Illustrated procedure for covalent immobilisation of dyes to microcellulose fibers

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(Please also consult the original publications for the required amounts of materials and the procedures)

Chemicals and materials

HCl (37%; M = 36,46 g/mol)

H_2SO_4 (98%; M = 98,08 g/mol)

NaOH (pearls, MW = 40,00 g/mol)

Na_2CO_3 (MW = 105.99 g/mol)

pH indicator dye of choice (GJM- 492, GJM 503, GJM-534)

pH-insensitive dye Remazol Brillantblau R (RBBR, MW = 626.54, purity ~50%)

SigmaCell microcellulose fibres (S3504, Sigma-Aldrich Type 20, 20 μm)

Glassware and utensils

Glass dish or plastic tray (# is depending on the amount of different cellulose materials to be coloured separately)

Schott bottles 250 ml (4)

Mortar and pestle (1)

Graduated cylinder (10 ml, 100 ml, 250 ml)

Beaker 1.5 L or bigger (1)

Magnetic stirrer, plus magnetic stirring bar, length > 5cm

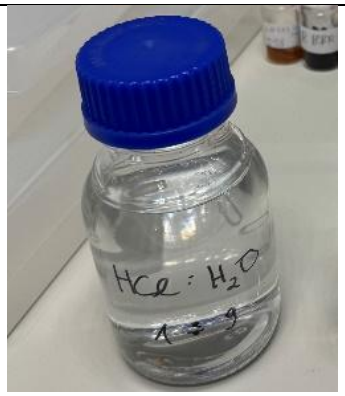
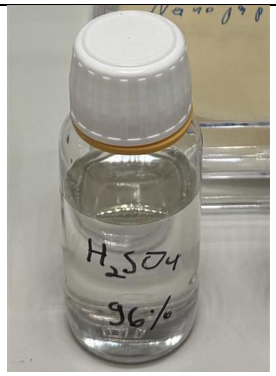
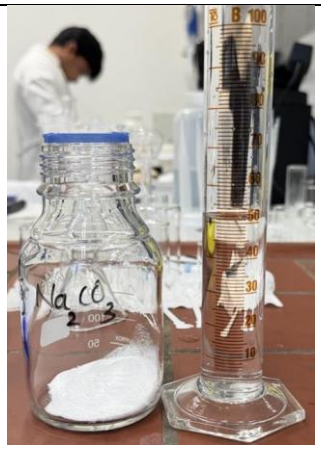
Glass/plastic pipettes

Buchner funnel, ca. 10 cm diameter

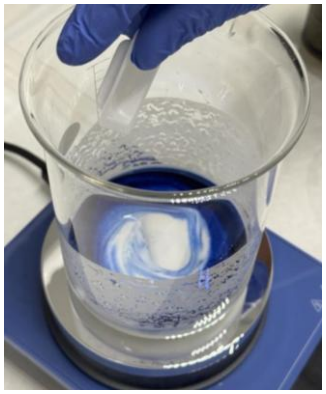
Suction flask 250 ml

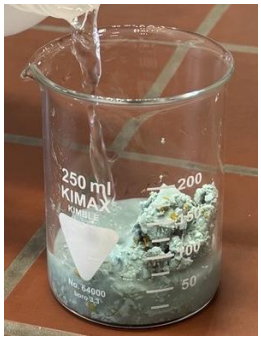
Filter paper

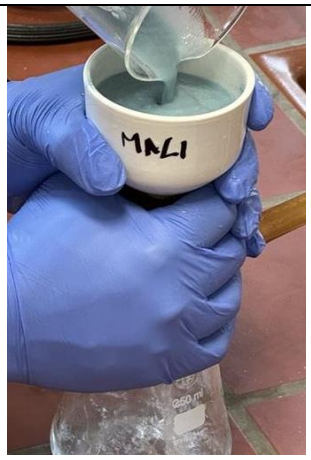
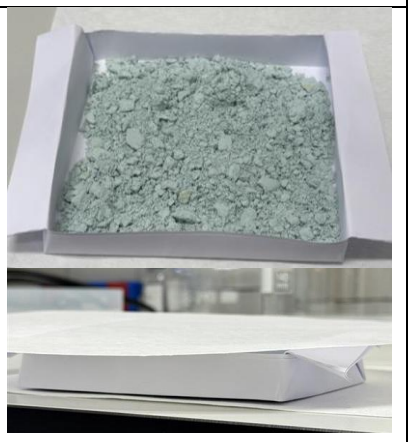
Vacuum pump

