Summer 8-10-2022

The Formation and Application of Polymeric Micro- and Nanoparticles

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ABSTRACT

Nano- and microparticles are used in the pharmaceutical industry for sustained release drug delivery systems. For example, polymeric particles are currently used as an FDA-approved drug delivery system for leuprolide acetate to treat prostate cancer[1]. Our drug of interest is CPDI-02 (formerly known as EP67)—a C5a-derived decapeptide agonist of the C5a Receptor (CD88) that activates mononuclear phagocytes to produce an immune response while potentially minimizing neutrophil-mediated toxicity[2]. Currently in the Vetro Lab, CPDI-02 is being tested on pigs and mice to treat methicillin-resistant *Staphylococcus aureus* (MRSA) infections and as the adjuvant for a vaccine for cytomegalovirus (CMV). This investigation explored formulation parameters that impact particle size and loading of CPDI-02 in a traditional oil-in-water (O/W) emulsion. We also explored adapting the formulation using microfluidic chips to generate nano- and microparticles and improve run-to-run consistency in particle size.

Tyr-Ser-Phe-Lys-Asp-Met-Pro-[Me-Leu]-[D-Ala]-Arg

Figure 1. Amino acid sequence of CDPI-02

BACKGROUND

Traditional Approaches to the Formation of Micro- and Nanoparticles

- Typically, emulsions are used to form polymeric micro- and nanoparticles
- The emulsion consists of two phases:
  - The dispersed phase (DP) is usually an organic solvent containing both the desired drug and a polymer—poly(lactic-co-glycolic acid) (PLGA)
  - The continuous phase (CP) is a solution of water and poly(vinyl alcohol) (PVA)—a surfactant that prevents aggregation and coalescence of the dispersed phase
- The amount of energy applied to create the emulsion determines the size of the particles obtained
- Sources of energy include shaking, vortexing, stirring, homogenization, and sonication

Limitations of the Traditional Approach

- Reproducibility and conformity in size
- Scalability

Microfluidic chip-based emulsion

- Herringbone: turbulence in the mixing region impacts nanoparticle size
- Droplet-generator: DP and CP flow rates causes sheer that impacts microdroplet size
- Advantage: Reproducibility and conformity in particle size

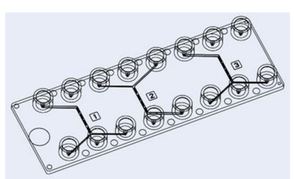

Figure 2. Fluidic 187 Herringbone

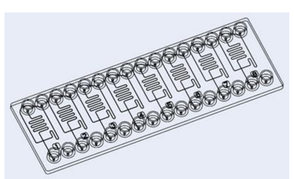

Figure 3. Fluidic 440 Droplet-generator

SPECIFIC AIMS

1. Determine if changes in viscosity (polymer concentration) affects particle size
2. Determine if the amount of energy applied affects particle size
3. Determine if changes in viscosity affects drug encapsulation
4. Evaluate parameters that impact particle size obtained using microfluidic chips

METHODS

- Form CP by making a filtered 2% (m/v) solution of PVA
- Form DP by dissolving PLGA in 1 mL dichloromethane (DCM), acetonitrile, or ethyl acetate
- Dissolve 5 mg CPDI-02 in 300 µL methanol and combine with DP to form a cosolvent oil phase
- For traditional emulsion: add 1 mL 2% PVA to DP and vortex (1,000 RPM) to form an O/W emulsion
- For microfluidic emulsion: pump DP and CP through chip using syringe pumps
- Transfer emulsion to a beaker with CP and evaporate organic solvent with stirring (300 RPM) until particles harden overnight
- Centrifuge and wash with D/I to remove PVA and recover harden particles (3X)
- Lyophilize for 48 hours
- Determine particle size using NanoSight (nanoparticles) or Nexcelcom (microparticles)
- Assay loading (if applicable) using microBCA. Known mass of particles was dissolved in 300 µL DMSO to disrupt polymer matrix, then stirred overnight in 0.05 M NaOH and 0.5% SDS to digest polymer

