Novel pH-responsive hybrid peptide block copolymers for intracellular delivery applications

Angela MH Leung, Vanessa KW Ng, Michael KC Tam*

*Department of Chemical Engineering, Waterloo Institute for Nanotechnology, University of Waterloo, 200 University Avenue West, Waterloo, Ontario, Canada, N2L 3G1, *mkctam@uwaterloo.ca*

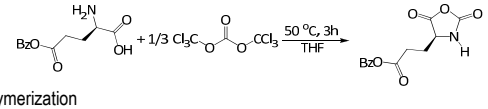
Research Objectives

- Synthesize pH-responsive block copolymers composed of glutamic acid and 2-(diethylamino)ethyl methacrylate
- Characterize the block copolymer self-assembly and pH-responsive behaviour
- Investigate complexation of polymer with DNA and a model drug
- Test in vitro delivery of complexes to mammalian cell cultures

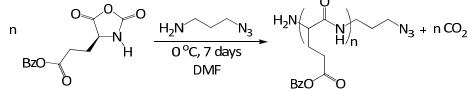
Synthesis

Ring Opening Polymerization of N-Carboxyanhyrides

N-carboxyanhydride synthesis by phosgenation of γ-benzyl-L-glutamate
- FTIR (KBr pellet): 1850 cm^{-1}, 1790 cm^{-1} (anhydride)
- ^{1}H NMR (d_6-DMSO): δ 7.33 (m, 5H, C_6H_5), 5.06 (m, 2H, bz-CH_2), 4.43 (dd, 1H, CHR), 2.33 (m, 2H, CH_2COO), 1.90 (m, 2H, CH_2CH)

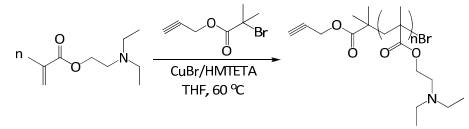


Polymerization
- FTIR (KBr pellet): 1660 cm^{-1}, 1555 cm^{-1} (polypeptide)
- Block length and PDI: 20, 1.10 (GPC), 15 (^{1}H NMR)



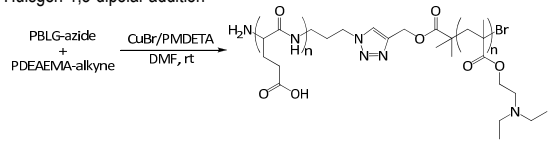
Atom Transfer Radical Polymerization

- Block length and PDI: 40, 1.06 (GPC), 35 (^{1}H NMR)



Block Synthesis by Click Chemistry

Huisgen-1,3-dipolar addition

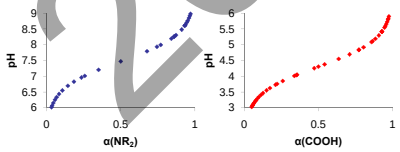


Instrumentation

Instrument	Properties
Gel Permeation Chromatography (GPC)	Molecular weight and distribution
Fourier Transform Infrared Spectroscopy (FTIR)	Functional groups
Nuclear Magnetic Resonance (^{1}H NMR)	Chemical structure
Potentiometric Titration	Acid/base titration and solution conductivity
Zetasizer	Particle size and zeta potential
Dynamic Light Scattering (DLS)	Critical micelle concentration, radius of gyration and intensity
Static Light Scattering (SLS)	Hydrodynamic radius
Gel Electrophoresis	DNA, RNA and protein separation under applied electric field
Fluorescence Microscopy	Imaging under light and UV

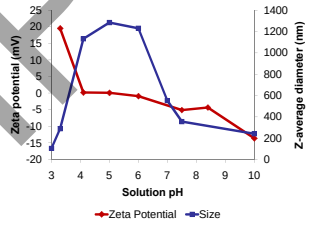
pH-Responsive Behaviour

Degree of protonation of polymer based on potentiometric titration
- pK_a of carboxyl group = 4.3
- pK_a of amine group = 7.5



Self-Assembly

pH-Responsive Assembly



pH effect on size and surface charge
- Measured using zetasizer
- Polymer concentration of 200 μg/ml in 25 mM buffer solutions
- Isoelectric point around pH 5.1
- Large size increase around pH 4-6, where surface charge is closest to 0, due to polymer aggregation

Physical Characterization by Light Scattering

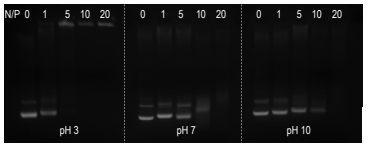
Static and dynamic light scattering to investigate conformational behaviour at different pHs
- Critical micelle concentration investigated using light scattering intensity
- Radius of gyration and hydrodynamic radius found to look into type of structure formed
- Values are averages over a range of concentrations from 0 μg/ml to 500 μg/ml

Parameter	pH 3	pH 7	pH 10
CMC (μg/mL)	~200	~50	~10
R_g (nm)	79.27 ± 5.48	192.63 ± 10.70	107.90 ± 4.58
R_h (nm)	59.40	102.40	62.35
R_g/R_h	1.31	1.89	1.74
Possible Structure	Vesicle	Gaussian Chain	Gaussian Chain

Gene Delivery

DNA Complexation

Agarose gel electrophoresis assay at different pH and N/P ratios

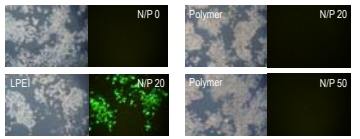


- Examined electrophoretic mobility of complexes at different ratios of polymer to DNA
- Better complexation with DNA at lower pH, due to greater cationic charge from PDEAEMA segment

Polyplex formation
- Polymer condenses DNA to a particle diameter ranging from 120 – 170 nm, depending on solution pH

In Vitro Gene Delivery

Delivery of polyplexes to neuroblastoma cells (N2a)



- Used pIRES-eGFP plasmid
- Viewed under fluorescence microscopy (10 x)
- LPEI used as positive control
- No gene expression observed with polymer

Concluding Remarks and Future Plans

- Block copolymer of glutamate and DEAEMA synthesized using NCA-ring opening polymerization, ATRP and Huisgen-1,3-dipolar addition
- Polymer formed vesicles and gaussian chains based on pH
- Polymer condenses DNA at higher N/P ratios
- Further studies on the polymer self-assembly based on pH and concentration are necessary
- Additional tests needed on the delivery of therapeutic agents

Acknowledgements

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