# The Mucus Barrier: Understanding and Recreating a Suitable Analog for Absorption Studies

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## 1. Introduction

- Mucus gel lines the surface of the small intestine, protecting it from infections and physical damage.
- Commercial mucin, or freshly harvested mucus is not suitable for absorption studies as it can kill cells, due to its contaminated nature
- It is valuable to invent a reproducible method to make up mucus that is suitable for further research

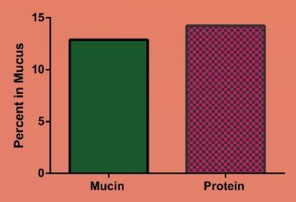
## 2. Aims

- To mimic native mucus construction and behaviour in a normal gut
- Identify key properties (rheology and permeation), and composition of mucus; as a standard to meet.

## 3. Methods

- The components used to reconstruct mucus are separated from native mucus into mucin (main gelling component), proteins, fats, and DNA.
- The components were then reconstituted to understand its role in mucus.
- The procedure to separate mucus is described by Friedl et al (2013), Pierce BCA (Bradford) Protein Assay is according to manufacturer's instruction and Periodic Acid – Schiffs (PAS) Assay is described by Mantle and Allen (1978).

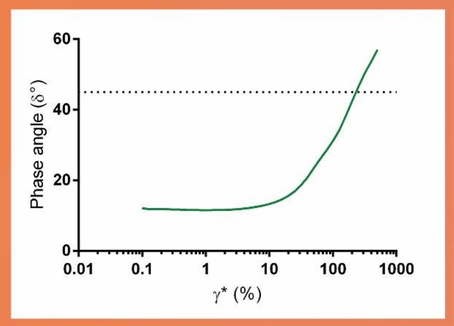
## 4. Results




### Composition

Bradford protein assay –estimated total proteins in mucus is 14.2%
PAS assay – estimated mucin in mucus is 12.9%

Figure 1 – Percentage of mucin and protein estimated through the PAS assay and the Bradford assay respectively. PAS assay uses commercially produced mucin as standard, while the Bradford assay uses bovine serum albumin as standard.

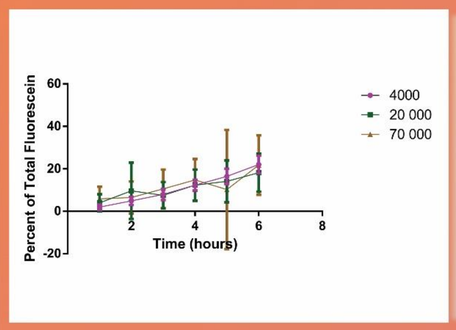



### Rheology

As more shear force is added onto the mucus gel, interactions get broken and it allows the gel to flow.

When the phase angle increases above 45°, it becomes less of a gel but more of a sticky liquid.

Figure 2 – The measures of phase angle (δ) in degrees of small intestine mucus, with a gradually increasing amount of force exerted (γ*) on the mucus gel. Phase angle is used to characterise the state of a material; 0° to 45° means it is a gel, 45° to 90° means it is a viscous liquid

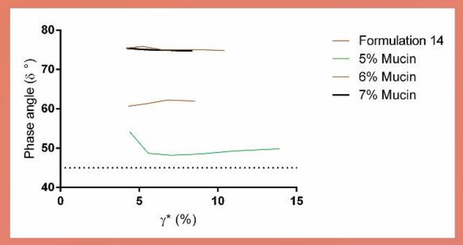



### Permeation

Mucus gel is a network of molecules with "spaces between the fibres"

Permeation - the rate that mucus allows different sized molecules to pass through

Figure 3 – The relative percentage of permeated particles of several molecular weight across small intestine mucus with their standard error bars. There is no significant difference in the permeation of Fluoroscein molecules with molecular weight of 4000, 20 000, and 70 000.




### Mucus Reconstruction

These reconstructed mucus formulations did not form a gel, their phase angles are above 45°.

Figure 4 – The Phase angles (rheological properties) of constructed mucus; Formulation 14 (5% mucin, 1% protein, 1% lipid, 0.02% DNA), 5%, 6%, and 7% mucin in solution

## 5. Discussion

- **Composition** - Protein and Mucin measured through the Bradford and PAS assays showed that the mucus sampled contained more protein and mucin than described in other studies.
- **Rheology** - In small intestine mucus, the phase angle remains under 45°, until the force applied is great enough to make it flow. Ideally, the reconstructed mucus should attain this consistency.
- **Permeation** - As there is no difference in permeation for the molecules tested, a different range should be tested in the future.
- **Mucus Reconstruction** - The mucus formulations tested did not form a gel as their phase angles are >45°. Even by increasing the concentration of mucin to 7%, above the accepted 5% in the normal gut, the reflected phase angles are still >45°, meaning it is more of a sticky liquid rather than a gel.
  It would be worth testing mucin at 13% with 14% protein as measured using PAS and Bradford assays.

## 6. Conclusion

- The reconstruction of mucus is not simple and the results of the experiments points towards a need to understand the complex role each component has in mucus, and modify current extraction techniques.
- Several methods to recombine mucus constructs were also tried and tested. These results provide a good reference for future researches.

## 7. References

- MANTLE, M. & ALLEN, A. 1978. A Colorimetric Assay for Glycoproteins Based on the Periodic Acid/Schiff Stain. Biochemical Society Transactions, 6, 607.
- FRIEDL, H., DÜNNHAUPT, S., HINTZEN, F., WALDNER, C., PARIKH, S., PEARSON, J. P., WILCOX, M. D. & BERNKOP-SCHNÜRCH, A. 2013. Development and Evaluation of a Novel Mucus Diffusion Test System Approved by Self-Nanoemulsifying Drug Delivery Systems. Journal of Pharmaceutical Sciences, 102, 4406-4413.

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