Influence of medium composition on oxygen transfer rate in animal cell culture

D. Toye, A. Galifi, T.Salmon, P. Marchot, E. Verdin, M. Crine

LGC CHEMICAL ENGINEERING

gsk GlaxoSmithKline Biologicals

Université de Liège

Introduction

- **Culture of animal cells in suspension in bubble columns**
  - Oxygenation of culture medium by gas bubbling (air – O_2)
  - Hydrodynamic stress on cells adhering on bubbles (bubble breakage and coalescence)
  - Foam formation
- **Additives**
  - Reduce cell adherence (Pluronic F68)
  - Increase bubble stability (PVP)
  - Limit formation of foam (antifoam agent)
- **Influence on liquid properties** (viscosity, surface tension)
  => influence on oxygen transfer rate, k_La
  (increased resistance, smaller bubbles, coalescence phenomenon)

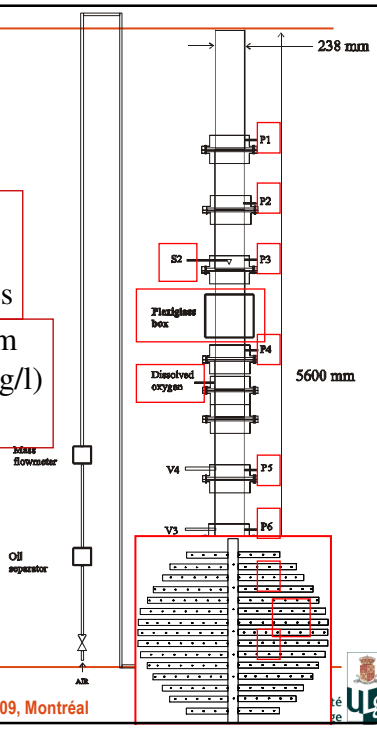
Scope of the study

- **Discriminate the influence of additives on**
  - Mass transfer coefficient, k_L
  - Interfacial surface area, a

- **Experimental approach**
  - **Synthetic liquid culture medium**
    (aqueous solution containing electrolyte and proteins)
  - **Experimental measurements**
    - Liquid phase properties : viscosity, surface tension
    - Gas phase distribution : bubble diameter, holdup
    - Global oxygen transfer rate : k_La
  - **Integrated analysis** of all experimental data
    => effects on k_L and on a and resulting effect on k_La

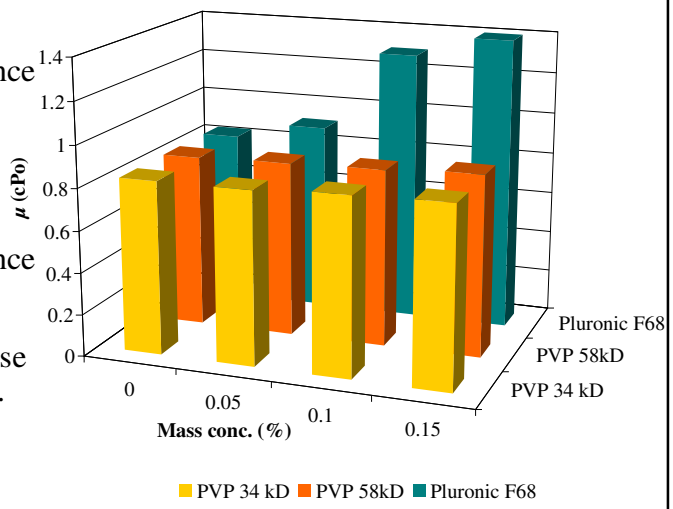
Experimental setup

- Bubble column (transparent PVC)
  H = 5.6 m – I.D. = 0.24 m
- Gas = air
  u_G = 0.4 – 2 cm/s (0.05 – 0.25 vvm)
  Stainless steel sparger : 203x1 mm holes
- Liquid phase = synthetic culture medium
  Water + NaCl (9g/l) + Albumin (100 mg/l)
  + additives (cell protection + antifoam)
- S1 – S2 : Optical probes (saphire tips)
- Plexiglas box
- P1-P8 : Pressure probes
- Dissolved oxygen probe



