Using PolyNIPA Based Polymers to Create pH and Temperature Sensitive Indicators

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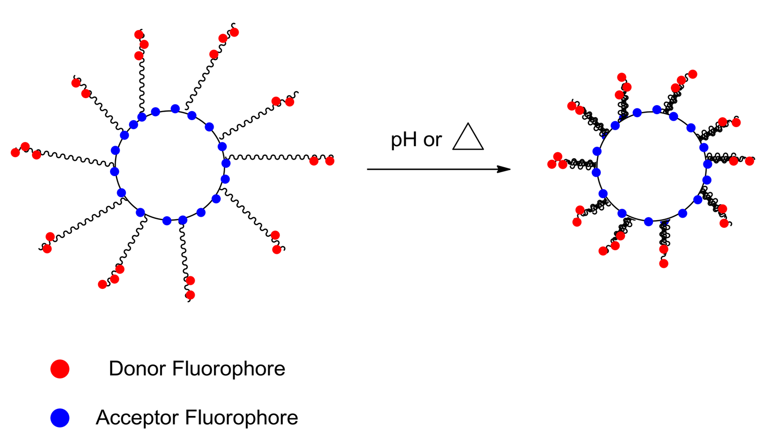
Introduction

Extensive research has been done recently on environmentally sensitive polymers, since they have properties that could have potential applications in biomedical fields and in industry. These polymers are of interest as they shrink and swell due to changing environmental conditions.[1] This research is focused on the synthesis of the fluorescent indicators that are sensitive to pH and temperature. The synthesis involves growing poly(N-isopropylacrylamide), polyNIPA ,based polymers from a silica surface, by the process of reversible addition fragmentation chain transfer, RAFT, polymerization. The indicators measure the swelling and shrinking of the polymer by fluorescence resonance energy transfer, FRET. [2,3]

Indicator Design



The indicators are silica particles that have polyNIPA based polymers grown from them. There are donor fluorophores on the end of the polymers and acceptor fluorophores on the surface of the particle. The indicators are designed so that when there is enough of a temperature or pH change it induces a phase change. This will cause the copolymer to collapse and have a compact configuration on the surface. This configuration brings the donor and acceptor fluorophores close enough for FRET. In FRET, there is an induced-dipole, induced-dipole interaction where energy is transferred from a donor fluorophore to an acceptor fluorophore. These fluorophores are typically different colors. FRET is very sensitive to distance, so the molecules need to be very close to each other. [2,3]

