

# Rheology and phase behavior of dense casein micelle dispersions

A. Bouchoux\*, B. Debbou\*, G. Gésan-Guiziou\*,  
M.-H. Famelart\*, J.-L. Doublier#, B. Cabane§



\*STLO, INRA - Agrocampus Ouest UMR 1253, F-35000 Rennes, France

#BIA, INRA UR1268, F-44300 Nantes, France

§PMMH, CNRS UMR 7636, ESPCI, F-75231 Paris, France

## (i) The casein micelle (briefly)

. **Composition** |  $\alpha_{s1}$ -,  $\alpha_{s2}$ -,  $\beta$ -,  $\kappa$ - caseins  
8% minerals ( $\text{Ca}^{2+}$ , phosphate)

. **Large globular aggregates**

Diameter ~ 50-500 nm (30-40% polydisperse)  
(average 200 nm)

. **Soft / "Sponge-like" object**

Voluminosity ~ 4.4 mL/g

. **Structure / ~"core-shell" particles**

"Shell" : polyelectrolyte brush  $\kappa$ -casein

"Core" : Homogeneous web caseins  
+ CaP nanoclusters

. **"Dynamic" colloid / in constant equilibrium with its environment**

Strongly sensitive to pH, T, salts, etc...

Native state  $\rightarrow$  pH 6.7 / I  $\approx$  100 mM

. **(and a fascinating object...)**



Milk proteins = 80% of caseins (25 g/L)

[Dalglish et al., Int. Dairy J., 2004]

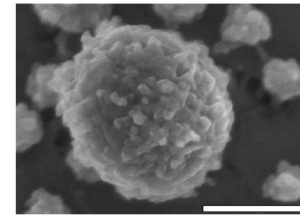
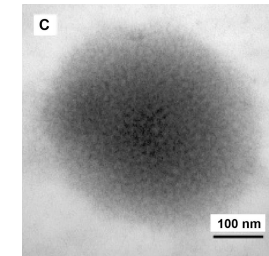
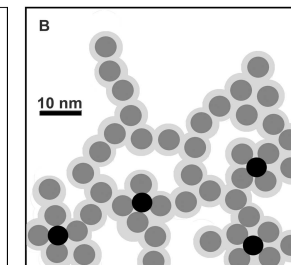
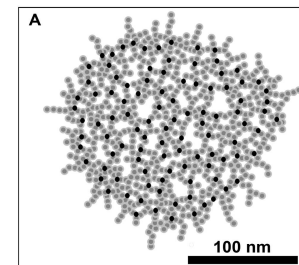
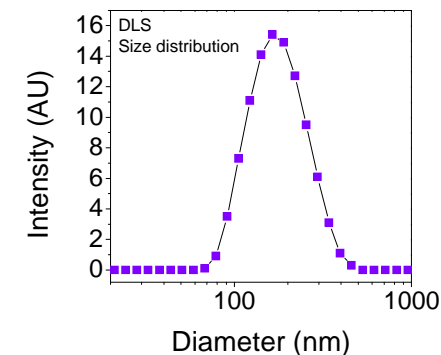


Fig. 2. Electron micrograph of an individual casein micelle, as for Fig. 1. Note the apparent connection between the main micelle and a subsidiary structure, which may be a part of the micelle being dissociated. Note the ring of smaller particles which may represent pieces dissociated from the micelle. Scale bar = 200nm.



[McMahon et al., J Dairy Sc., 2008]



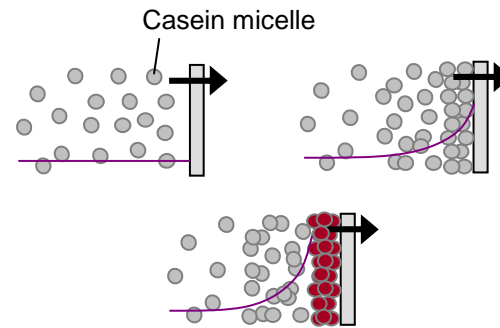
[McMahon et al., J. Dairy Sc., 2008]

● Calcium phosphate nanoclusters  
● Casein

## (ii) Why this study ? Rheology, phase behavior, high concentrations...

### ■ Dairy processing

#### . Membrane filtration



#### . Spray drying



- **Concentrated / highly concentrated states**
- **Performances** depend on the **evolving structure** and **properties** of the dispersions during the concentration process

