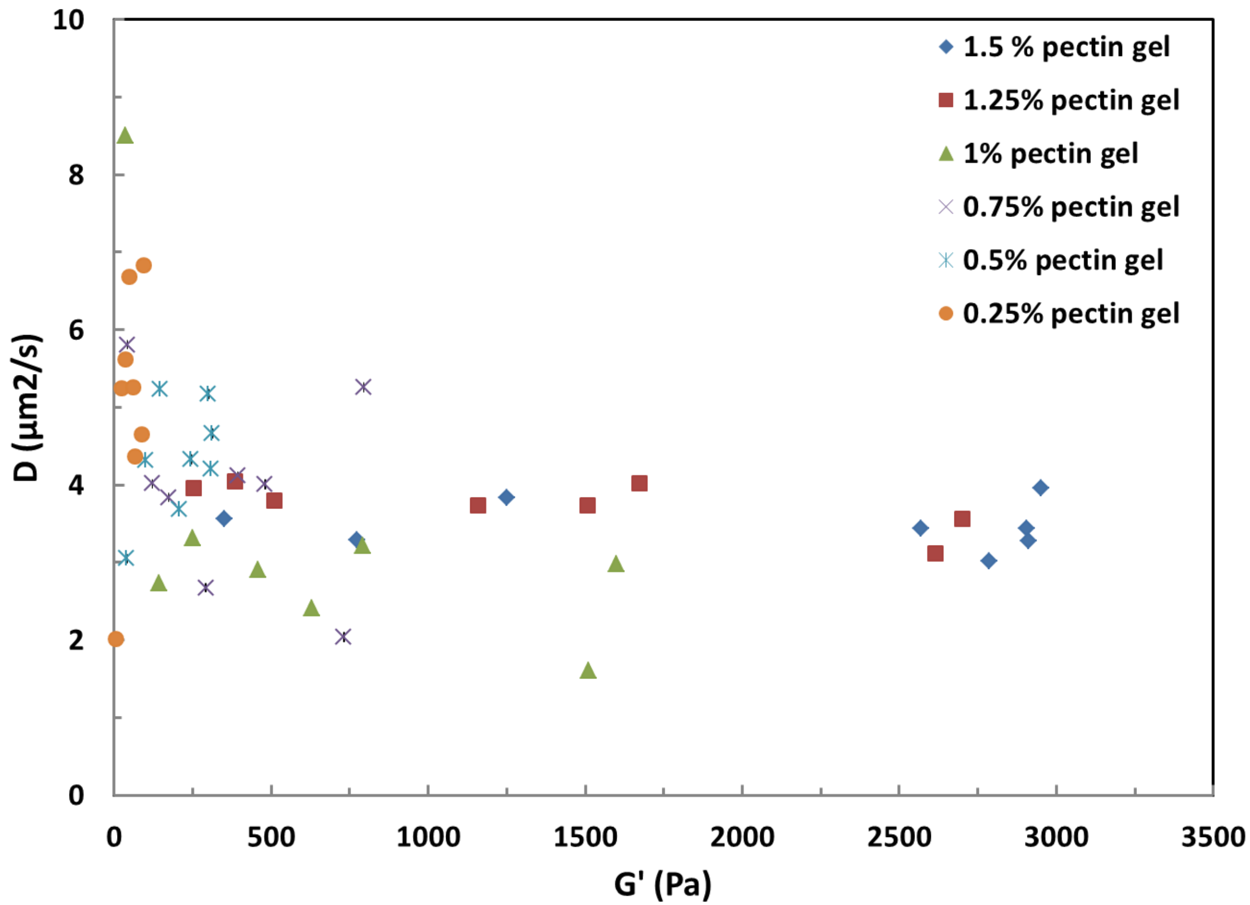
V. Results – During pectin gel formation (1.5-0.25%)



Higher D values obtained during the first steps of gelation