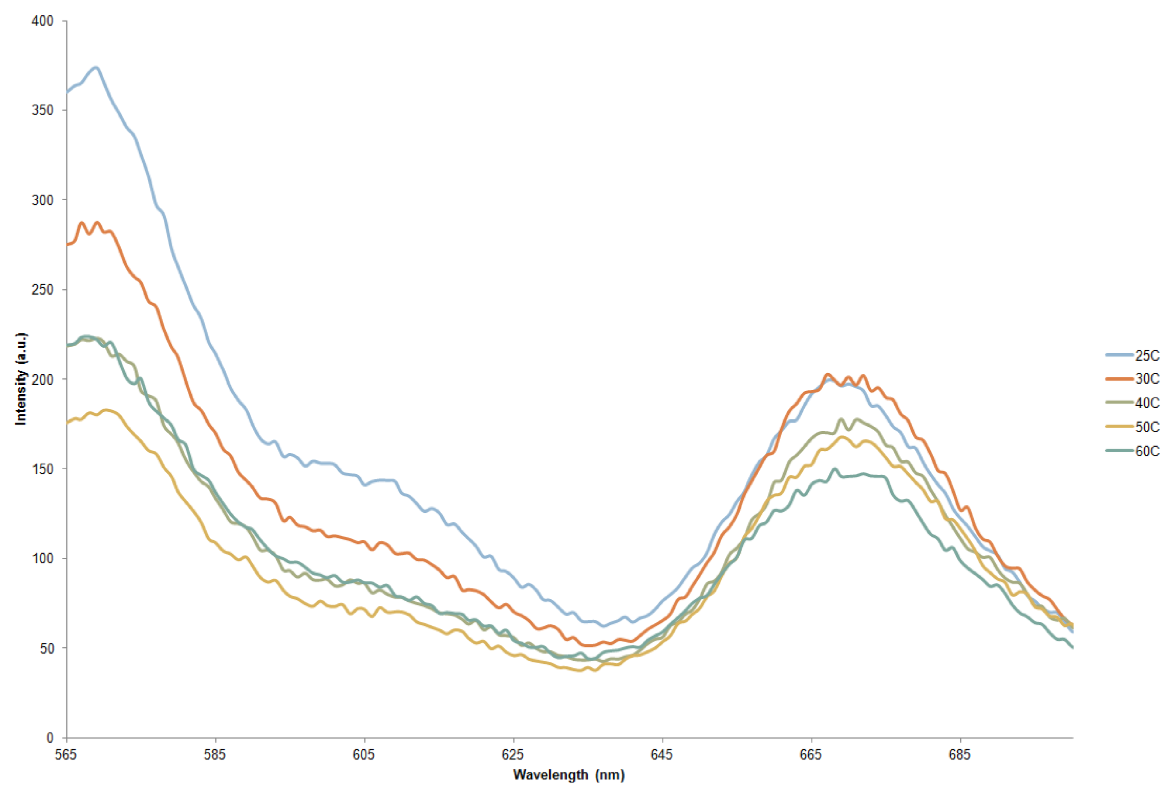
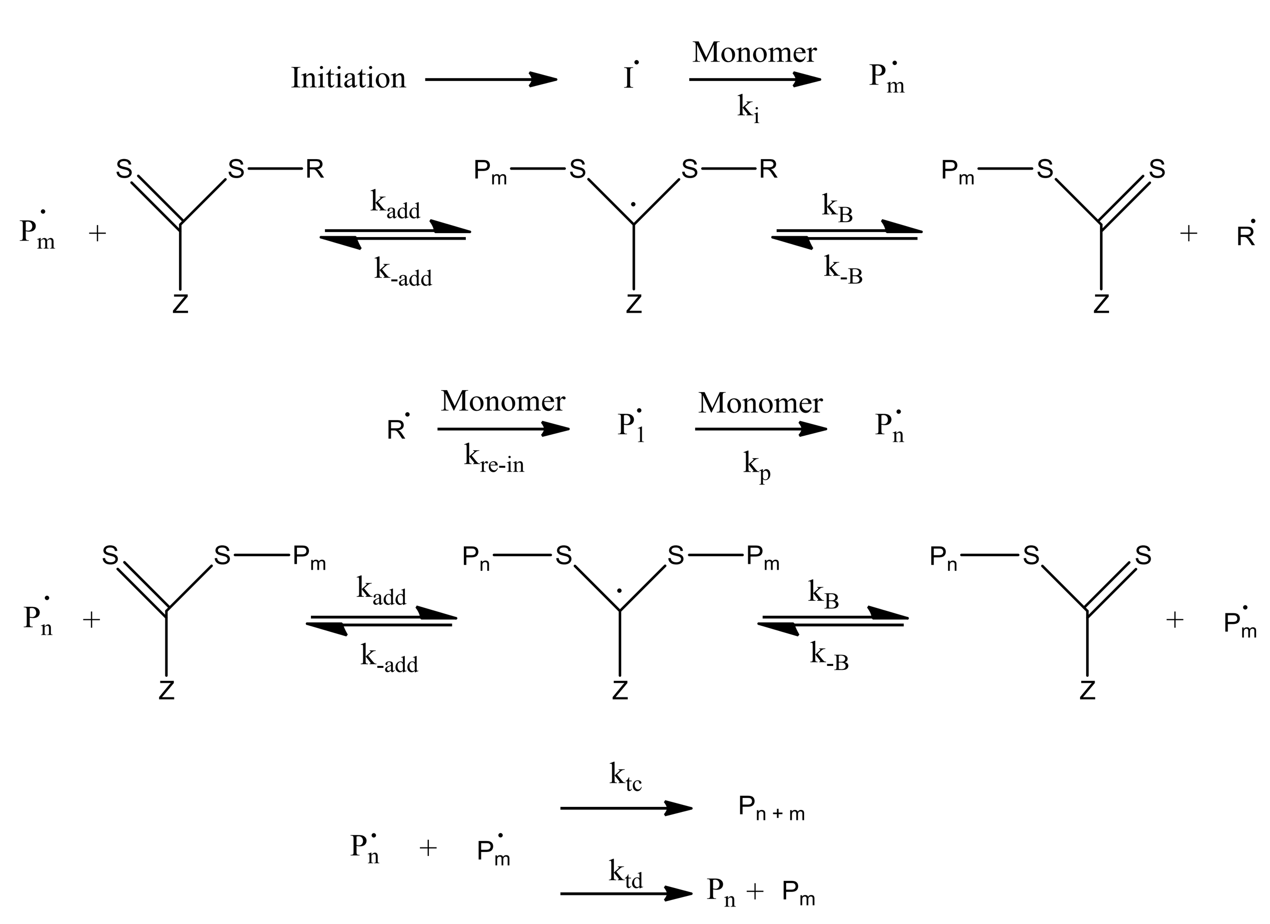


