POLBIONICA

We print organs. We do science not fiction!

TINTBIONIC® METHACRYLATE SOLUTIONS

INSTRUCTION FOR THE PREPARATION OF METHACRYLATE SOLUTIONS

GELMA HAMA CHIMA ALGMA

The instructions are intended as auxiliary recommendations.
The user can make appropriate changes, taking into account his hardware resources.

LAB EQUIPMENT:

- hotplate magnetic stirrer
- stirring element
- thermocouple
- water bath (crystalliser + demineralised water)
- measuring cylinder / serological pipette
- metal spatula
- measuring vessel
- analytical balance (weighing)
- stand with a metal foot
- demineralised water wash bottle
- beaker set
- funnel
- pH meter
- vacuum pump
- 0.22 µm bottle/syringe filter

METHODOLOGY

1. Set the crystalliser with an appropriately sized volume (criteria below) on a hotplate magnetic stirrer.

Criteria for crystalliser selection:

- The crystalliser must be a glass vessel with a well-defined, flat, uniform bottom.
- The dimensions of the crystalliser must be selected so that, ideally, its bottom coincides in size with the hotplate of the magnetic stirrer.
- The volume of water inside the vessel must be closely related to the amount of solution heated. It must cover the entire height of the column of liquid in the vessel in which the solution will be heated.

Top up the crystalliser with demineralised water. Then, using the foot-mounted stand, immerse the thermocouple connected to the stirrer (according to the manufacturer's instructions) in such a way that:

- The thermocouple does not touch the wall or bottom of the crystalliser,
- The immersion of the thermocouple is not less than 2 cm from the end of the thermocouple (optimally — halfway up the water column),
- It does not interfere with manoeuvring inside the water bath,
- It is located in the plane of the hotplate of the stirrer

> ***Note:*** *Failure to comply with the above may result in distorted temperature measurements and deviations from good laboratory practice.*

Then, set the temperature to 50°C on the magnetic stirrer and start the hotplate. Further work using the prepared water bath should only be continued once the set temperature has stabilised.

> ***Note:*** *Depending on the size of the crystalliser, the volume of water contained in it and the heating power of the stirrer, the warm-up time may be extended. Due to the protection systems of the magnetic stirrers, stirring errors can occur during heating due to the medium heating up too slowly in relation to the heating power. If such an error occurs, the magnetic stirrer must be restarted, and the temperature set again. The heating process can be accelerated by initially setting the temperature higher than the target temperature, waiting until the hotplate has warmed up and then reducing it to the target one. Moreover, the temperature can rise sharply above the set value due to the control characteristics of the heating process. If this happens, turn off the heating, wait until the expected value is reached and only then start the heating.*

2. The following is required to prepare the solution in sequence:

a. Weigh **X g** of methacrylate into a labelled, sterile dark glass bottle or one protected from light, e.g. with aluminium foil (both are best) of suitable capacity (the bottle size should be selected according to the volume of the target solution in such a way as to minimise the loss of material by depositing on the walls).

Diagram of the bottle description on the (wet-proof) label on the wall of the vessel:

- From the top right corner downwards: 1. sample name and concentration

- 2. m_1= mass of vessel with cap,
- 3. m_2= mass of vessel with solution,
- 4. m_3= pH of final solution (4)
- Top left corner — sample preparation date,
- Lower left corner — preparer's initials.

b. Inside the vessel, place a stirring element, the size and shape of which should ensure smooth and uninterrupted mixing of the solution.

c. Using a syringe or serological pipette, measure **X ml** of sterile PBSx1 solution **(dissolve the methacrylated chitosan in 1% acetic acid!)** and then cap with a stopper.

d. Place the prepared suspension in a heated water bath, allow the solution to warm internally (usually 5-10 min) and then gradually increase the stirring speed until **400 rpm is reached**. Particular attention must be paid to the smooth movement of the stirring element to prevent foaming of the solution and ensure even distribution of the material in the solution.

e. Keep stirring until a clear and homogeneous solution is obtained.

> ***Note:*** *If solution foaming occurs, reduce the stirring speed to **100 rpm as a first step, and if this does not work, stop stirring completely** and wait for the foaming to subside. To do this, it is advisable to gently move the bottle in the Z-axis so that all the foam is part of the prepared solution.*

In addition, when planning work with a solution, it is important to consider the type of solution concentration you want to obtain (percentage, mass/volume, etc.). The right choice of this parameter can determine the success of your experiment. An overview of the quantities of reactants used depending on the type of concentration is given in the table below:

No.	Concentration	Solvent quantity (ml)	Methacrylate mass (g)	Bottle size
1	20%	8	2	25 ml
2	20% m/V	10	2	25 ml
3	20% m/m	10	2	25 ml

Table 1. *The proportion of reagents used for a concentration of 20% depending on the concentration choice*

An overview diagram of the prepared water bath set-up is shown below. The red arrow indicates placing the vessel in the water bath, provided that the water bath has been heated to the set temperature. When placing the bottle in the water bath, the stirring function should be switched off and only activated when it is ensured that the bottle sits securely on the bottom of the vessel.

