

Plastic, naturally! by Christelle Lam Ching Wang

Biotechnologies

Experiment

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Project summary

For this project, I decided to develop a system for the controlled release of medication using polyhydroxybutyrate (PHB), a biodegradable plastic. I will manufacture PHB microspheres containing vitamin A and test their release rate in vitro.

Project report

I. INTRODUCTION

PHB, a biodegradable plastic synthesized by bacteria, has gained a lot of attention since it offers an alternative to man-made synthetic plastics. PHB has various biomedical applications, including the manufacturing of surgical bandages, sutures and medical powder used in ostomy bags, and bone replacement procedures. It is also used for the controlled release of medication. Medications in PHB microspheres are gradually released into the body. Since PHB is biocompatible and non-toxic, the host accepts it without any difficulty.

2. LITERATURE REVIEW

2.1 PHB is manufactured by partially starving bacteria. Scientists create a disequilibrium by decreasing the amount of nutrients—such as nitrogen, sulphur, phosphate, iron, magnesium, potassium or oxygen—available to the bacteria, while continuing to provide them with predetermined quantities of carbon. As a result, the excess carbon is produced in the form of PHB. In order to prevent the bacteria from consuming their PHB reserves, they are dehydrated under vacuum at low temperatures. In other words they are lyophilized, or freeze-dried.

2.2 The controlled release aspect makes it possible to control the dose and the time required to release the medication. Combining a biomaterial—in this case, the PHB—with a medication makes it possible to protect implants from infections associated with foreign bodies and to expand the therapeutic potential of the medication's components.

2.3 The biodegradation of PHB is possible thanks to microbial enzymes that decompose it. The ultimate by-products of its biodegradation are carbon dioxide (CO₂) and water (H₂O).

3. ISSUE AND HYPOTHESIS

Currently, medications such as anticancer drugs produce side-effects because they are not sufficiently selective. For this reason, the aim of this project was to manufacture PHB microspheres using the solvent evaporation method, with a view to manufacturing others that contain medication.

The hypothesis was as follows: The PHB microspheres would measure 50 μm , since a magnetic stirrer would be used instead of a homogenizer.

4. METHODOLOGY

The solvent evaporation method will be used to prepare the 50- μm microspheres. 30 mL of a chloroform solution with a 0.178% PHB concentration will be dissolved in 80 mL of chloroform. Then, 10 mL of this mixture will be added to 50 mL of distilled water containing 0.05 g of a methyl cellulose emulsifier. The mixture will be stirred at room temperature at 2000 rpm, using a mechanical stirrer and an ultrasonic disintegrator (power: 250 W; operating frequency: 50 KHz). The solvent will be left to evaporate under these conditions over a period of two hours. Finally, the particles will be separated by centrifugation at 4000 rpm for 10 minutes and rinsed twice with distilled water.

The same process will be used to prepare the 50- μm microspheres containing vitamin A. The only difference is that 0.3 g of vitamin A will be added to the chloroform solution with a 0.178% PHB concentration.

A spectrophotometer will be used to verify whether the vitamin has been encapsulated in the PHB microspheres. Following centrifugation and rinsing with distilled water, 0.950 mL of the microsphere suspension containing vitamin A will be agitated in a vortex. The spectra will be analyzed and compared with those of the PHB microspheres.

Micrographs will be taken with an electron microscope to determine the size of the microspheres. The following steps represent the preparation of microspheres for microscopy. Over a period of four hours, 100 μL of aqueous suspension of particles will be dried on a metal rack at room temperature. The samples will then be covered with gold by means of sputation and placed in an electron microscope. The diameter of the particles in the micrographs will then be measured.

5. RESULTS

See appendix.

6. DISCUSSION

The micrographs reveal that the prepared solution was not properly purified since it contains too much methyl cellulose. Contrary to my initial hypothesis, in which I suggested that the microspheres would measure 50 μm , the size of the manufactured microspheres range between 9.21 μm and 27.2 μm . In order to manufacture PHB microspheres containing medication, 0.1 g of medication is added to the chloroform solution.

7. CONCLUSION

By following this procedure, I was able to manufacture PHB microspheres using the solvent evaporation method. I would be able to produce a system for the controlled release of vitamin A by adding 0.1 g of vitamin A. Finally, in order to test the microspheres in vitro, I could suspend them in a USP type-II dissolution and calculate the release rate using a Hewlett-Packard diode spectrophotometer.

The remaining results will be available during the exhibition.