Preparation of Aggregation Stable Block Copolymer Nanoparticles for Simultaneous Drug Delivery and Imaging

M. Gindy and R. Prud'homme*

*Princeton University, Princeton, NJ, USA, mgindy@princeton.edu

ABSTRACT

We present a novel process for the simultaneous encapsulation of active pharmaceutical compounds (β-carotene, α-tocopherol succinate) and imaging agents (dodecanethiol gold nanoparticles and 7-amino-4-methyl coumarin) based on diblock copolymer self assembly in the diffusion limited regime. Uniform particles with tunable sizes from 50-300 nm and long term stability are prepared at high concentrations of incorporated compound. The degree of agent loading is varied independently of particle size, allowing for precise control of system parameters.

Keywords: block copolymer, nanoparticles, drug delivery, imaging

1 INTRODUCTION

The development of polymer based nanoparticles as novel drug delivery systems has been extensively studied in recent years[1-3]. The utility of nanoparticles for delivery of therapeutic agents results from their unique chemical composition, which allows for the incorporation of hydrophobic solutes within the nanoparticle core, potentially imparting reduced cytotoxicity and extending drug circulation *in vivo*[4, 5]. Most recently, nanoparticles have found employment as sensing and image enhancement agents. In particular, nanoparticles based on gold chemistry have been utilized in a variety of biomedical applications including diagnostic assays, thermal ablation, radiotherapy enhancement as well as drug and gene delivery[6, 7]. Utilizing these converging technologies, we have developed a novel nanoparticle based vehicle for the simultaneous delivery of therapeutic and imaging agents and combinations thereof. The nanoparticle delivery vehicle, based on a biocompatible, biodegradable block copolymer, relies on passive targeting through the enhanced permeability and retention (EPR) effect for delivery to tumor cells[8, 9]. This form of drug delivery improves the therapeutic response to drugs and allows for concurrent monitoring of drug uptake.

We have previously designed and detailed a nanoparticle formation process based on rapid micromixing to produce high supersaturations, which we term Flash NanoPreciptiation[10-12]. This work introduces a novel approach that expands the applications of the Flash NanoPrecipitation process to pre-formed solutes in conjunction with molecularly dissolved compounds, and permits the formation of nanoparticle formulations with limited recrystallization and stable size distributions.

2 EXPERIMENTAL METHODS

In the Flash NanoPrecipitation process, a tangential flow mixing cell is used to provide rapid micromixing of block copolymer, drug solute and imaging agent. The process depends upon the tuning of kinetics of solute precipitation and block copolymer assembly on the nanoparticle surface. Amphiphilic block copolymers in solution spontaneously self-assemble when the solvent quality for one block is selectively decreased. The use of impinging jets provides mixing timescales that are shorter than the timescale for nucleation and growth of dissolved solute particles, which then allows for the colloidal stabilization of nucleated particles by block copolymer self-assembly on the surface. This process yields nanoparticles with size distributions, morphologies, and drug loading efficiencies not attainable through other technologies[13, 14].

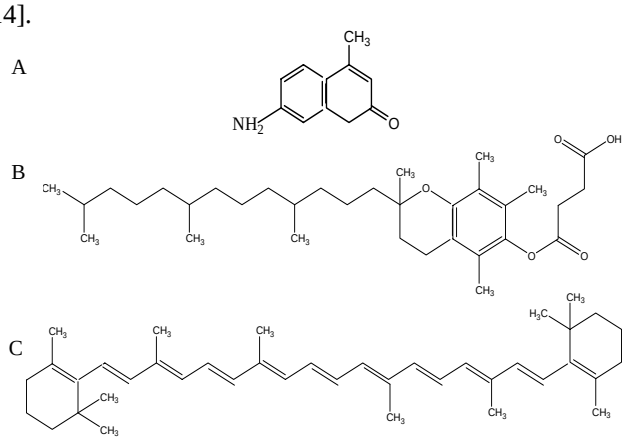

Figure 1: Chemical structures of (A) 7-amino-4-methyl coumarin (B) α-tocopherol succinate and (C) β-carotene.

In this work, an amphiphilic diblock copolymer, poly(ethylene glycol)-*b*-poly(ε-caprolactone) (5,000-b-6,000 g/mole) (PEG(5k)-*b*-PCL(6k)) is used to encapsulate a model hydrophobic drug (β-carotene) and two types of imaging agents; dodecanethiol-capped gold nanoparticles synthesized per the method of Brust *et al.*[15], and a coumarin derivative, 7-amino-4-methyl coumarin covalently conjugated to α-tochopherol succinate (Figure 1). The reaction of 7-aminio-4-methyl coumarin with α-

tochopherol serves to enhance the hydrophobicity of the coumarin molecule and increase the encapsulation efficiency of the fluorescent probe within the hydrophobic core of the polymer nanoparticles.