**How the micelles interact ?**  
**How interactions influence the properties of the dispersions ?**

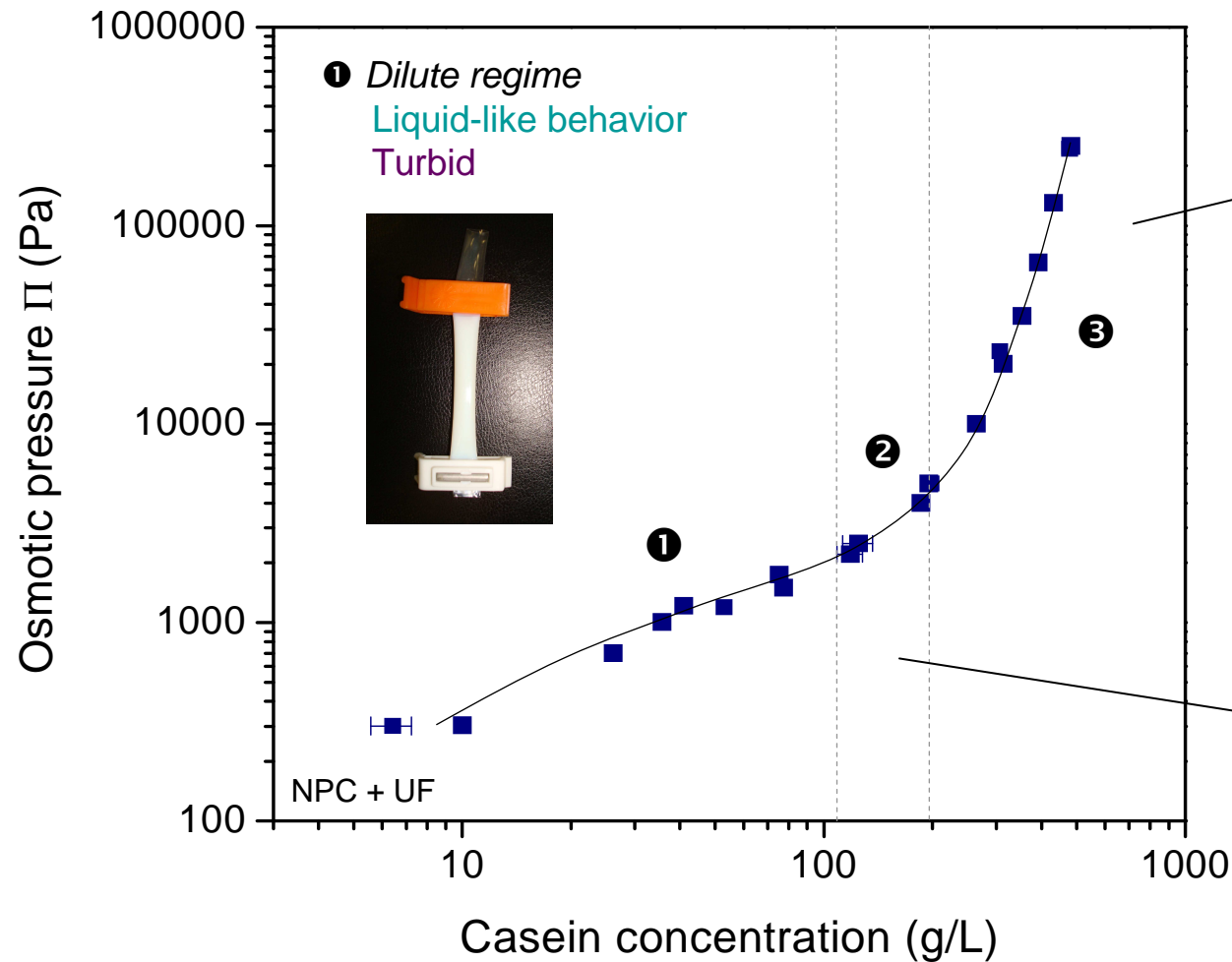
#### **Model system**

- . **Micelle only** (no "serum" proteins)  
NPC = Native PhosphoCaseinate powder
- . **"Native" solvent** :  
UF = Ultrafiltrate of skimmed milk

### (iii) Osmotic stress experiments

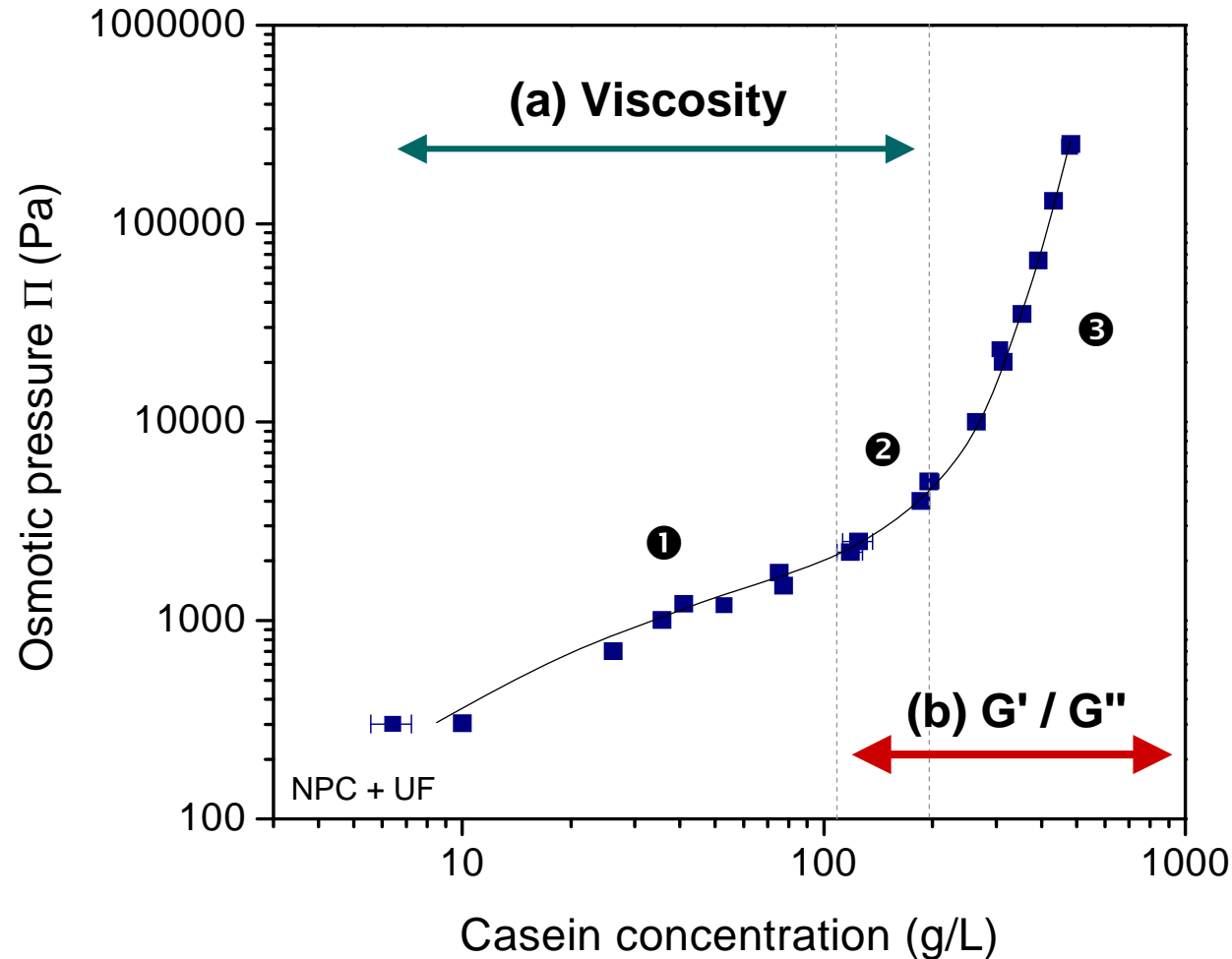
[Bouchoux et al., Biophys. J., 2009]

- $\Pi$  = balance of all interactions in the system



### (iii) What about rheology ?

- Can we learn more from the **flow** and **dynamic properties** of the dispersions ?



#### . QUESTIONS :

##### (a) $\eta$ vs. $\dot{\gamma}$ , C

- Casein micelles as hard spheres ?
- Effect of particles "softness" / deformability ?

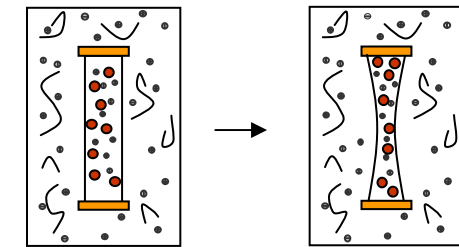
##### (b) $G' / G''$ vs. $\omega$ , C

- Still hard spheres ?
- What forces cause the resistance to deformation ?
- Intermicelle adhesions ?