Figure 1: Graph of the Wavelength vs. Intensity. This graph is of a synthesis of poly(NIPA-*co*-AEMA) on silica. The FRET ratio is taken at different temperatures

RAFT Mechanism

The synthesis involves growing polyNIPA from a silica surface, by the process of reversible addition fragmentation chain transfer, RAFT, polymerization. RAFT is a type of radical polymerization. It uses a chain transfer agent to create a thiocarbinylthio compound which reacts with monomer to create the polymer through a reversible chain-transfer process. An initiator is needed to start the process. [4,5]



Data Charts

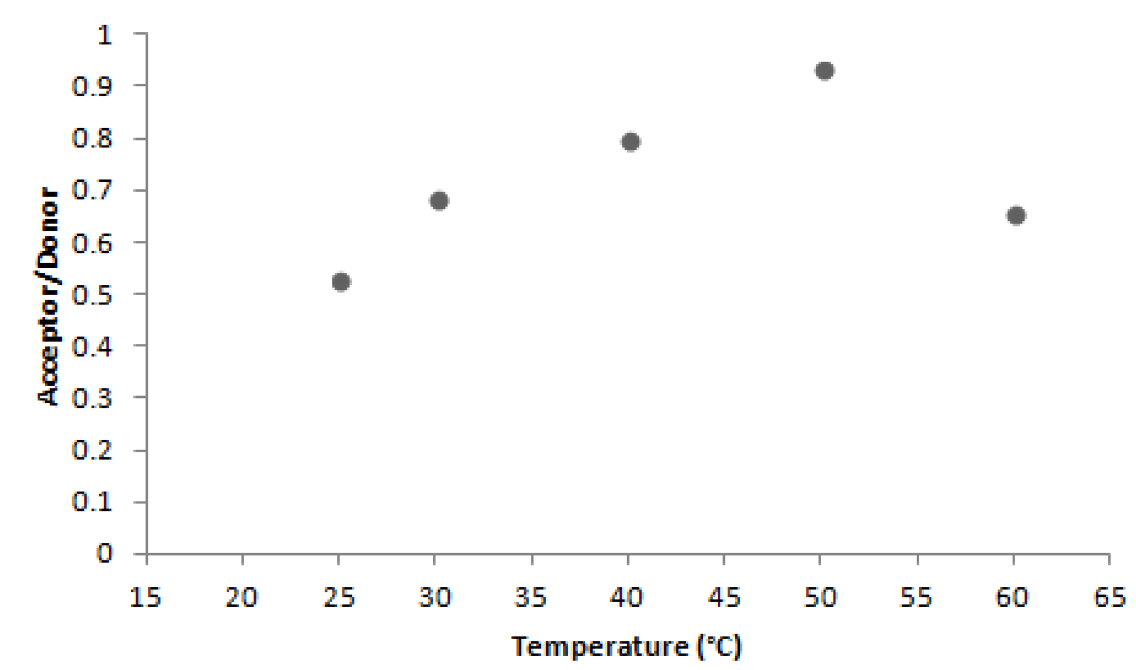


Figure 2: Graph of Temperature vs. the ratio of Acceptor/Donor Fluorescence. This graph is of the poly(NIPA-*co*-AEMA) on silica from Figure 1.