EXPERIMENTS

- Varied viscosity comparing impact on size (350 mg PLGA /mL solvent and 100 mg PLGA/ mL solvent)
- Varied energy by increasing vortex time (30 sec versus 60 sec) to determine impact on size
- Varied viscosity comparing impact on encapsulation (350 mg PLGA /mL solvent and 100 mg PLGA/ mL solvent)
- Attempt to replicate above using microfluidic chips

RESULTS

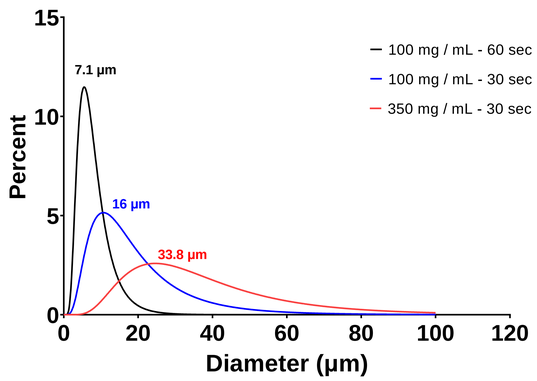

Figure 4. Traditional emulsion. Increasing viscosity (350 mg PLGA / mL solvent compared to 100 mg PLGA / mL solvent) produced larger microparticles. Higher energy (60 sec vortex) produced smaller microparticles compared to 30 sec vortex with 100 mg PLGA / mL solvent emulsions

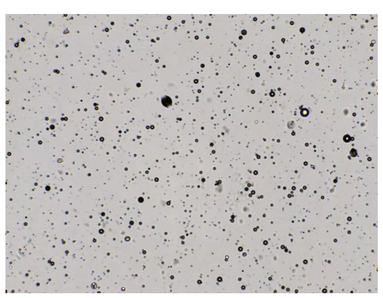

Figure 5. Image of microparticles (100 mg PLGA/ mL DCM) using Nexcelcom cell counter

Table 1. Increasing viscosity of the DP improves encapsulation efficiency of CPDI-02

Condition	Encapsulation (%)
100 mg PLGA	12.0
350 mg PLGA	83.2
350 mg PLGA (0.1 M NaCl)	81.5

$\sigma \leq 0.05\%$ for n = 3 internal replicates

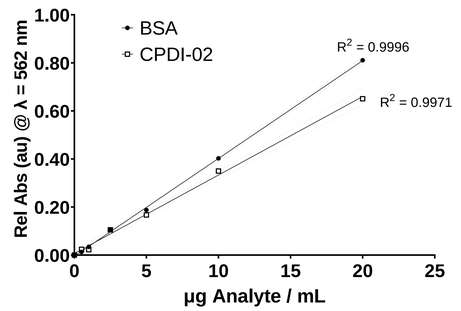

Figure 6. microBCA calibration curve used in determination of CPDI-02

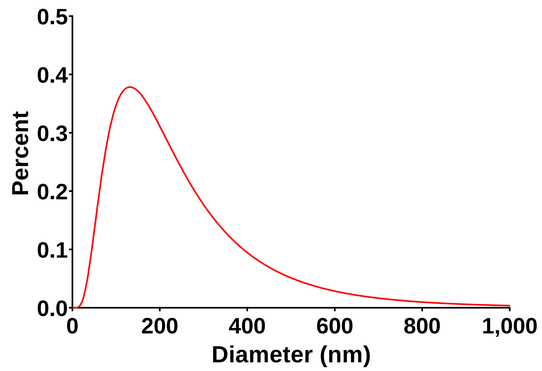

Figure 7. NanoSight sizing of nanoparticles produced using a herringbone microfluidic chip (40 mg PLGA / mL acetonitrile with DP rate of 1 mL/min and CP rate of 9 mL/min)

CONCLUSIONS

Formulation parameters can significantly impact particle size and loading

Microfluidic droplet-generator chip-based emulsions

- Experienced problems with assembly, back-pressure, fluid flow, and precipitation
- Attempting to use new chip design for future experiments

REFERENCES

[1]C.L. Ventola. Progress in Nanomedicine: Approved and Investigational Nanodrugs. *P&T,* 2017; 42(12): 742 - 755.

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