## (iv) Experimental

### ■ Sample preparation

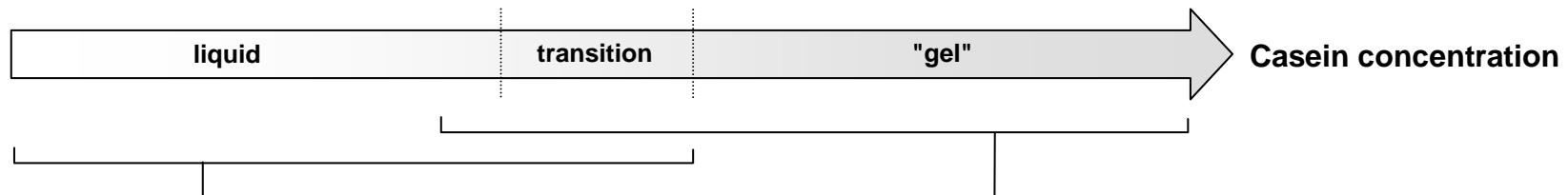
- Casein micelles in native solvent : NPC powder + UF
  - Low concentrations ( $C < 180$  g/L) -> Simple mixing
  - High concentrations ( $C > 180$  g/L) -> Osmotic stress



Osmotic stress / PEG35000

- [ Caseins not structured in micelles : Sodium caseinate (SC) powder + UF ]

### ■ Rheology ( $T = 20^\circ\text{C}$ )



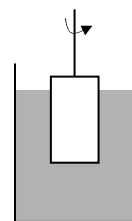
#### (a) Viscosity



Contraves  
Low-Shear 30



TA  
AR2000



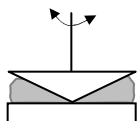
Couette  
geometry

$$\dot{\gamma} = 10^{-1} - 10^3 \text{ s}^{-1}$$

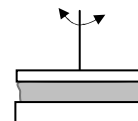
#### (b) $G' / G''$



TA  
AR2000



Cone-  
plate

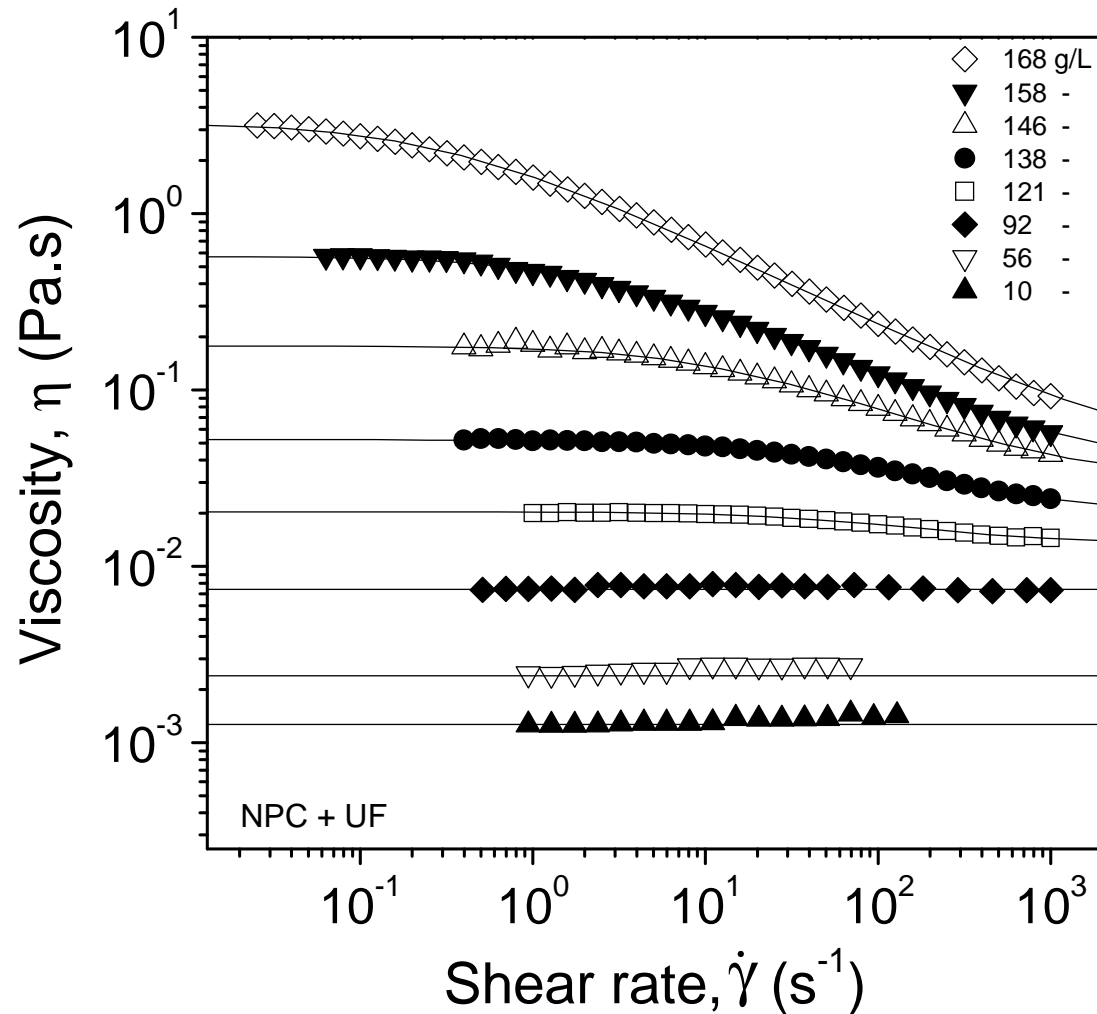


Parallel  
plates

- $f = 10^{-2} - 10$  Hz
- Linear region



# (v-a) Viscous flow / Casein micelles as hard spheres?



• Shear-thinning above 100 g/L

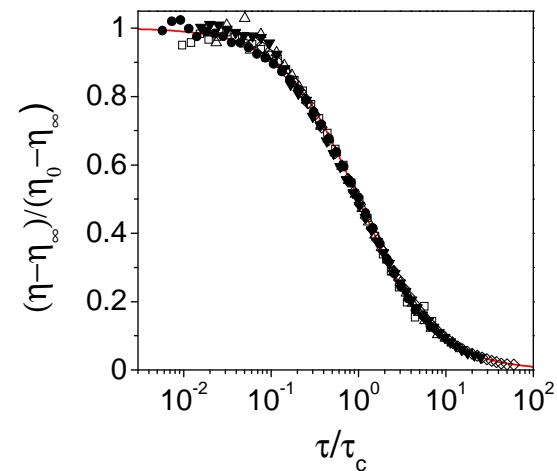
• Common phenomenon for the hard-sphere fluid

[De Kruif et al., J. Chem. Phys., 1985]

[Krieger & Dougherty, J. Rheol, 1959]

