Hydroxyethyl Methacrylate Microsphere Synthesis for Bionanoparticle Encapsulation

Costanoan Immunotherapies is researching the use of bionanoparticles, developed by Polybatics, to develop specialized immunotherapies. The polyhydroxybutyrate (PHB) bionanoparticle is a biopolymer inclusion that has been modified to display proteins on its surface in a uniform manner. However, researchers have run into the following issues:

- PHB bionanoparticles aggregate over time
- Proteins conjugated to the surface of the PHB bionanoparticles are not designed to endure harsh conditions
- Purifying target antibodies

Opportunity: Develop a recipe and experimental system that produces 40-80 µm polyhydroxyethyl methacrylate (pHEMA) microsphere for bionanoparticle encapsulation. Encapsulation will protect the PHB bionanoparticles and prevent them from forming aggregates.

Application

- Chromatography resin

Encapsulated Bionanoparticles

pHEMA Microsphere (Not shown to scale)

Bionanoparticles

Figure 1: Illustration of the encapsulated bionanoparticles inside a pHEMA microsphere. Bionanoparticles are absorbed onto the pHEMA surface and encapsulated within the hydrogel.

Project Objectives

- Generate pHEMA microsphere synthesis schematic
- Construct system for polymerization reaction
- Synthesize 40-80 µm pHEMA microspheres

Chemical, Biological & Environmental Engineering

HYDROXYETHYL METHACRYLATE MICROSPHERE SYNTHESIS

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Polymerization Reaction

![Polymerization reaction mechanism](image)

- HEMA Monomer
- pHEMA Chain 1
- pHEMA Chain 2
- EDGMA
- APS/TEMED

Reaction System

![Experimental system for the polymerization reaction](image)

A 100 mL Schott bottle is used as the main vessel. A Caframo BDC250 impeller stirrer is used to mix reagents. Reagents were added to the vessel through a septum. Nitrogen gas is flowed into the system to expel the vessel of oxygen.

Figure 2: Polymerization reaction mechanism of the pHEMA microspheres. Ammonium persulfate (APS) and tetramethylethylenediamine (TEMED) react and form polymerization radicals. The radicals and hydroxyethyl methacrylate (HEMA) form pHEMA chains. Ethylene glycol dimethacrylate (EDGMA) crosslinks pHEMA chains to form a porous microsphere structure.

Figure 3: Experimental system for the polymerization reaction.

Figure 4: The SEM images above are shown to compare the uniformity of the PHB bionanoparticles (A) and the lack of uniformity currently present in the pHEMA microspheres (B). Image 1A shows the PHB bionanoparticles in an aggregate state, however, it is visible that each PHB bionanoparticle is similar in shape and size, ranging mostly between 150-400 nm in diameter. Image 1B shows the product of pHEMA microsphere synthesis using impeller stirrer; the microspheres lack uniformity in size and showed aggregation. Images 1C and 1D are close up images of a pHEMA microsphere aggregate synthesized using a magnetic stirrer.

Impeller Physics Determines Microsphere Size

![Impeller physics diagram](image)

- Inertial Region
- Viscous Region

- Reynolds's Number = \( \frac{ND^3}{\eta} \)
- Weber's Number = \( \frac{pN^2D^2}{\gamma} \)
- Shinnar's Correlation: \( d = \frac{C_{microm}}{Re^{0.25}We^{-0.5}} \)

- \( d \) - mean particle diameter
- \( N \) - impeller velocity
- \( D \) - impeller diameter
- \( \eta \) - interfacial tension
- \( p \) - continuous phase density
- \( Re \) - Reynolds number

Conclusions and Future Work

- Aggregation inhibited accurate microsphere diameter measurements
- Type of impeller and mixing affects pHEMA microsphere formation and size
- Impeller stirrer is preferred over magnetic stirrer for optimal radial and axial mixing
- Determine source of aggregation
- Perform additional trials at different impeller speeds to determine statistical significance of bead diameter differences
- Perform additional trials with different impellers to observe their effects on pHEMA microsphere uniformity
- Perform PHB bionanoparticle encapsulation

Acknowledgements

Dr. Skip - For providing insight regarding the polymerization
Diba Behnoudfar - For assisting with polymerization research
and lab techniques
Miranda Raper - For providing lab equipment training
Bonan Yu - For assisting with lab techniques
Andrew Brickman - For guidance in equipment selection
Polybatics - For providing PHB bionanoparticles
Dr. Harding - For providing project guidance

References