Liquid phase properties : viscosity

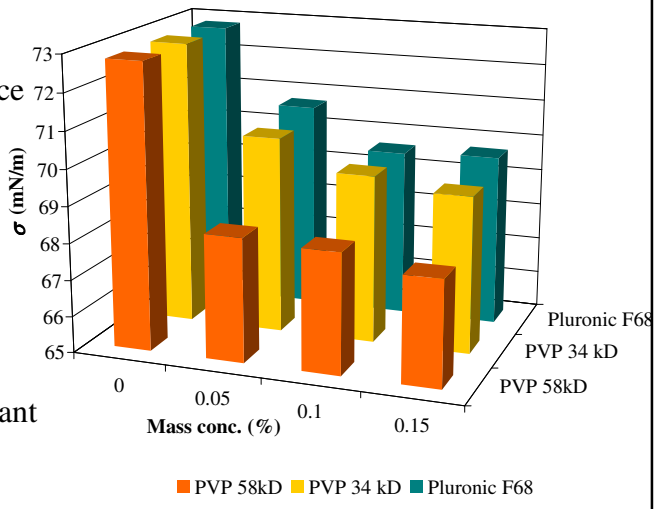
- **NaCl and Albumin**
  => Negligible influence
  ($\mu_{medium} \approx \mu_{water}$)
- **PVP**
  (34kD and 58kD)
  => Negligible influence
- **Pluronic F68**
  => Significant increase
  (conc. = 0.15% mass.
  => +70% rel.)



Increased resistance to transfer expected with Pluronic F68

Liquid phase properties : surface tension

- **NaCl and Albumin**
  => Negligible influence
  ($\sigma_{medium} \approx \sigma_{water}$)
- **PVP 34kD**
  & **Pluronic F68**
  => Low surfactant effect
- **PVP 58kD**
  => Significant surfactant effect (-7% rel.)



Smallest bubbles (highest gas holdup) are expected with PVP 58kD

Liquid phase properties : Expected effects on oxygen transfer rate

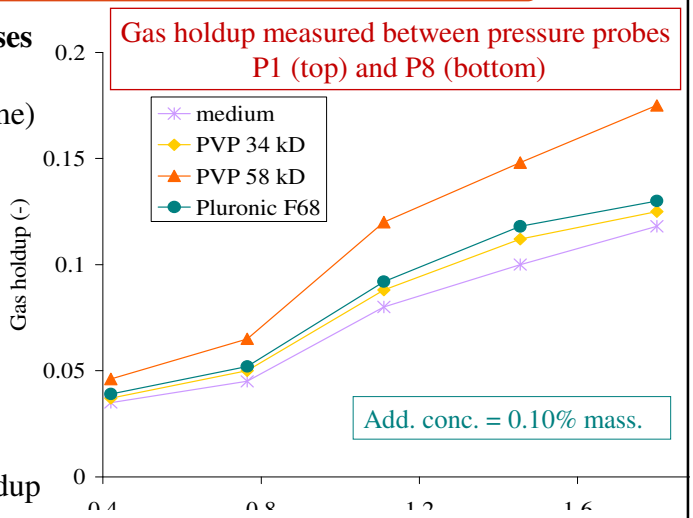
- **NaCl** (9 g/l) and **Albumin** (100 mg/l)
  => Negligible influence on liquid properties
- **Pluronic F68**
  => Significant increase of viscosity & low surfactant effect
  => **Significant negative effect** on oxygen transfer rate
- **PVP 34kD**
  => Negligible effect on viscosity & low surfactant effect
  => **Limited** (+ or -) **effect** on oxygen transfer rate
- **PVP 58kD**
  => Negligible effect on viscosity & significant surfactant effect
  => **Significant positive effect** on oxygen transfer rate

Gas phase properties : global holdup

Gas holdup **increases** linearly **with** u_G (homogenous regime)

PVP 34kD & **Pluronic F68**
=> Small increase of gas holdup (< 10% rel.)

PVP 58kD
=> Significant increase of gas holdup (max +50% rel.)



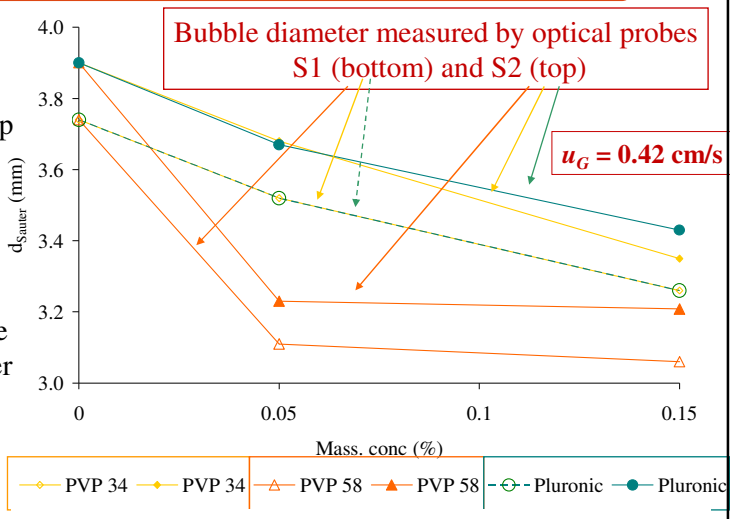
Good agreement with measured surfactant effects