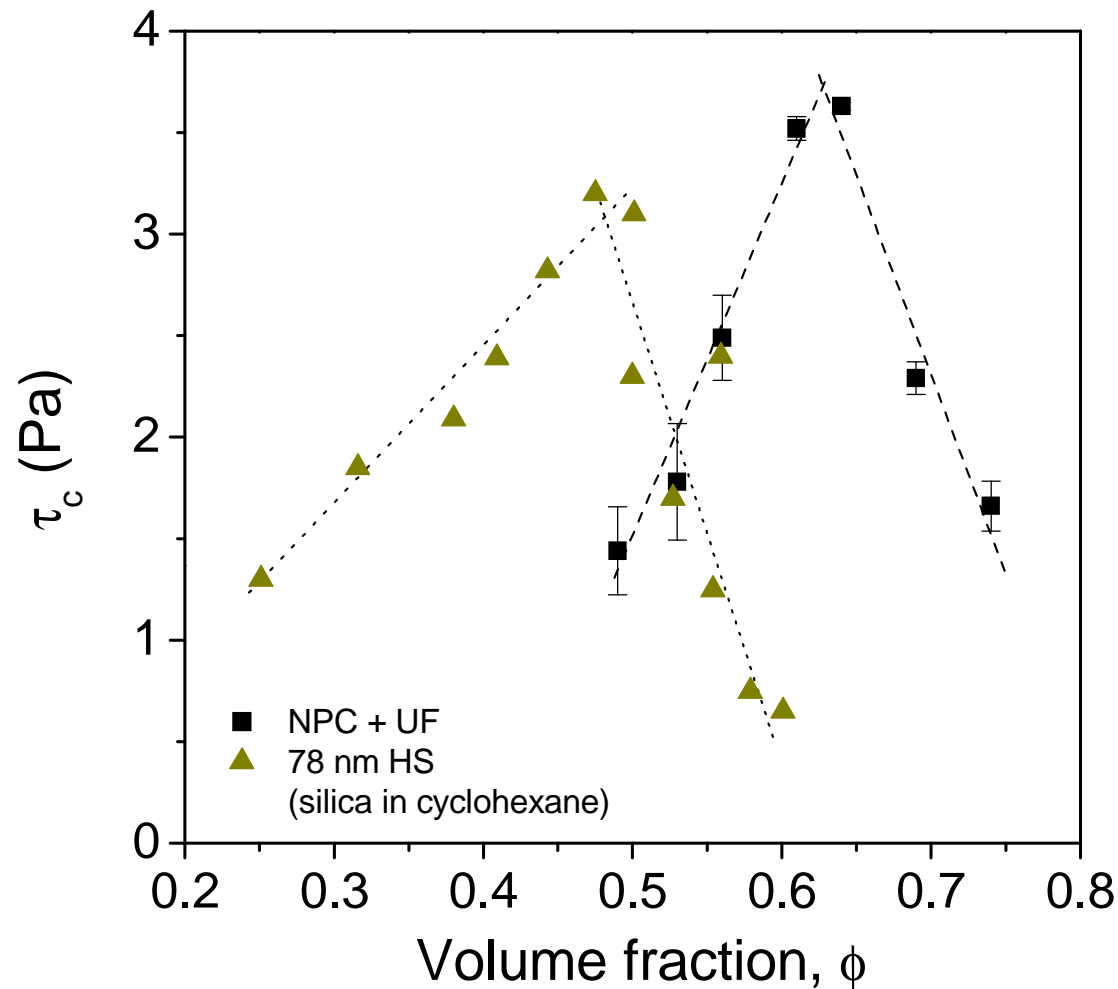
• "Simple" Krieger's model

$$\frac{\eta - \eta_{\infty}}{\eta_0 - \eta_{\infty}} = \frac{1}{1 + \tau/\tau_c}$$



NPC+UF ~ Hard sphere fluid

## (v-a) Viscous flow / Casein micelles as hard spheres?



$$\phi = 4.4 \times 10^{-3} * C(\text{g/L})$$

### Characteristic shear stress $\tau_c$ , NPC vs. monodisperse HS

[De Kruif et al., J. Chem. Phys., 1985]

- Similar values of  $\tau_c$
- Similar behavior:
  - Maximum in  $\tau_c$  (disorder-order)
  - Decrease to 0 (close-packing)

- Shift in volume fractions

#### Polydispersity

Random Close Packing  $\phi_{rcp}$

Monodisperse  $\phi_{rcp} = 0.64$

40% polydisperse  $\phi_{rcp} \approx 0.78$

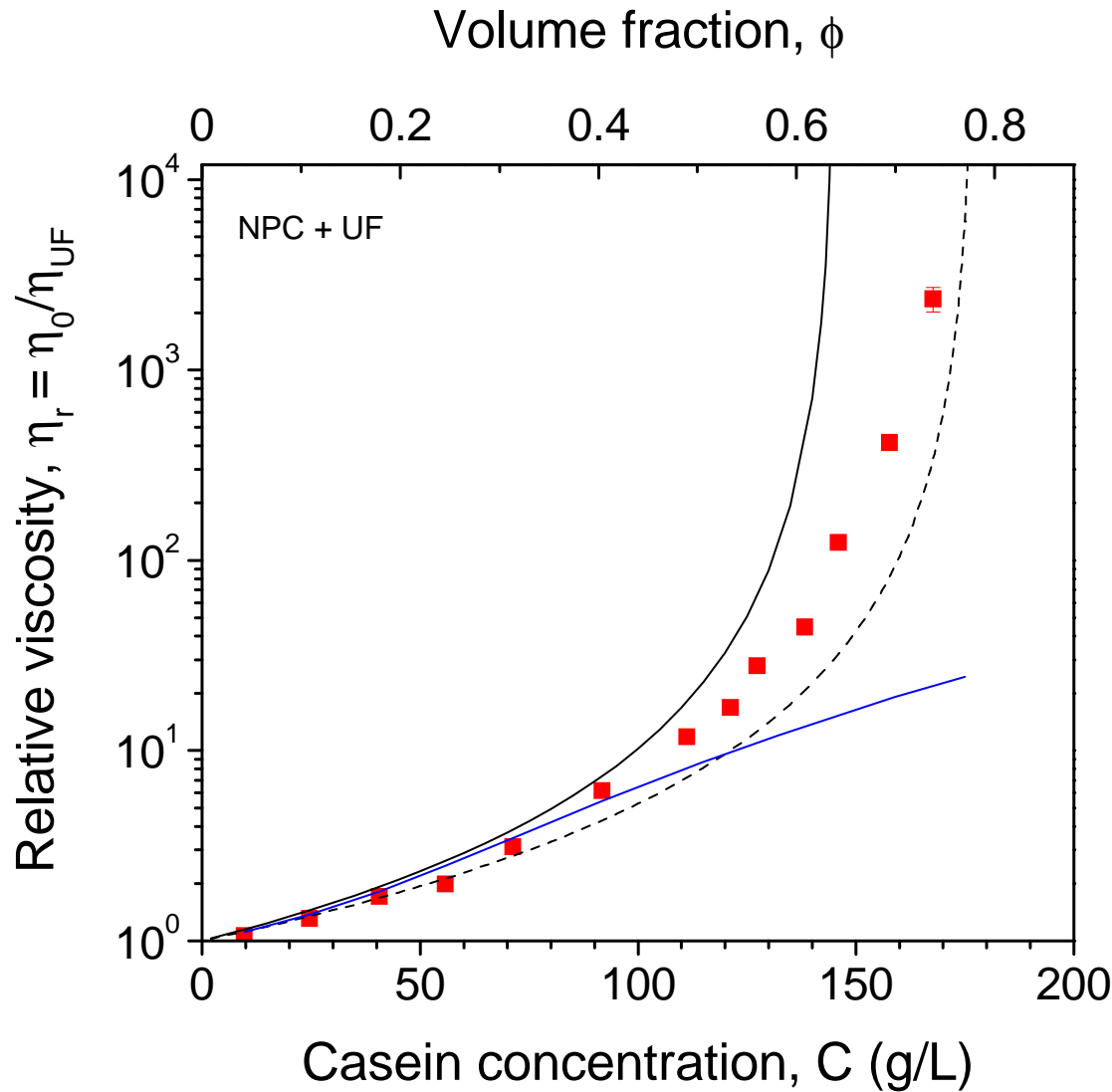
#### Soft spheres

[Russel, Colloidal Dispersions, 1989]