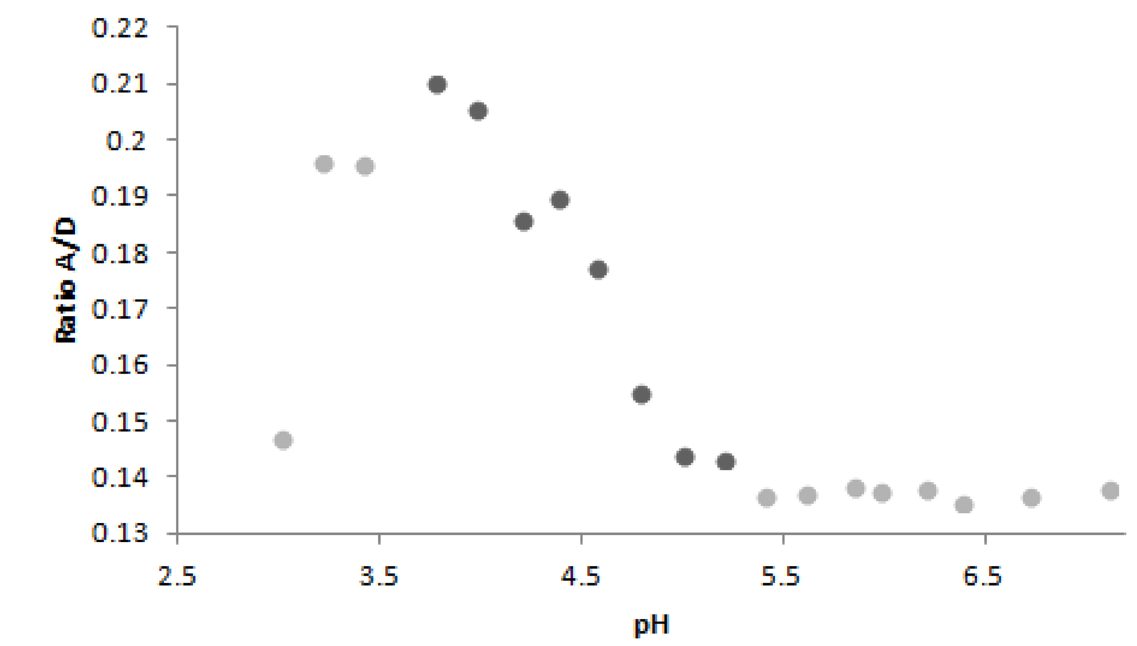


Figure 3: Graph of pH vs. the ratio of Acceptor/Donor Fluorescence. This graph is of a 5% solution poly(NIPA-*ran*-AEMA-*ran*-MAA) at 30 degrees Celsius.

Results and Discussion

A poly(NIPA-*co*-AEMA) polymer grafted on silica was successfully synthesized (**Figure 1** and **Figure 2**). The fluorophores used were Alexa Fluoro 555 carboxylic acid succinimidyl ester on the silica surface and Alexa Fluoro 647 carboxylic acid succinimidyl ester on the polymer chains. The indicator had a significant change in FRET ratio (0.53-0.93) between ~20-50 degrees Celsius. This shows that in this temperature range the acceptor fluorophore was able to transfer more of its absorbed energy as temperature increased. The polymers shrunk and it brought the donor and acceptor closer together. The results show that the ratio increases at a fairly linear pace until between 50-60 degrees Celsius, where the thermal quenching becomes too much. This polymer was successful at having a significant FRET change in temperature, however there was not a monomer that would be effected by a pH change. A liquid poly(NIPA-*ran*-AEMA-*ran*-MAA) polymer was successfully synthesized (**Figure 3**). The fluorophores used were Alexa Fluoro 555 carboxylic acid succinimidyl ester and Alexa Fluoro 647 carboxylic acid succinimidyl ester randomly distributed on the polymer chains. The polymer had a successful change in FRET ratio (0.14-0.21) in the pH range of approximately 3.75-5.25. The problem is that ratio change is not very much as well as these results could not be demonstrated on silica particles.

Conclusion

There was success at making a polyNIPA based polymer that was sensitive to pH or temperature. Unfortunately a polyNIPA based polymer that was sensitive to both pH and temperature that was grafted on silica particles was unable to be synthesized. Once this is achieved, further research can be with metal ions using this type of polymer indicator to create metal ion sensors.

Acknowledgements

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References:

1. Nur, H.; Pinkrah, V.; Mitchell, J.; Benée, L.; Snowden, M.; *Synthesis and properties of polyelectrolyte microgel particles* Advances in Colloid and Interface Science **2010**, *158*, 15-20
2. Ha, T.; Enderle, T.; Ogletree, D.F.; Chemla, D.S.; Selvin, P.R.; Weiss, S.; *Probing the interaction between two single molecules: fluorescence resonance energy transfer between a single donor and a single acceptor* Proc. Natl. Acad. Sci. **1996**, *93*, 6264–68
3. Ha, T.; Tinnefeld, P.; *Photophysics of Fluorescent Probes for Single-Molecule Biophysics and Super-Resolution Imaging* Annu. Rev. Phys. Chem. **2012**, *63*, 595–617
4. Moad, G.; Rizzardo, E.; Thang, S.; *Modern Polymerization Techniques* Material Matters **2010**, *5*, 2-8
5. Perrier, S.; Takolpuckdee, P.; *Macromolecular Design via Reversible Addition–Fragmentation Chain Transfer (RAFT)/Xanthates(MADIX) Polymerization* Journal of Polymer Science: Part A: Polymer Chemistry **2005**, *43*, 5347–5393