Testing Sputum in Cystic Fibrosis Patients using Rheology: the Ageing Effect

Étienne Ghiringhelli,[1] Matthieu Robert de Saint Vincent,[1] Boubou Camara,[2,3] Sébastien Quétant,[2,3] Christophe Pison,[2,3] and Jérémy Patarin[1]

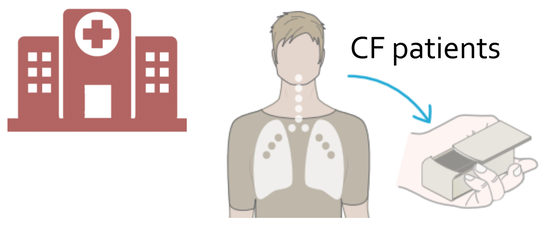
1 Rheonova, Grenoble, France, contact@rheonova.fr; 2 CHU Grenoble Alpes, Pôle Thorax et Vaisseaux, Clinique Universitaire de Pneumologie, Grenoble, France; 3 Univ. Grenoble-Alpes, LBFA-U1055, Grenoble, France

Context

Sputum rheology investigates mechanical properties of the mucus, *i.e.* elasticity and viscosity. Rheology is proposed as a prospective tool to **follow the status of patients with cystic fibrosis** (CF), and to **assess the individual efficiency of current and future treatments** during clinical practice (endpoints). However, this characterisation may involve sample ageing during their transient storage from collection to measurement. To better define rheological assessment protocols, the possible effect of **ageing** of sputum samples on their rheological properties is investigated.

Protocol

Sample Collection



> 1 mL sample; N = 17

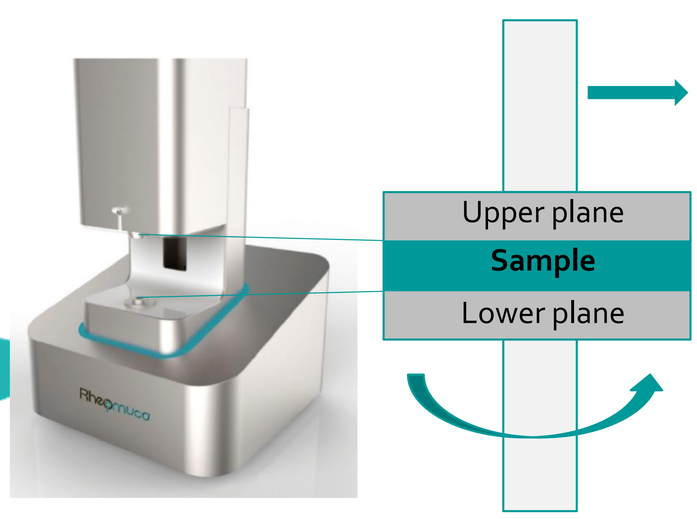
Sample Preparation

Sputum is composed of **highly viscoelastic mucus plugs** included in a **less viscoelastic matrix**. This heterogeneity induces **artefacts** in rheology measurements.

French patent FR 1757108, filed 26 July 2017

Rheological Measurements

Rheology is the study of the flow of matter that exhibits a combination of **elastic, viscous and plastic behaviours** by combining elasticity and fluid mechanics.



In phase contribution:

- **G′ elastic modulus** $G' = \frac{\tau}{\gamma}\cos\delta$

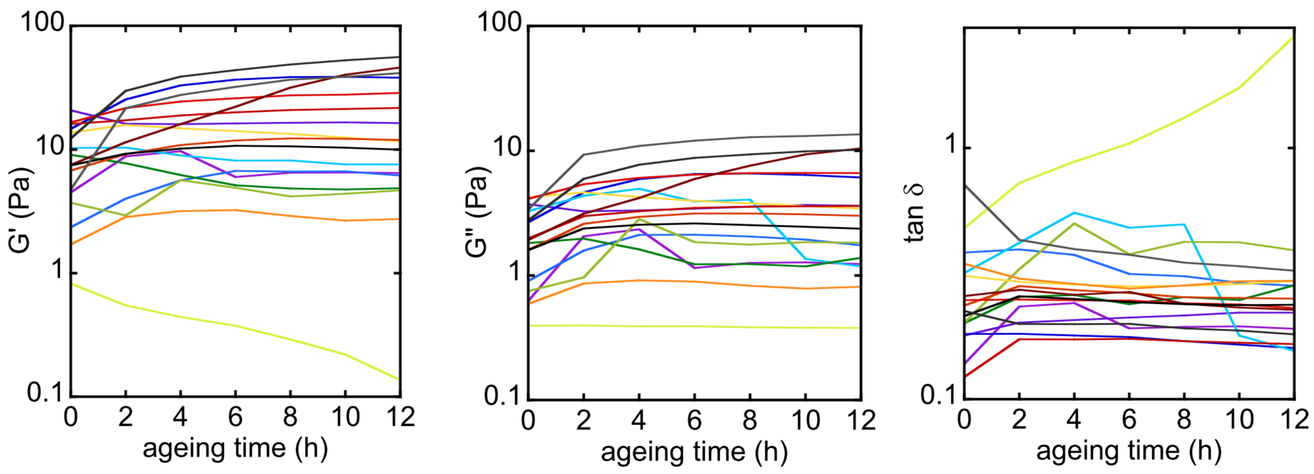
Out of phase contribution:

