Tuning Molecular Weight of Poly(NIPAAm-co-HIPAAm-co-SAKIPAAm) to Improve Biomarker Enrichment

CNF Summer Student: Maxim Sokolov
Student Affiliation: University of California, Berkeley

Summer Program(s): 2024 Cornell NanoScale Facility International Research Experiences for Undergraduates (CNF iREU) Program at National Institute for Materials Science (NIMS), Japan

Principal Investigator(s): Dr. Mitsuhiro Ebara, Smart Polymers Group, National Institute for Materials Science (NIMS), Tsukuba, Ibaraki, Japan

Mentor(s): Dr. Ahmed Nabil, Soichiro Yoshizumi, Smart Polymers Group, NIMS

Primary Source(s) of Research Funding: NSF Awards to Cornell OISE-2246252 (IRES) and NNCI-2025233 (NNCI)

Contact: msokolov@berkeley.edu, ebara.mitsuhiro@nims.go.jp, tolba.ahmednabil@nims.go.jp, ousoui1203@gmail.com

Summer Program Website: https://cnf.cornell.edu/education/reu/2024

Abstract:

"Smart" polymers, those that can reversibly change physio-chemical properties in response to an outside stimulus (e.g. ionic concentration, temperature, pH) have been studied for a range of biomedical applications. Among them, temperature responsive polymers have been studied best, bearing applications in early disease detection through biomarker enrichment, drug delivery, and cell sheet engineering. The parameters enabling these polymers to perform their tasks most effectively have not been fully explored. We synthesized poly(NIPAAm-co-HIPAAm-co-SAKIPAAm) in varying conditions to determine a relationship between reaction parameters and molecular weight.

Summary of Research:

Background and Motivation. Viruses contain characteristic proteins which, as biomarkers for disease, can be used in diagnosis. Low biomarker concentration can make detection difficult, potentially causing false negative test results. Additionally, underdeveloped communities are often limited to cheaper, less powerful tests. By conjugating an antibody selective for a disease biomarker to a temperature responsive polymer, the polymer can be precipitated out of solution, increasing the concentration of the sample and increasing the power of the applied test. This process is called biomarker enrichment. The polymer does not need to be cleaved from the antibody and biomarker in order to analyze the sample, allowing for easy testing after polymer introduction. Poly(NIPAAmco-HIPAAm-co-SAKIPAAm) is used because PNIPAAm is well studied, and HIPAAm converts to SAKIPAAm, which readily conjugates with antibodies through click chemistry [1].

Different monomer ratios and molecular weights will affect the temperature at which the polymer will precipitate out of solution, the lower critical solution temperature (LCST). They will also affect the polymer's ability to enrich biomarkers. Low molecular weight polymers are susceptible to agglomeration, and larger ones may tangle [2]. Too many functional sites will cause competition between antibodies, and too few will not be effective. There exists a set of optimal parameters for enrichment, so it

ID	In Feed (M %)		DBCO acid (mg)	CDT (mM)	AIBN (mM)	N+H conc. (M)	Time (h)	NMR Comp (M %)			GPC M_W (g/mol)	Đ
	N	H						N	H	SAKI		
A1	70	30	-	94.4	18.9	4	24	78.5	21.5	-	10403	1.06
A2	70	27	36	94.4	18.9	4	24	78.5	7	14.5	10825	1.05
A3	70	28.5	18	94.4	18.9	4	24	78.5	10.9	10.6	10821	1.05
B1	70	30	-	47.2	9.4	4	24	76.5	23.5	-	17063	1.16
B2	70	27	36	47.2	9.4	4	24	76.5	8.5	15	17151	1.16
B3	70	28.5	18	47.2	9.4	4	24	76.5	18.5	5	16761	1.15
C1	70	30	-	23.6	4.7	4	24	81	19	-	33060	1.37
C2	70	27	36	23.6	4.7	4	24	100			33506	1.30
C3	70	28.5	18	23.6	4.7	4	24	81	8.2	10.8		
D1	70	30	-	11.8	2.4	4	21	71.5	28.5	-	13783	1.16
*E1	90	10	-	47.2	4.8	2	8	86	14	-	4806	1.14
F1	70	30	-	23.6	2.4	4	8	72	28	-	7936	1.10
F2	70	30	-	15.7	1.6	6	8	79	21	-	10702	1.12
G1	50	50	-	23.6	2.4	4	8	63	37	-	8036	1.13
G2	50	50	-	15.7	1.6	6	8	53.5	46.5	-	12181	1.15
H3	70	30	-	23.6	2.4	4	51	77	23	-		
H4	70	30	-	23.6	2.4	4	94	79.5	20.5	-		

Table 1: Tabulated reaction parameters and characterization results for all samples.