Gas phase properties : bubble diameter

Bubble diameter **increases** (+5% rel.) from bottom to top (pressure ↘ ≠ coalescence)

PVP 34kD & **Pluronic F68** => Small decrease of bubble diameter

PVP 58kD => Significant decrease of bubble diameter



Good agreement with measured surfactant effects

Gas phase properties : Effects on interfacial surface area

- **Pluronic F68** & **PVP34kD**

  => Small increase of gas holdup
  & Small decrease of bubble diameter
  => **Low effect** on interfacial surface area

- **PVP 58kD**

  => Significant increase of gas holdup
  & Significant decrease of bubble diameter
  => **Significant positive effect** on interfacial surface area

Surface tension has a **major influence** on interfacial surface area

Viscosity has a **negligible effect** on interfacial surface area

Oxygen transfer rate

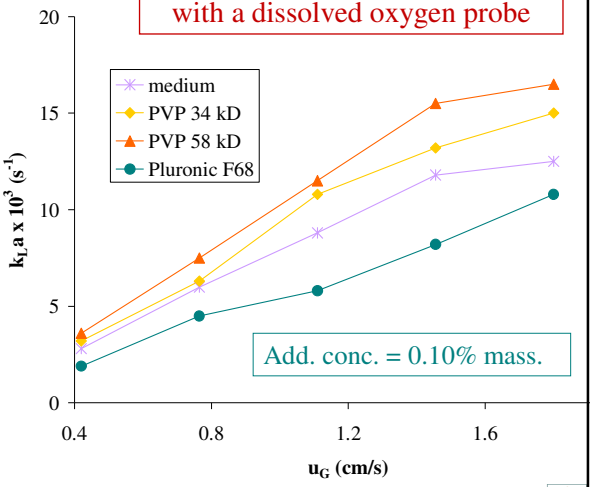
k_La **increases** linearly **with** u_G

Pluronic F68
=> **Significant negative** effect on k_La

PVP 34kD
=> **Low positive** effect on k_La

PVP 58kD
=> **Significant positive** effect on k_La

k_La measured by the "gas in – gas out method" with a dissolved oxygen probe

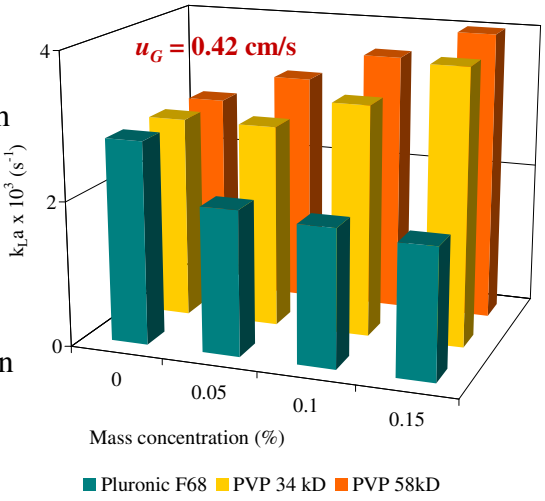


Oxygen transfer rate

Influence of additive concentration on k_La

PVP 34kD
& **PVP 58kD**
(positive effect)
=> k_La **increases** with concentration

Pluronic F68
(negative effect)
=> k_La **decreases** with concentration



Result synthesis

Additive	Viscosity	Surface tension	Gas holdup	Bubble diameter	$k_L a$
PVP 34 kD	=	↘	↗	↗	↗
PVP 58 kD	=	↘↘	↗↗	↗↗	↗↗
Pluronic F68	↗	↘	↗	↗	↘

The increased resistance induced by a viscosity increase is not easily counterbalanced by an interfacial surface area increase due to surfactant effect

Gas phase properties mainly depend on surface tension

Concluding remarks

- Very low gas superficial velocities (0.4 to 2 cm/s) and aeration rates (0.5 to 0.25 vvm)
  => **No coalescence phenomenon**

- Synthetic culture medium without any cell
  => **No time evolution of medium composition**

More experiments are required before drawing any definite conclusions

Thank you for your attention