- **G″ viscous modulus** $G'' = \frac{\tau}{\gamma}\sin\delta$

- **tan δ phase tangent** $\tan\delta = \frac{G''}{G'}$

tan δ < 1 → Gel (solid-like) behaviour
tan δ > 1 → Viscous (liquid-like) behaviour

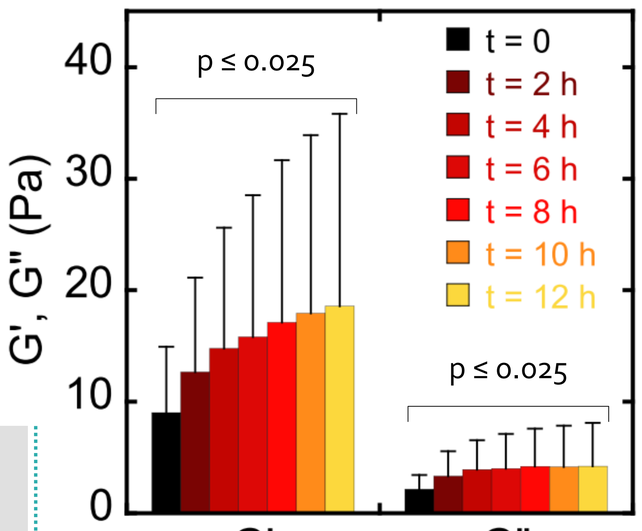
0.5% strain @ 1 Hz & **4°C** (1 min) → G′, G″, tan δ in the **linear regime** every 2 h

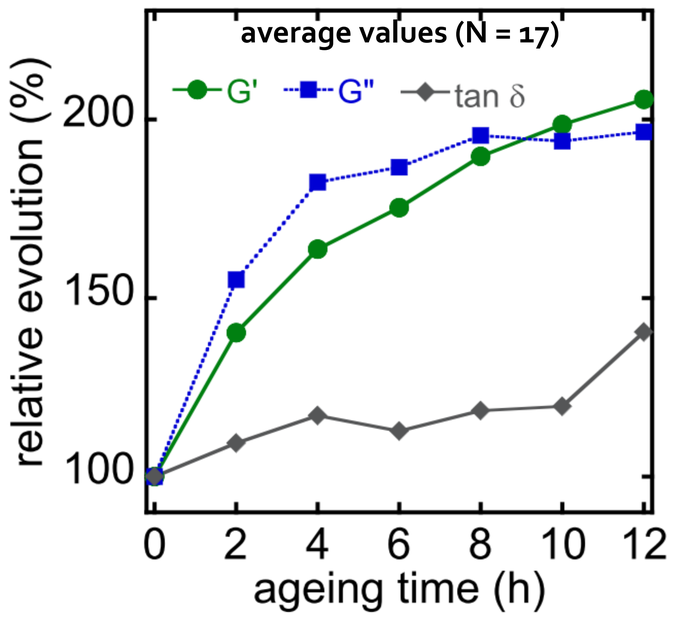


Individual evolution:

- G′ and G″ consistently increase over time, while tan δ essentially remains constant.
- G′ and G″ increases gradually slow down within 4–6 h.

Results





Sputum ageing (4°C):

- G′ and G″ both increase with **similar dynamics** (initially fast, slows down after 4-6 h).
- G′ and G″ both increase in **similar proportions** (x2 within 8-10 h).
- tan δ remains constant.

Concluding Remarks

1. **Mucus globally thickens** when stored at 4°C. This thickening effect mainly occurs within the first 4–6 h after sputum collection. However, **no significant gelation or fluidisation** is observed, suggesting that this thickening is not related to evaporation.

2. Since the sputum viscoelasticity evolves with storage time, **care must be taken to minimise it and/or to define unified protocols** if rheology is to be used as a clinical marker.

Perspectives

Measurements performed at 4°C mimic the standard sample storage conditions. Other relevant storage conditions (*e.g.*, at room temperature (20°C), within controlled humidity, after freezing/thawing cycles) would call for complementary investigations to understand the possible changes in the mucus microstructure.

contact@rheonova.fr