NPC+UF ~ Polydisperse  
Hard sphere fluid



# (v-a) Viscous flow / Casein micelles as hard spheres?



.  $\phi < 0.35$  : HS model

$$\eta_{r,0} = 1 + 2.5\phi + 4\phi^2 + 42\phi^3$$

[De Kruif et al., J. Chem. Phys., 1985]

.  $\phi > 0.35$  : Quemada's equation

$$\eta_r = (1 - \phi/\phi_{\max})^{-2}$$

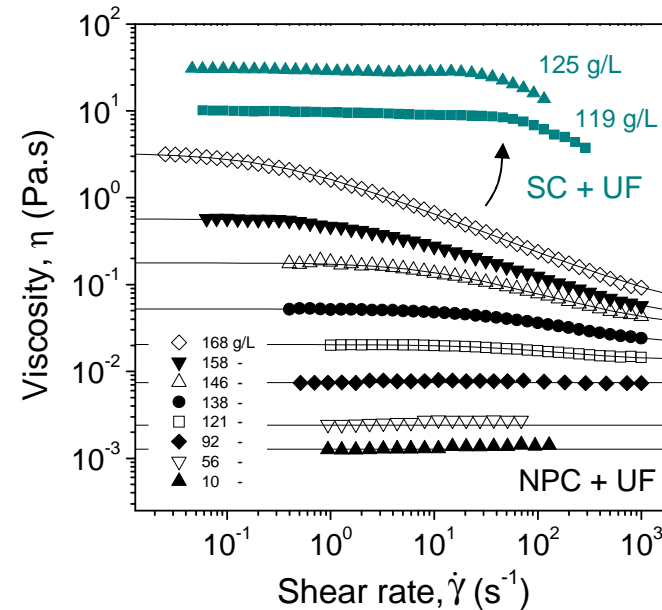
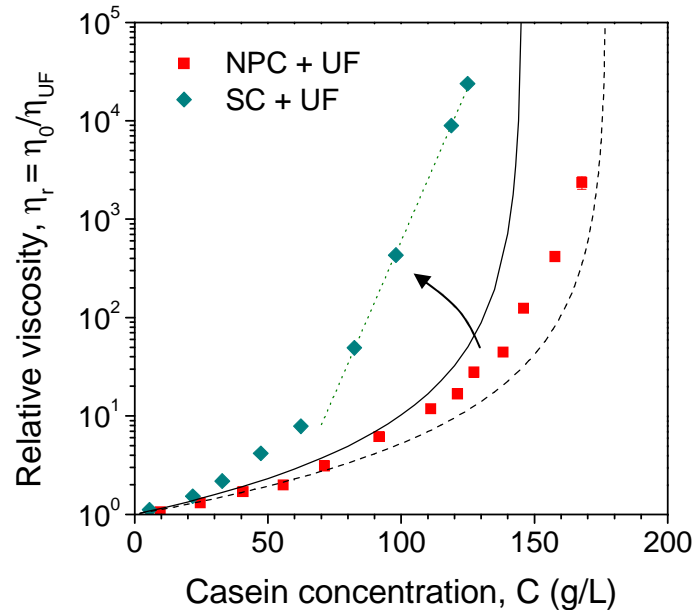
—  $\phi_{\max} = 0.64$

- - -  $\phi_{\max} = 0.78$

NPC+UF ~ Polydisperse  
Hard sphere fluid

# (v-a) Viscous flow / Casein micelles as hard spheres?

- And when the micelle structure is lost ? (Sodium Caseinate - SC)



• Sodium caseinate dispersions = associating polymer / "hyper-entanglements"

- Resistance to flow of "native" casein micelle dispersions at C < 180 g/L

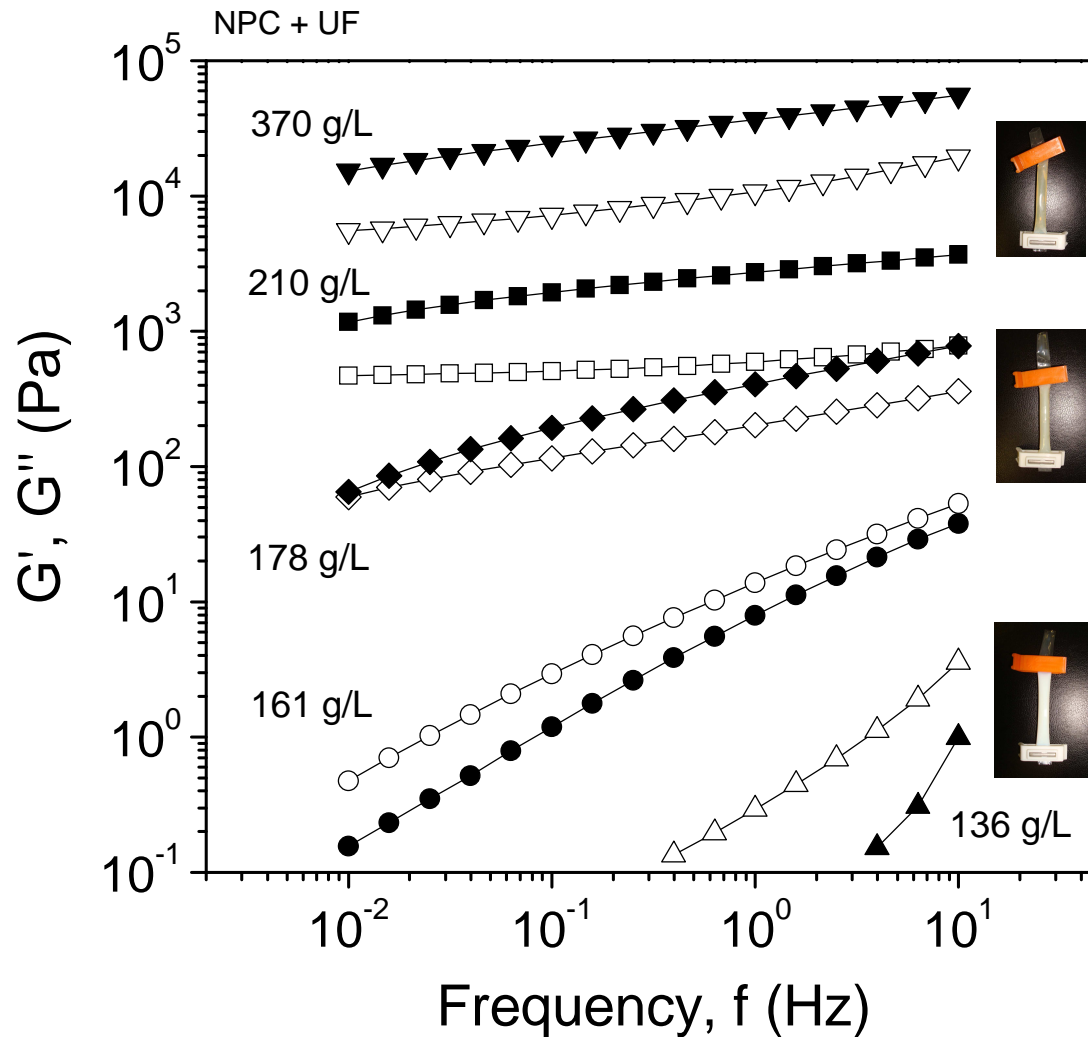
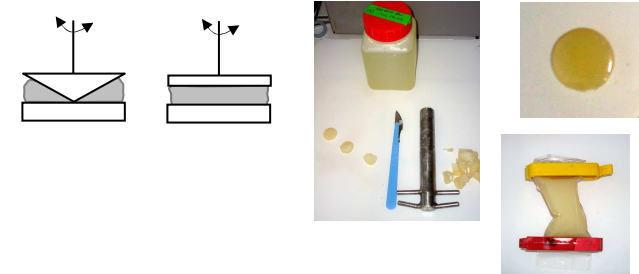
~ 40% polydisperse hard sphere fluid