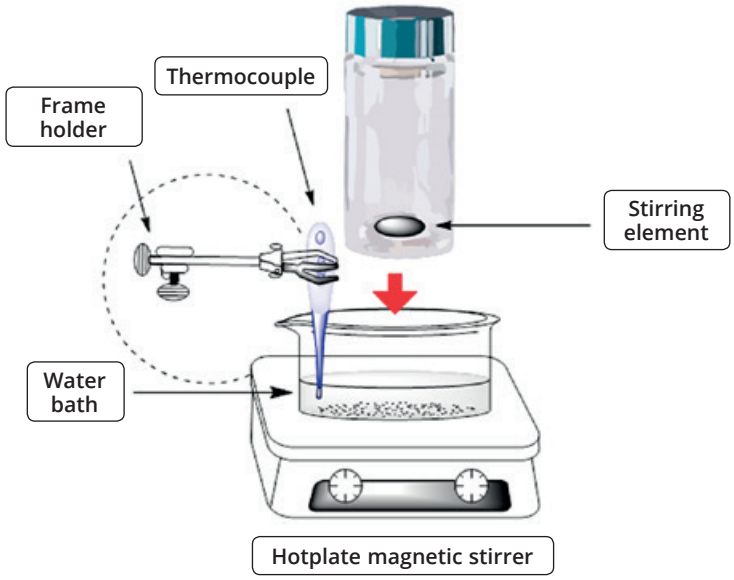

Figure 1. *An overview of the reaction set*

3. To the resulting clear solution, add an appropriate amount of photoinitiator (e.g. LAP), seal the bottle tightly and return it to the water bath. Stir until the photoinitiator is fully dissolved

   Note: ***All volumes and weights of materials used are recorded to three significant figures. In addition, be sure to note down your observations on the material received so that any concerns can be verified during the result analysing. If the material, from an observer's point of view, raises any doubts, these should be reported by e-mail to the laboratory manager and the chemistry team before use, including the batch number and collection date.***

4. Subsequently, measure the pH (according to the pH meter manufacturer's instructions) of the resulting solution, making sure to use the appropriate measuring probe depending on the viscosity of the solution. Note the result. If the pH value of the resulting solution deviates from the intended one, adjust the pH value with 5M NaOH or 5M HCl, making sure to mix the solution thoroughly after adding each reagent dose. Record the volume of solution used and the final pH.

No.	Methacrylate	pH	Temperature [°C]	Percentage concentration [%]
1	GELMA	7	50	1-20
2	GELGMA	7	50	1-20
3	ALGMA	5-6	20	1-5
4	CHIMA	5-6*	50	1 lub 2
5	HAMA	5-6	20	1 lub 2

Table 2. *Suggested temperature, pH and percentage concentration of methacrylate solutions*

* Methacrylated chitosan should be dissolved 'acidically' in a 1% acetic acid solution. Once dissolved, it can be neutralised to the pH given in the table

   Note: ***the pH of the solution must be defined before preparing the solution. Its value will limit the solution's biological, physical and chemical properties. In addition, before each use, ensure that the pH of the solution does not deviate from the expected value and adjust it if necessary (see point 4).***

5. Next, transfer the heated material quantitatively to a bottle filter with a pore size of 0.22 µm and filter (according to the manufacturer's recommendations and good laboratory practice) until the entire solution has percolated.

6. Once the filtration is complete, move the filter bottle with the fitted filter under the laminar flow cabinet, unscrew the filter from the top of the bottle and secure the whole thing with a stopper.

   Note: ***During filtering, the solution may foam again. In this case, wait until the foaming subsides before measuring the volume.***

7. Reheat the solution, if necessary, in a water bath, transfer it under a laminar flow cabinet and, using a sterile syringe fitted with a needle, determine the volume of the solution obtained after filtration. Record the volume in the research dossier to determine material loss.

8. The prepared material from the syringe is ready for use, which should be noted on the bottle each time.

9. Store the solution at <4°C for no longer than 14 days after preparation.

10. In order to recreate the material during the storage period, the water bath must be prepared again according to points 1 and 2 and the parameters contained therein as part of this procedure.