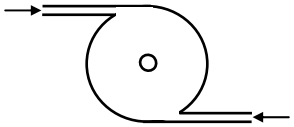

Figure 2: Schematic diagram for a tangential flow mixing cell which permits unequal stream velocity mixing. The mixer effluent is collected through the center outlet stream.

The drug and imaging agents are dissolved or suspended in a water-miscible organic solvent such as tetrahydrofuran (THF) or dimethylsulfoxide (DMSO) and subsequently mixed against an opposing water stream using a tangential flow mixing cell (Figure 2). The collision of the two incoming jets provides a region of high intensity micromixing where solute supersaturation is rapidly achieved and precipitation of drug, imaging agent and block copolymer occurs without diffusional limitations. The result is the formation of polymer protected nanoparticles encapsulating both the targeted drug and imaging agent.

3 RESULTS AND DISCUSSION

We have successfully encapsulated dodecanethiol-capped gold nanoparticles with an initial diameter of 2-5 nm in poly(ethylene glycol)-*b*-poly(ε-caprolactone) (5,000-b-6,000 g/mole) diblock copolymer nanoparticles using a tangential flow vortex mixer. High resolution transmission electron microscopy (TEM) photographs (Figure 3) of the resulting nanoparticles confirm the stabilization of gold nanoparticle clusters by block copolymer assembly onto the cluster surface.

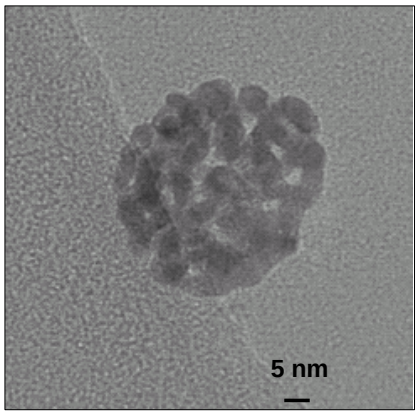

Figure 3: TEM image of block copolymer stabilized dodecane-thiol nanoparticles prepared using a tangential flow vortex mixer.

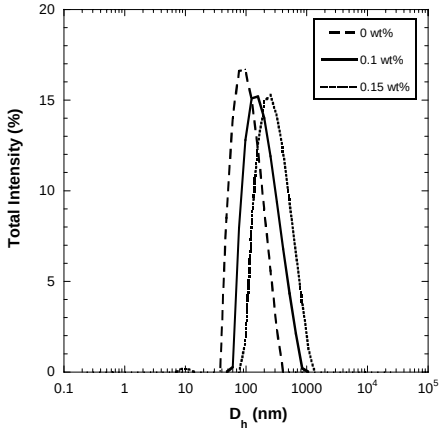

Figure 4: Size distributions of nanoparticles composed of dodecanethiol gold /PEG(5k)-*b*-PCL(6k) at 0.02/0.1 w/w%, with PCL(3.2k) concentration as indicated.

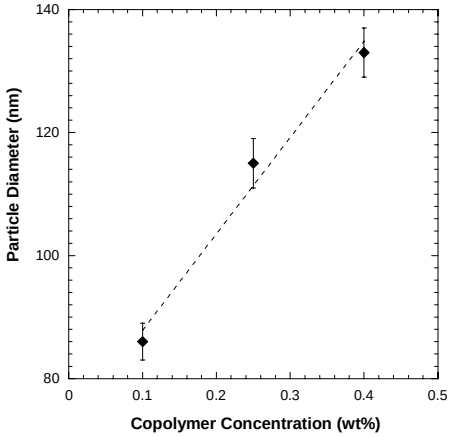

Figure 5: Particle size as a function of block copolymer concentration. Nanoparticles composed of dodecanethiol gold /PEG(5k)-*b*-PCL(6k) at 0.02w% gold, with PEG(5k)-*b*-PCL(6k) concentration as indicated.

Particle size can be specified *a priori* and attained through the precise specification of process inputs. The addition of a second nucleating agent, poly(ε-caprolactone) (3,200 g/mole), is used to yield expanded particle cores and is demonstrated to result in an increase in particle diameter as a function of homopolymer concentration. Particle size is varied uniformly between 80 and 300 nm with the addition of poly(ε-caprolactone), as determined by dynamic light scattering (DLS), while maintaining constant dodecanethiol gold and block copolymer concentrations (Figure 4). Further manipulation of particle diameter is attained by adjusting the ratio of stabilizing copolymer to dodecanethiol gold, with larger particle diameters resulting from an increase in block copolymer concentration (Figure 5).