→ The micelles interact through excluded volume effects only

In accordance with short-range interactions through the  $\kappa$ -casein brush

The schematic shows a cluster of grey spheres of varying sizes, representing the polydisperse nature of casein micelles. The spheres are arranged in a way that suggests interactions between them, consistent with the text's description of excluded volume effects.

# (v-b) Viscoelastic properties

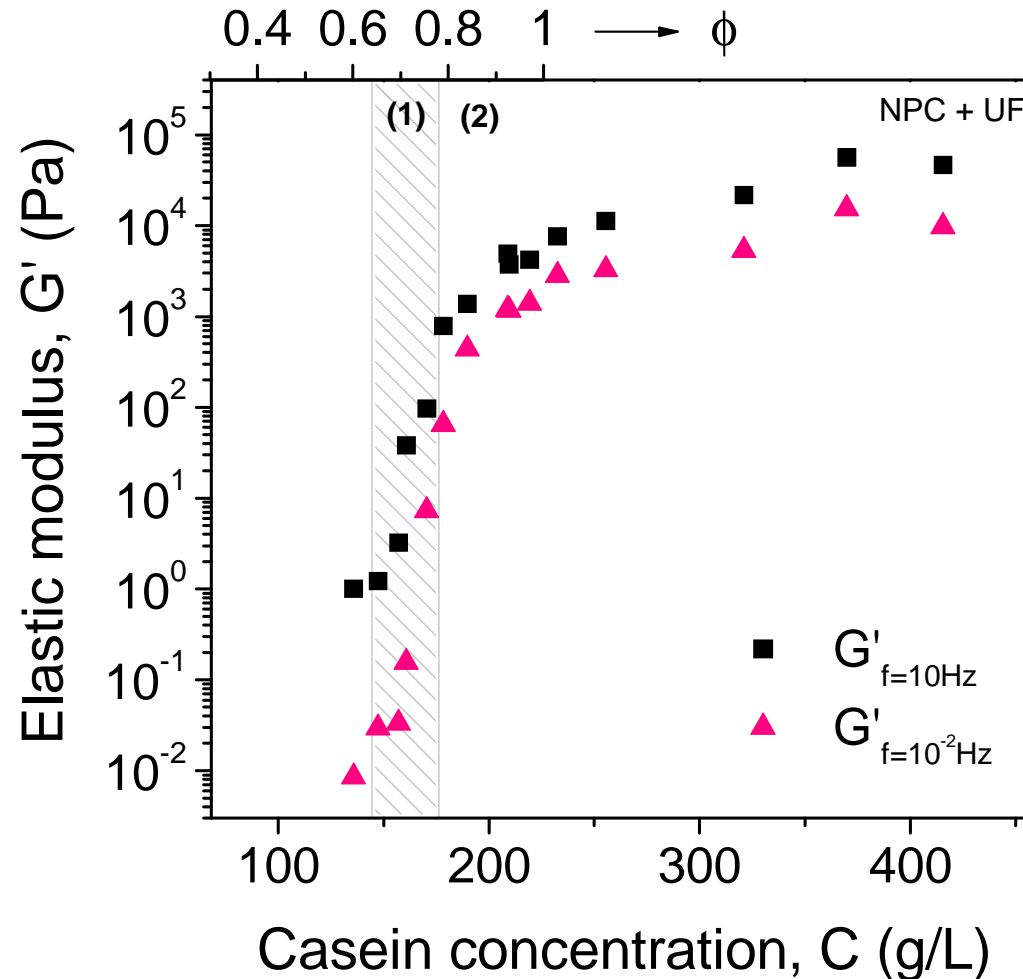


$G' \gg G''$   
Very low frequency dep.

$G'/G''$  crossover  
Lower frequency dep.

Viscoelastic fluid  
Strong frequency dependence

# (v-b) Viscoelastic properties



.  $G'$  vs.  $C$  → 2 regimes

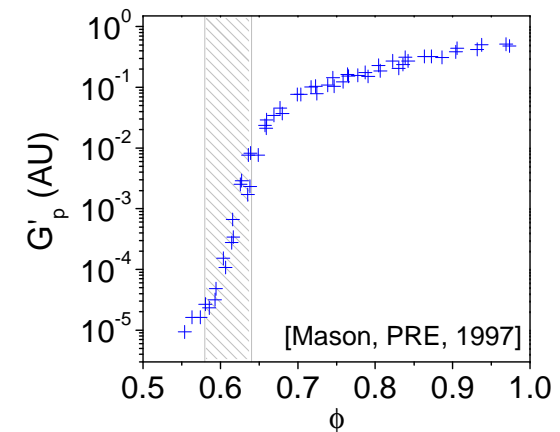
(1)  $C < 178$  g/L,  $\phi < 0.78$

- Micelles are still separated
- Dramatic increase in  $G'$

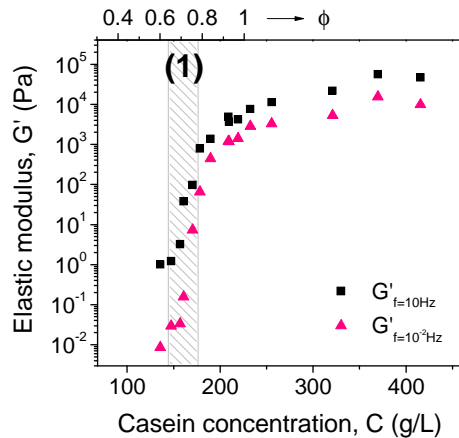
(2)  $C > 178$  g/L,  $\phi > 0.78$

- Micelles deform and deswell
- Increase in  $G'$  with  $C$  is less pronounced

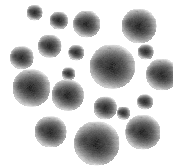
Similar behavior with monodisperse emulsions (!)



