



Thesis project:

Mucosal rheology in the airways
of patients with severe lung disease

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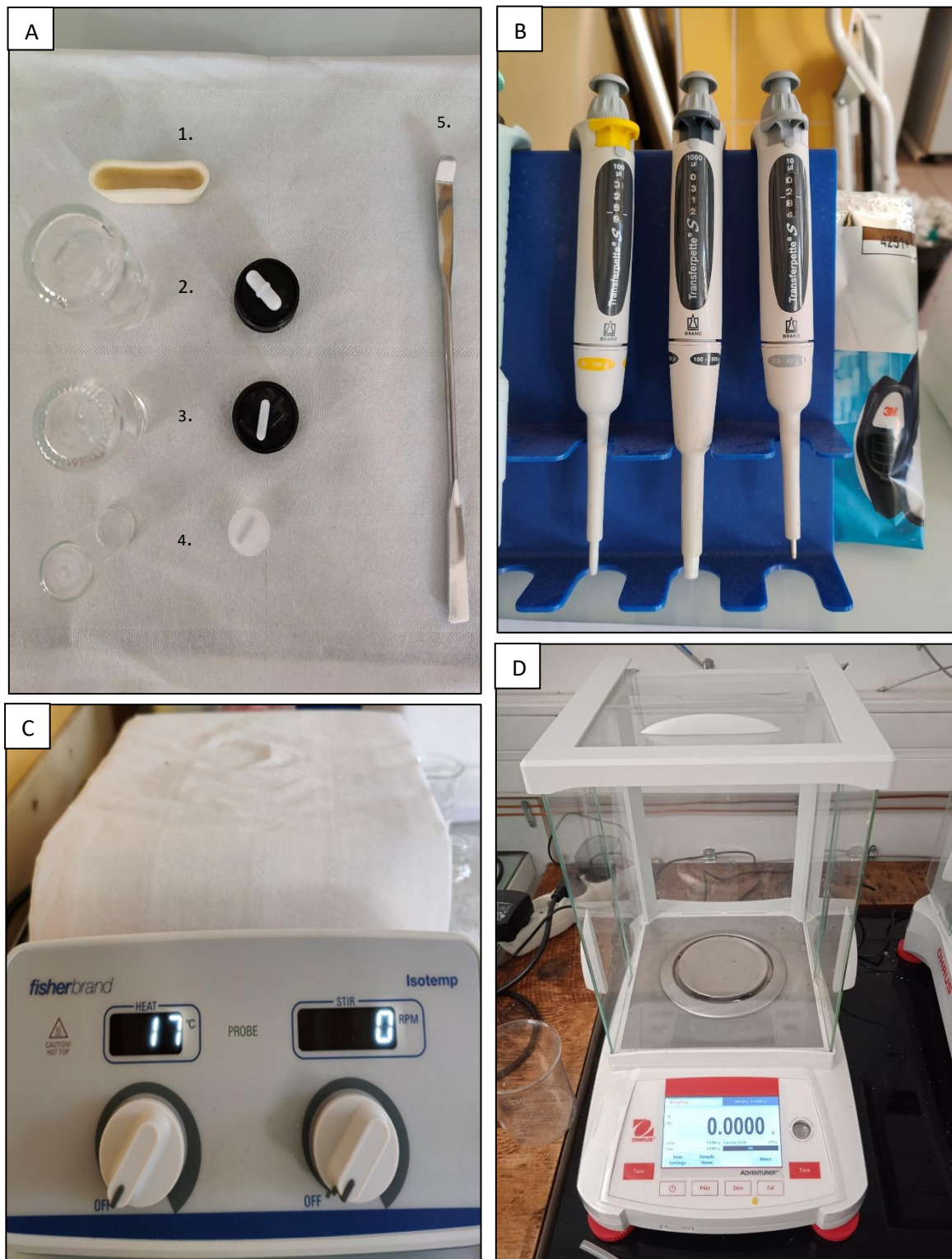
DOE 3:

This appendix, introduced in Chapter n. 3, aims to present the experimental procedure followed to develop DOE3, showing tools, components and basic steps for organizing the reconstruction of synthetic lung mucosa.

