

Highly Controlled Open RAFT Polymerizations by Enzyme Degassing

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STATEMENT OF PURPOSE: Polymer based biomaterials remain the most promising class of biomaterials because of their versatility and relative ease of synthesis coupled with low costs. Controlled radical polymerization (CRP) techniques such as atom transfer radical polymerization (ATRP) have provided means to synthesize well defined macromolecules¹. However, one of the major drawbacks with CRP's is their intolerance to oxygen. Oxygen interacts with the propagating center in any radical polymerization and neutralizes the catalyst in any ATRP reaction. This limitation makes polymerizations more challenging to conduct in high throughput as well as the ability to maintain fine control over the process.

Our group recently reported the use of the enzyme Glucose Oxidase (GO_X) to remove oxygen from controlled RAFT polymerizations (Enz-RAFT). GO_X has been extensively used as an initiator in polymerization reactions but we exploited its ability to degas oxygen as the activity of enzyme is very high and can remove dissolved oxygen faster than diffusion from surface can occur, even at very low enzyme concentrations ($<1\mu\text{M}$).

This simple, yet robust technique enables combinatorial polymer synthesis in microtiter plates on the bench top without the need of highly specialized synthesizers and at much lower volumes than is possible by any other technique.

METHODS:

A stock solution of RAFT agent in a mixture of methanol/PBS (30 v/v) was made and the pH was adjusted to 7.0. In a typical polymerization, the RAFT agent (32 mg, 0.134 mmol) was added to deinitiated monomer (3.36 mmol) at 25 equivalents to 1 ratio. Glucose and VA-044 (initiator) were added in PBS and the solution was made up to a final monomer concentration of 2 M with methanol and PBS such that the final ratio of methanol: PBS is 1:4. For GO_X degassed polymerizations, a solution of GO_X in PBS (100 mM) was added, and the solution was left at room temperature for 10 min to degas before heating at 45°C overnight. Samples were collected by simply removing the lid and pipetting out $30\mu\text{L}$ of solution. Reactions were performed in $300\mu\text{L}$ solutions on 96 well plates or $40\mu\text{L}$ solutions on low-volume 384 well plates. NMR was used to measure the percent conversion while SEC (size exclusion chromatography) was used to determine the molecular weight of the polymer samples^{1,2}.

RESULTS:

- Regardless of the solution volume, a linear evolution of molecular weight with conversion and very low dispersities ($D < 1.1$) was observed
- GO_X maintained its activity at higher monomer concentrations (1.6 M)
- We were able to synthesize polymers (conversion $>80\%$) with various degrees of polymerizations (ranging from 20-200) using acrylamide, methacrylamide and methacrylate monomers
- Technique expanded to synthesize diblock and triblock copolymers without intermediate purification
- Clean shifts in molecular weight distribution with low dispersity ($D < 1.2$) was observed by SEC for each block co-polymer (this data not provided in abstract)

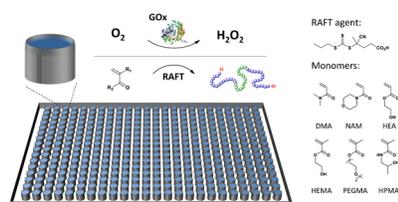


Figure 1: Representation of enzyme-degassed RAFT polymerizations performed in 384 well plates, showing the structure of RAFT agent and monomers investigated

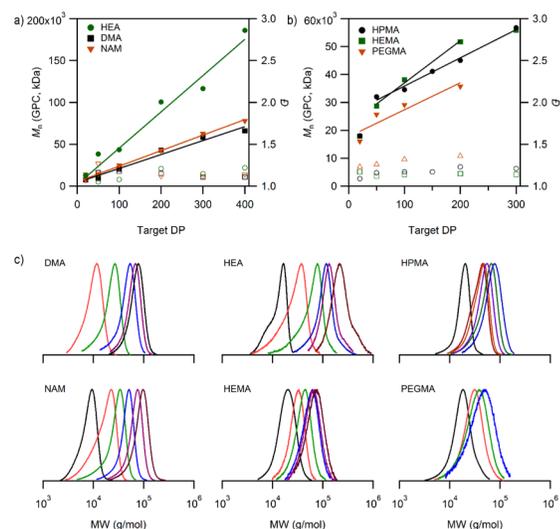


Figure 2: a, b) Molecular weight (closed symbols) and dispersity (open symbols) of homopolymers DMA, NAM, and HEA (a), and for HEMA, PEGMA, and HPMA (b). c) GPC traces for each polymerization. DMA, HEA, NAM, HEMA, and PEGMA polymerizations (0.5M monomer at 45°C for 20 h with a $[\text{RAFT}]/[\text{VA044}]$ ratio of 10, and HPMA polymerizations were conducted at 1M monomer at 50°C for 20 h with a $[\text{RAFT}]/[\text{VA044}]$ ratio of 5.

CONCLUSIONS:

- Novel technique to generate large libraries of polymers without complicated robotics or degassing steps
- Polymers can be made in extremely low volumes ($40\mu\text{L}$) and at low concentrations (0.5 M)
- Block copolymers can be created by simple addition of subsequent monomer to the same vessel

References:

- 1) Gormley AG, Chapman R, *Macromolecules*, 2014;47, 8541-8547
- 2) Chapman R, Gormley AG, *Angewandte Chemie International Edition*, 2016;55, 4500-4503)