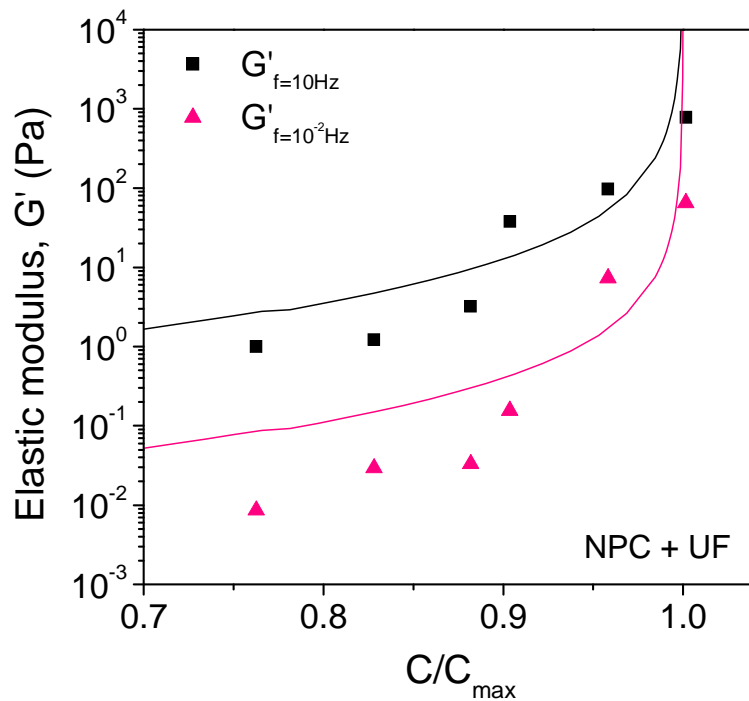
# (v-b) Viscoelastic properties



- (1)  $C < 178 \text{ g/L}$ ,  $\phi < 0.78$
- Micelles are still separated
  - Dramatic increase in  $G'$



**Hard sphere fluid**  
 "Classical" models of  
 Lionberger & Russel  
 [J. Rheol, 1994]



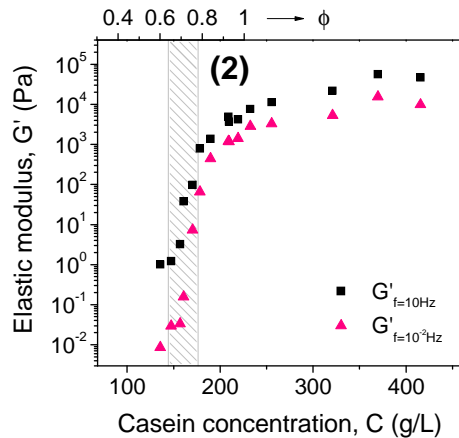
- Calculations**
- No adjusted parameter
  - Spheres of radius 100 nm
  - $C_{\max} = 178 \text{ g/L}$

$$\frac{G'_{\infty} d^3}{k_B T} = \left( \frac{d^2 \omega}{D_S^0(\phi)} \right)^{1/2} \frac{24 \phi^2}{5 \pi} g(1)$$

$$g(1) = \begin{cases} \frac{1 - (1/2)\phi}{(1 - \phi)^3}, & \phi < 0.5 \\ \frac{0.78}{0.64 - \phi}, & \phi \geq 0.5 \end{cases}$$

$$D_S^0 = D_0(1 - 1.56\phi)(1 - 0.27\phi)$$

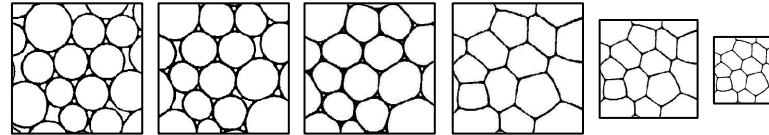
# (v-b) Viscoelastic properties



(2)  $C > 178 \text{ g/L}$ ,  $\phi > 0.78$

- Micelles deform and deswell
- Increase in  $G'$  is less pronounced

→ 2 possible sources of elastic resistance



- Resistance to deformation of the micelle "itself"

- . Facets are created upon compression
- . Comparable to emulsions that resist to shear stress through the elasticity of their interfaces

→ Spring network model [Mason, PRE, 1997]

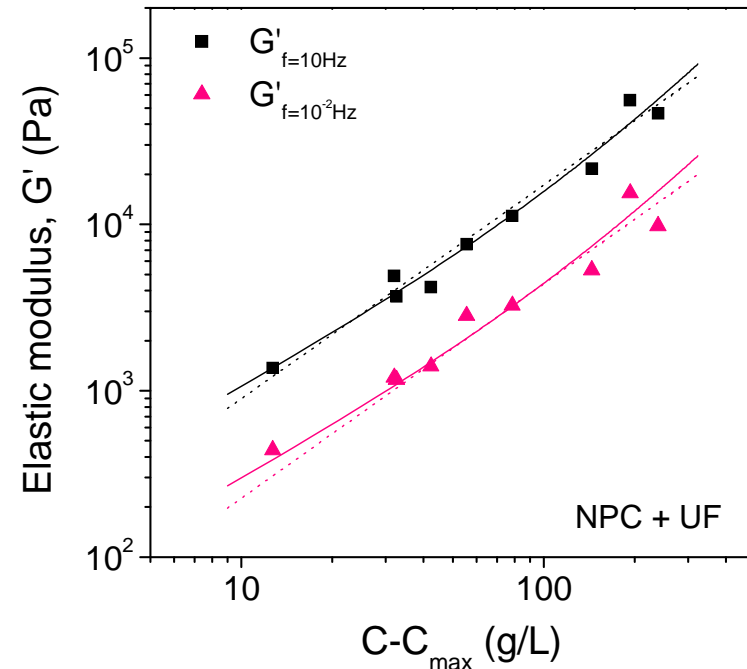
$$G' \sim C(C - C_{\max})$$

- Resistance of bonds between micelles

- . Intermicelle bonds are formed (gel is partly "irreversible") [Bouchoux et al., Biophys. J., 2009]
- . Number of bonds  $\propto (C - C_{\max})$