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RECONSTRUCTION TOOLS



(Fig.A) 1. Ceramic pot to weight the component 2. Bottle (T) complete of stirrer and cup 3. Bottle (-) complete of stirrer and cup 4. Glass vial complete of stirrer and cup. - (Fig.B) Micropipette 1000, 100, 10 µl. (Fig.C) Magnetic Stirrer at controlled rpm and T - (Fig.D) Precision Balance



(Fig.E) Precision Rheometer "Anton Paar MCR 302".

COMPONENTS of RECONSTRUCTED MUCUS:



Sodium chloride chemicals.
TSCHM
(code: 27810295)



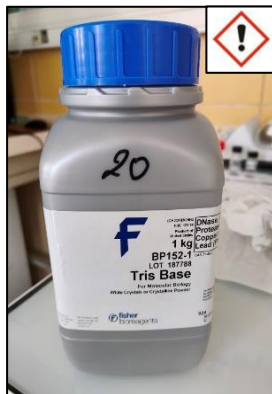
Mucin from bovine submaxillary glands.
Sigma Aldrich
(code: M3895)



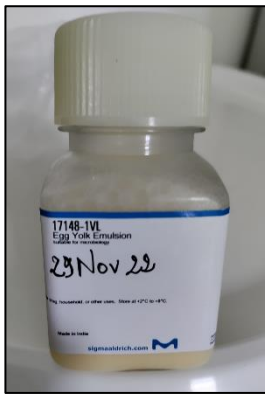
Potassium Chloride, Extra Pure, SLR, Eur. Ph.
Thermo Fisher scientific
(code: P/4240/60)



Mucin from porcine stomach.
Sigma Aldrich
(code: M2378)



Tris Base (White Crystals or Crystalline Powder/Molecular Biology).
Fisher BioReagents
(code: BP152-1)



Egg Yolk Emulsion.
Sigma Aldrich
(code: 17148-1VL)



Deoxyribonucleic acid, low molecular weight from salmon sperm.
Sigma Aldrich
(code: 31149-10G-F)



Actigum.
Cargill
(code: CS 6)



*Deionised water.
(taken from the laboratory
deioniser)*
*Demineralised water.
(taken from the tank filled by
the laboratory distiller)*

EXPERIENCES PROCEDURE

Procedure to perform the reconstruction of (g) of reconstructed pulmonary mucus without DNA.

RECONSTRUCTED MUCUS - FIRST DAY

Operative condition 25 °C, 1 Atm

Y SOLUTION

1. Weight a bottle with inside the stirrer and a cap (g).
2. With the help of a micropipette of 1000 µl fill the bottle at pt.1 with (ml) of DEIONIZED H₂O (Better fill with a quantity of nearly 500 µl less than how we had to add, perform a check with the balance and then add the Δ resulting from this measure and the value desired).
3. Briefly add following the order reported under constant stirring at 800 rpm :
 - NaCl (Sigma) (POWDER little spatula) (mg)
 - KCl (Sigma) (POWDER little spatula) (mg)
 - Actigum (POWDER little spatula) (mg)

VIP. Use ceramics cup to weight the element with little spatula
4. Stir for 24 h before to set it into the fridge

RECONSTRUCTED MUCUS (NO DNA) - SECOND DAY

Operative condition 25 °C, 1 Atm

X SOLUTION

1. Weight a bottle with inside the stirrer and a cap (g).
2. With the help of a micropipette of 1000 µl fill the bottle at pt.1 with (ml) of DEIONIZED H₂O (Better fill with a quantity of nearly 500 µl less than how we had to add, perform a check with the balance and then add the Δ resulting from this measure and the value desired).
3. Briefly add following the order reported under constant stirring at 800 rpm :
 - MUC5AC (POWDER little spatula) (mg)
 - MUC5B (POWDER little spatula) (mg)