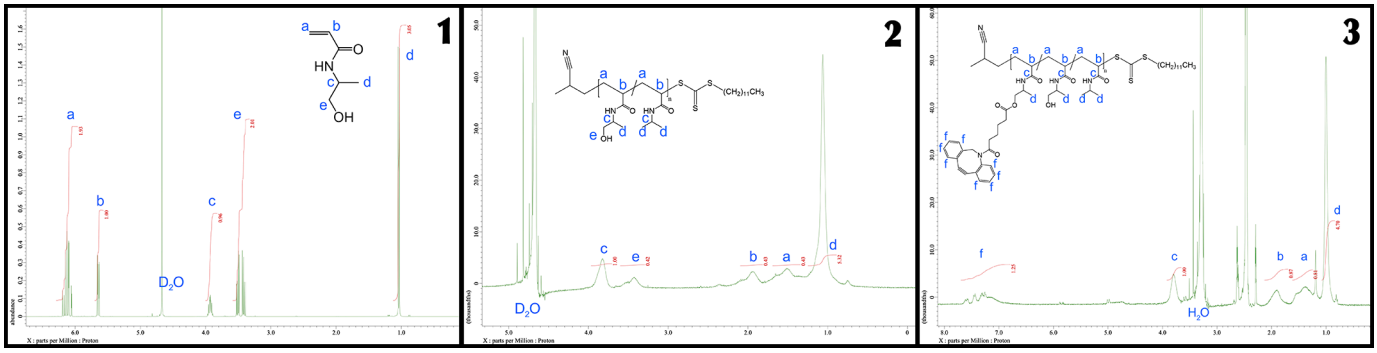

Figure 1: HIPAAm 1H-NMR data. Figure 2: poly(NIPAAm-co-HIPAAm) 1H-NMR data for sample A1. Figure 3: poly(NIPAAm-co-HIPAAm-co-SAKIPAAm) 1H-NMR data for sample A2..

is worthwhile to investigate how these parameters affect the polymer's efficacy, and how reaction conditions affect these parameters.

Goal 3 of the United Nations' 2030 Agenda for Sustainable Development is to "ensure healthy lives and promote well-being for all at all ages." Underdeveloped areas have demonstrated a clear need for low-cost, portable, and fast disease detection. Our group's Smart-Ex biomarker enrichment technology is simple to use, inexpensive, and allows testing in a variety of conditions, helping meet these global demands.

Materials and Methods. NIPAAm monomer was recrystallized from stock solution under hexane, then vacuum dried. HIPAAm monomer was synthesized according to [3], then the monomers were polymerized by reversible addition fragmentation chain transfer (RAFT) polymerization using cyanomethyldodecyl trithiocarbonate (CDT) as chain transfer agent (CTA) and azobisisobutyronitrile (AIBN) as radical initiator. Polymerizations were performed in various schemes. In the first block, 4M of NIPAAm and HIPAAm monomer in ethanol at a 7:3 molar ratio was used. CDT and AIBN were fixed in a 5:1 ratio. CTA molarity varied inversely to target molecular weight, constant of proportionality k=472. After 24h in a 60°C oil bath, the polymer was dissolved in tetrahydrofuran, reprecipitated in diethyl ether, and vacuum dried. According to the process described in [4], some HIPAAm units in the polymer were converted to SAKIPAAm, and the sample was again dried.

The second trial involved a variation in molarity of the monomers in ethanol at 2M, 4M, and 6M as well as NIPAAm/HIPAAm molar ratios of 9/1, 7/3, and 5/5 for a total of nine samples. They were polymerized for 8 hours at 60°C, dissolved in THF, reprecipitated in diethyl ether, and dried. HIPAAm monomer was characterized with hydrogen nuclear magnetic resonance spectroscopy (1H-NMR, JEOL ECS 400, 300MHz) and polymerized samples were characterized with gel permeation chromatography (GPC, Shimadzu Nexera, solvent: DMF containing 10 mmol/L LiCl, standard: poly(styrene)).

Results and Discussion. HIPAAm monomer NMR data are shown in Figure 1. Representative NMR data for poly(NIPAAm-co-HIPAAm) and poly(NIPAAm-co-HIPAAm-co-SAKIPAAm) are shown in Figure 2 and Figure 3, respectively. The results of polymer synthesis are shown in Table 1. Two of the 2M samples were lost due to equipment malfunction, and two of the 90/10 molar ratio samples failed to polymerize. The remaining 90/10 sample had an exceptionally low yield. NMR failed for sample C2.

The peaks indicate that HIPAAm, poly(NIPAAm-co-HIPAAm) and poly(NIPAAm-co-HIPAAm-co-SAKIPAAm) were successfully synthesized. Additionally, results from the second block of polymerizations suggest molarity has a positive effect on polymer length, and monomer ratio has no effect. RAFT agent and initiator concentration have a negative impact on molecular weight, as predicted. HIPAAm generally exists in a lower percentage in polymer than in initial monomer concentration, according to NMR data.

Halving the amount of DBCO acid decreases the amount of SAKI groups present, but not necessarily by half. Overall, variation between samples is high, despite similar reaction conditions.

Conclusions:

Poly(NIPAAm-co-HIPAAm-co-SAKIPAAm) was successfully synthesized at different molecular weights. Poly(NIPAAm-co-HIPAAm) was successfully synthesized at different molecular weights in different reaction conditions. Time and reaction molarity have a positive impact on molecular weight. RAFT agent and initiator concentration have a negative impact on molecular weight, and monomer ratio has no impact on molecular weight. Significant variation exists between monomer input and NMR results. Stronger, quantitative relationships are difficult to form due to this uncertainty as well as measurement uncertainty. Further work conjugating these polymers with antibodies is ongoing.

References:

[1] https://doi.org/10.1039/c9sc03368h.
[2] https://doi.org/10.3390/polym8110380.
[3] https://doi.org/10.1021/bm050829b.
[4] https://doi.org/10.1039/D1BM00349F.