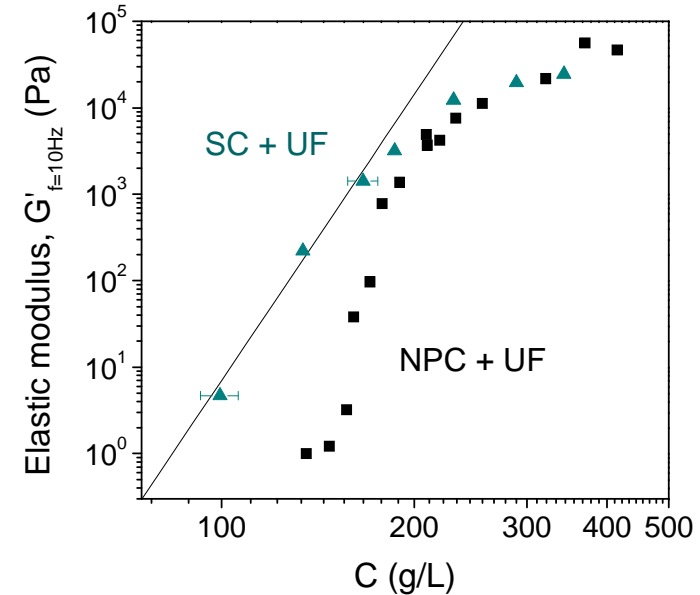
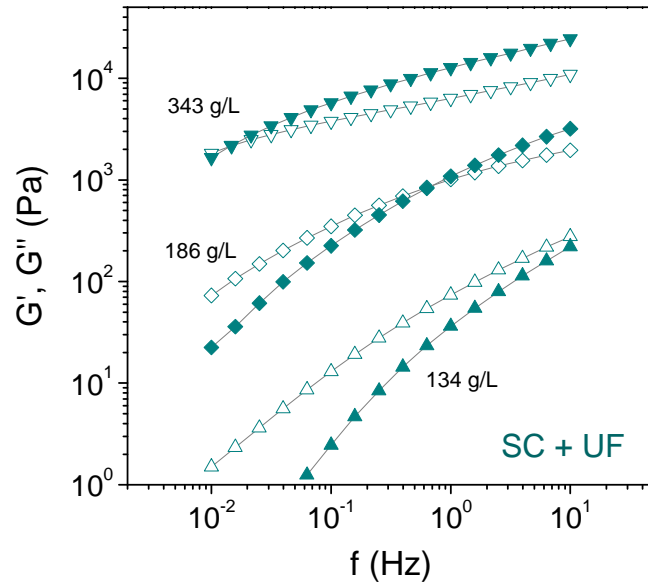
→ Percolation theory

$$G' \sim (C - C_{\max})^n \quad (\text{with } n = 1.3)$$



## (v-b) Viscoelastic properties

- And when the micelle structure is lost ? (Sodium Caseinate - SC)

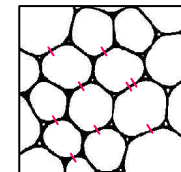


. Sodium caseinate dispersions = associating polymer / reversible gelation / no bonds

- Resistance to deformation of casein micelles dispersions

$C < 178 \text{ g/L} \rightarrow$  Viscoelastic behavior of the hard sphere fluid

$C > 178 \text{ g/L} \rightarrow$  Micelles deformation and intermicelle bonds



## (vi) Important conclusions / Answers to our questions...

### (a) Viscosity data

- . Casein micelles as hard spheres ?

**Yes**

- . Effect of particles "softness" / deformability ?

**Possibly; At high shear and approaching the transition**

### (b) Viscoelastic behavior

- . Still hard spheres ?

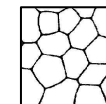
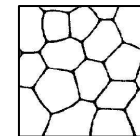
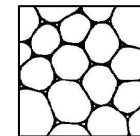
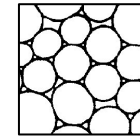
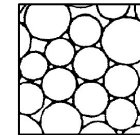
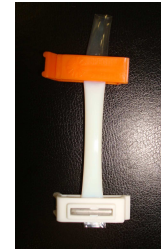
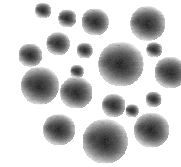
**Yes, until close packing at  $C = C_{\max} \approx 178$  g/L**

- . What forces cause the resistance to deformation after  $C_{\max}$  ?

**Combination of (i) Mechanical resistance of the micelle itself  
(ii) Formation of intermicelle bonds**

- > "Structure-dependent" forces that are absent in SC dispersions

**Real nature of these forces ?...**

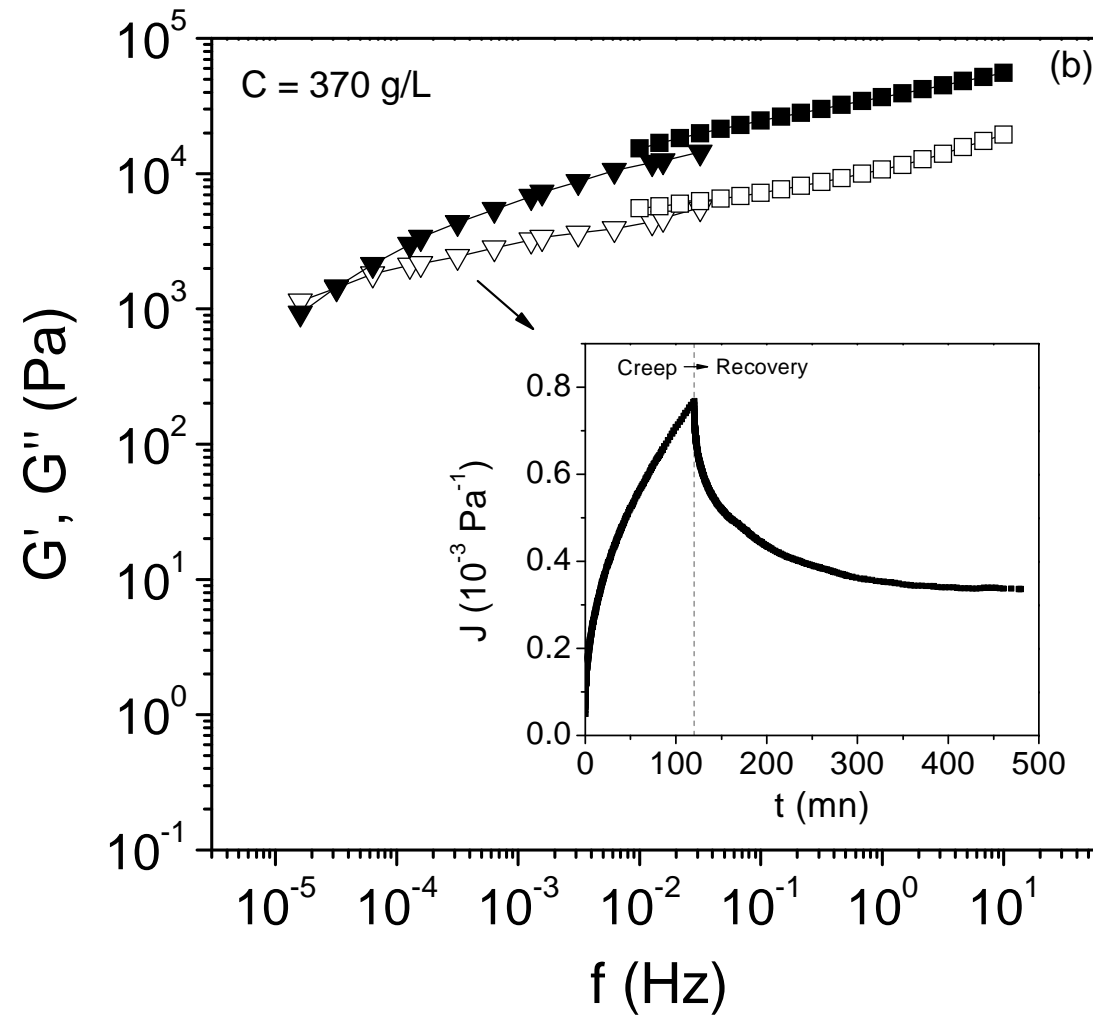




# Thank you



# (extra) Creep-Recovery tests



# (extra) $G'$ & $\Pi$ vs. $C$