Step	Procedure description	Scheme
1.	Prepare a diluted HCl solution by mixing 25 mL of HCl with 225 mL of distilled water, then transfer the solution to a Schott bottle	
2.	Place approximately 10 mL of 96% H_2SO_4 (taken directly from the original bottle) into a small airtight container for later use in the esterification of the respective pH indicator dye.	
3.	Prepare a 30% NaOH solution: Weigh 30 g of NaOH pellets and transfer them to a Schott bottle in the fume hood. Add 35 mL of distilled water and shake carefully for approximately 2–3 minutes due to the strongly exothermic reaction. Then add the remaining 35 mL of distilled water and shake carefully again. Allow the solution to cool to room temperature.	
4.	Weigh 12.5 g of Na_2CO_3 and dissolve it in 50 mL of distilled water in a Schott bottle.	

5.	Prepare the indicator dye solution: Weigh 50 mg of dye into a weighing container and transfer it to the mortar. Using a dropper or pipette, add 0.5 g of H_2SO_4 dropwise into the same weighing container. Transfer the acid from the container to the mortar, then begin gently moving the pestle over the dye–acid mixture. Mix until the material is completely liquefied and homogeneous. After homogenization, allow the mixture to stand for 30 minutes so that the sulfonation reaction can proceed. Do not press the pestle too firmly onto the dye, as the dye may stick to the bottom of the pestle.	
6.	Weigh 40 mg of the pH-insensitive dye RBBR into a weighing container (if required).	
7.	After 30 minutes have elapsed since step 5, gently stir the dye/acid mixture in the mortar with the pestle for 5 minutes.	
8.	Transfer 3.5 mL of 30% NaOH into a graduated cylinder.	

9.	Prepare a microcellulose suspension by weighing 25.0 g of SigmaCell microcellulose fibers (or other cellulose particles) into a 500 mL beaker containing 100 mL of distilled water. Stir the suspension for 1 hour to allow the microcellulose to swell in water. After 60 minutes of stirring, add the pH-insensitive dye RBBR (if required) in powder form directly from the weighing container into the beaker containing the microcellulose suspension, and continue stirring for 1 minute. The suspension should develop a light blue color.	 
10.	Then transfer the dye/acid solution directly from the mortar into the beaker. Rinse the mortar with the Na_2CO_3 solution to collect any remaining dye, and pour the rinsing solution into the beaker as well. Also add the 3.5 mL of 30% NaOH solution prepared in step 9 to the beaker. When using the pH indicator GJM-452, the final suspension will appear greenish to mustard-colored.	  

11.	Place the Büchner funnel on the suction flask and connect the flask to the vacuum pump. Insert the filter paper into the Büchner funnel. First, pour a small amount of distilled water onto the filter paper to secure it in place, then transfer the suspension from the beaker into the funnel.	
12.	Rinse the colored microcellulose fibers with distilled water five times. Then add a few drops of the HCl solution ($HCl:H_2O = 1:9$) and mix the material with a spatula inside the funnel. The microcellulose will change color from mustard to light blue when using the combination of the pH indicator GJM-452 and the pH-insensitive dye RBBR.	 
13.	Transfer the light blue microcellulose fibers to an empty beaker. Add 100 mL of distilled water and a few drops of the HCl solution, then stir until a homogeneous suspension is formed.	 

14.	Filter the suspension from the previous step through the Büchner funnel. Wash the suspension five times with distilled water. Repeat the process of resuspending the microcellulose in 100 mL of distilled water in a beaker, filtering it, and washing it with distilled water two more times. Then pour acetone onto the microcellulose in the funnel to aid drying. The microcellulose will obtain an even lighter shade of blue.	
15.	Transfer the microcellulose from the previous step into a beaker containing 100 mL of acetone and mix to obtain a homogeneous suspension. Then filter the suspension again and wash the microcellulose five times with acetone.	
16.	Transfer the colored microcellulose to a clean container that protects it from moisture and contamination. Cover it with a light, porous material, such as filter paper, to allow further drying, and leave it overnight.	

If you have questions/comments, please write to:

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