VIP. Use ceramics cup to weight the element with little spatula
VIP. VIP. For the MUC5AC morphology, similar to a cotton filament (really light and volatile), it is better to weight it performing compact little balls, so pressing it a little bit, managing to better enter it into the bottle avoiding the stickiness of the mucin on the bottle wall and preventing lost in the transfer of it.
4. Stir for 3 h
5. Using a micropipette mix solution Y into solution X
6. After 5 min. of continuous stirring, perform the **pH regulation** adding Trizma Base (POWDER little spatula), measuring the pH values, adding the base (taking note and add on the excel file the quantity of base added and the pH value before and after the addition), stirring for 5 min. at 800 rpm, measuring again the pH and so on till the neutrality of the sample.
7. With the help of micropipette of 100 and 10 µl add (µl) of Egg Yolk at the solution at pt.
8. With the help of micropipette of 1000, 100 and 10 µl add the remaining part of water so that the overall weight of the solution will be 10 g.
9. Stir for 5 min. at 800 rpm before performing the analysis.

RECONSTRUCTED MUCUS (WITH DNA) - SECOND DAY:

Operative condition 25 °C, 1 Atm

X SOLUTION

1. Weight a bottle with inside the stirrer and a cap (g).
2. With the help of a micropipette of 1000 µl fill the bottle at pt.1 with (ml) of DEIONIZED H₂O (Better fill with a quantity of nearly 500 µl less than how we had to add, perform a check with the balance and then add the Δ resulting from this measure and the value desired).
3. Briefly add following the order reported under constant stirring at 800 rpm :
 - MUC5AC (POWDER little spatula) (mg)
 - MUC5B (POWDER little spatula) (mg)
 - Trizma Base (POWDER little spatula) (mg)

VIP. Use ceramics cup to weight the element with little spatula
VIP. VIP. For the MUC5AC morphology, similar to a cotton filament (really light and volatile), it is better to weight it performing compact little balls, so pressing it a little bit, managing to better enter it into the bottle avoiding the stickiness of the mucin on the bottle wall and preventing lost in the transfer of it.
4. Stir for 3 h
5. Using a micropipette (1000 µl) mix solution Y into solution X
6. After 5 min. of continuous stirring, perform the **pH regulation** adding Trizma Base (POWDER little spatula), measuring the pH values, adding the base (taking note and add on the excel file the quantity of base added and the pH value before and after the addition), stirring for 5 min. at 800 rpm, measuring again the pH and so on till the neutrality of the sample.
7. With the help of micropipette of 100 and 10 µl add (µl) of Egg Yolk at the solution at pt.
8. With the help of micropipette of 1000, 100 and 10 µl add the remaining part of water so that the overall weight of the solution will be 10 g.
9. Stir for 5 min. at 800 rpm before performing the analysis.

CONSIDERATIONS

To gain a good repeatability of the measurements and in order to maintain in an optimum working state the Rheometer is necessary to pay attention to the following rules:

- The analysis has to be performed immediately after the end of the 5 min. at pt6.
- The analysis has to be performed at 32 °C and 1 Atm.
- The H₂O to use to maintain constant the humidity in the chamber has to be DISTILLATE.
- The humidity in the chamber of the Rheometer has to be constant.
- The two half of the chamber have not to touch the superior plate of the Rheometer. To avoid that is possible to regulate the high of the chamber wall.
- Once regulated the temperature, before performing the SAOS test, wait 5 min. so that the component into the sample will assume a stable configuration.
- Between an analysis and one other is necessary wait 15 min. so that the component into the sample will assume a stable configuration.
- Immediately after the analysis both superior and inferior plates, the two half of the cap and the glass wall of the chamber have to be cleaned and sterilised with DEIONIZED H₂O, Methanol, DEIONIZED H₂O and dried carefully with a absorbent paper.

This procedure is the latest one developed and is much simpler than the others two, so that it can guarantee greater repeatability. The decision to make two separate solutions was dictated by the convenience of making a Y solution from which quantities can be taken from time to time to be added to the X solutions, which are then reconstituted over the following days. This turns out to be another method to obviate the effect of ageing the entire mucosa (in fact mainly involving mucins). The preventive addition of Trizma base beforehand in the reconstruction of the X solution with DNA was done to avoid the precipitation of mucins due to the sudden drop in pH due to the addition of DNA. Ultimately, this operation mode allows the reconstruction of up to 4 different mucin samples per day, but is very component-intensive, using a large amount of them.