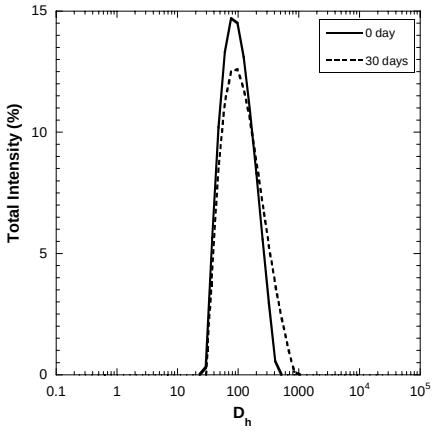

Figure 6: Size distributions of nanoparticles composed of β-carotene, dodecanethiol gold, and PEG(5k)-*b*-PCL(6k) at 0.02/0.05/0.1 w/w%, respectively. Particle size stability in 0.9% sodium chloride solution is demonstrated for a period of at least 4 weeks.

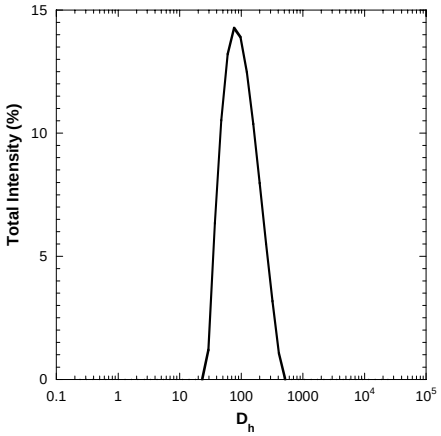

Figure 7: Size distributions of nanoparticles composed of 7-amino-4-methyl coumarin-α-tocopherol conjugate /PEG(5k)-*b*-PCL(6k) at 0.05/0.05w/w%, respectively.

The simultaneous encapsulation of β-carotene, a model hydrophobic drug agent, and dodecanethiol gold within polymer nanoparticles is also demonstrated. Particles with a mean diameter of 100 nm and a narrow size distribution, as determined by DLS, are obtained for a defined set of process input parameters. Particle stability in both deionized water and saline solution is established for a period of at least 4 weeks (Figure 6). Finally, the encapsulation of a water soluble fluorescent molecule, 7-amino-4-methyl coumarin, within the hydrophobic core of PEG(5k)-*b*-PCL(6k) nanoparticles is also demonstrated. Prior to encapsulation, the fluorescent probe is conjugated to α-tocopherol succinate through an ester linkage in order to provide enhanced hydrophobicity of the imaging agent, leading to more stable particle formulations. Particles with a mean diameter of 110 nm are produced at conditions specified in Figure 7.

4 CONCLUSION

The process of Flash NanoPrecipitation is successfully employed for the preparation of poly(ethylene glycol)-*b*-poly(ε-caprolactone) diblock copolymer nanoparticles encapsulating β-carotene, α-tochopherol succinate, dodecanethiol gold and 7-amino-4-methyl coumarin for the simultaneous delivery of drug and imaging agents. Nanoparticles with controlled-size, narrow particle size distributions, high encapsulation efficiencies, and long term stability are generated. Additionally, the amphiphilic polymer-based delivery vehicles detailed in the present work may be used for parenteral, nasal, subcutaneous, intramuscular, aerosol and oral administration.

REFERENCES

[1] Kataoka, K., A. Harada, and Y. Nagasaki, Advanced Drug Delivery Reviews, 2001. **47**(1): p. 113-131.

[2] Kazunori Kataoka, A.H., Yukio Nagasaki, Advanced Drug Delivery Reviews, 2001. **47**: p. 113–131.

[3] Kwon, G.S., Critical Reviews In Therapeutic Drug Carrier Systems, 1998. **15**(5): p. 481-512.

[4] Kwon, G.S., Critical Reviews In Therapeutic Drug Carrier Systems, 2003. **20**(5): p. 357-403.

[5] Adams, M.L., A. Lavasanifar, and G.S. Kwon, Journal Of Pharmaceutical Sciences, 2003. **92**(7): p. 1343-1355.

[6] Parak, W.J., et al., Nanotechnology, 2003. **14**(7): p. R15-R27.

[7] Tshikhudo, T.R., Z. Wang, and M. Brust, Materials Science And Technology, 2004. **20**(8): p. 980-984.

[8] Maeda, H., et al., International Immunopharmacology, 2003. **3**(3): p. 319-328.

[9] Seymour, L.W., Critical Reviews in Therapeutic Drug Carrier Systems, 1992. **9**(2): p. 135-187.

[10] Johnson, B.K. and R.K. Prud'homme, Australian Journal Of Chemistry, 2003. **56**(10): p. 1021-1024.

[11] Johnson, B.K. and R.K. Prud'homme, Physical Review Letters, 2003. **91**(11).

[12] Johnson, B.K. and R.K. Prud'homme, Abstracts Of Papers Of The American Chemical Society, 2003. **226**: p. U527-U527.

[13] Allen, C., et al., Journal of Controlled Release, 2000. **63**(3): p. 275-286.

[14] Kim, S.Y. and Y.M. Lee, Biomaterials, 2001. **22**: p. 1697-1704.

[15] Brust, M., et al., Journal Of The Chemical Society-Chemical Communications, 1994(7): p. 801-802.