Rapidly Self-Assembled, Hydrophobically-Modified Poly(oligo ethylene glycol methacrylate)-Based Nanogels for the Delivery of Poorly Water-Soluble Therapeutics

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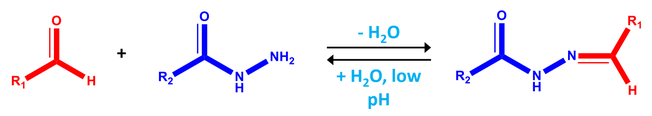
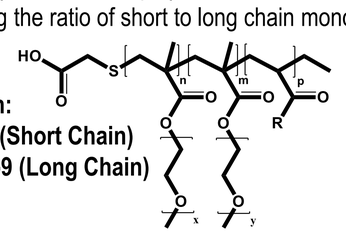
Background

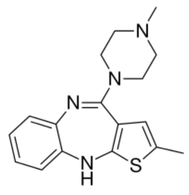
Nanogels, nanoscale networks of water-swollen, covalently cross-linked polymers, have the potential to be useful drug carriers as their hydrophilic nature enables long-term circulation in the body. Nanogels are also highly elastic and can deform to pass through tight junctions, such as those located in the blood-brain barrier (BBB). In addition, nanogel chemistry can be tuned to maximize drug uptake and target specific cell and tissue types. While several methods exist to fabricate nanogels, creating degradable and monodisperse nanogels has been a significant challenge. We recently addressed this challenge by developing a simple nanogel fabrication method that uses hydrazone chemistry to cross-link well-defined precursor polymers into nanogels[1]. The covalent hydrazone cross-link degrades slowly (over months) at physiological pH but faster (days-weeks) at acidic pH to clear the nanogel.



In this work, we aim to apply this chemistry to address two additional challenges with the use of nanogels for drug delivery. First, to enhance the hydrophilic nature of nanogels and thus promote higher circulation times nanogels will be fabricated using poly(oligoethylene glycol methacrylate) (POEGMA) precursor polymers. POEGMA, as a PEG-mimetic polymer, is highly hydrophilic and thus protein-repellent and does not elicit an immune response or induce cytotoxicity[2,3]. POEGMA polymers can also be rendered thermoresponsive by adjusting the ratio of short to long chain monomers[4].



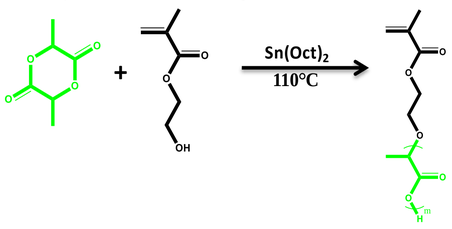
PEG chain length:
$M(EO)_2MA$: x = 2 (Short Chain)
$OEGMA_{475}$: y = 8-9 (Long Chain)



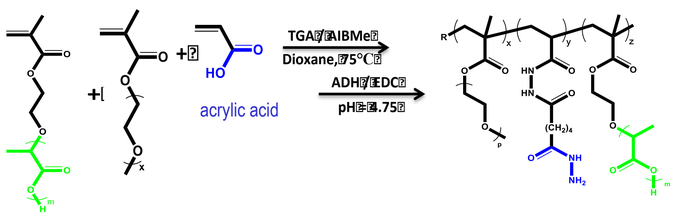
Second, the hydrophilic nature of nanogels is ill-suited to load and deliver many hydrophobic drugs of interest, including most antipsychotic drugs like olanzapine (right). Herein, we present a simple method to modify nanogel formulations with hydrophobic moieties to increase the uptake of such drugs. The rapid, thermally-driven self-assembly method produces monodisperse covalently cross-linked, yet degradable nanogels with high drug loading for hydrophobic therapeutics.

Experimental Methodology

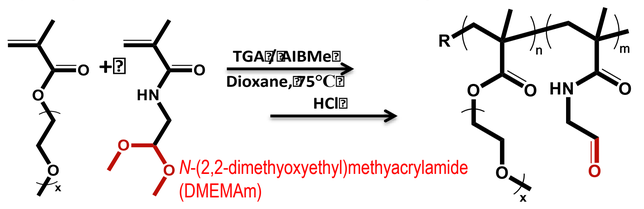
Synthesis of linear polymer precursors: Oligo(lactic acid methacrylate) (OLAMA) was synthesized using a tin-catalyzed ring-opening polymerization of L-lactide with hydroxylethyl methacrylate. OLAMA oligomers are prepared with either four ($OLAMA_4$) or eight ($OLAMA_8$) lactic acid repeats[5].



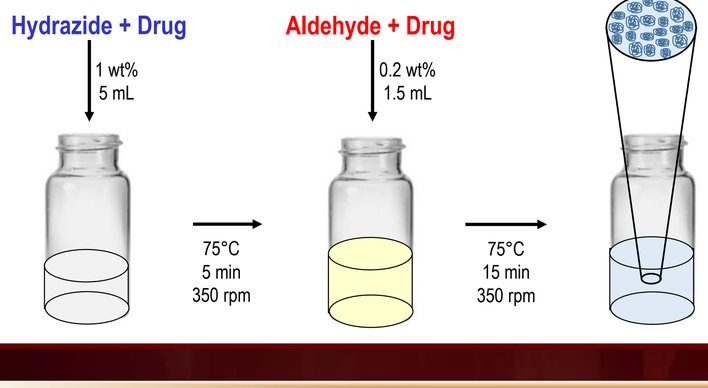
Hydrazide-functionalized polymers were prepared by copolymerizing POEGMA monomers with OLAMA and acrylic acid, then subsequently converting the carboxylic acid groups to hydrazide groups using carbodiimide chemistry[6].



Aldehyde-functionalized polymers were prepared by copolymerizing POEGMA monomers with an acetal monomer. Acid-catalyzed hydrolysis was subsequently used to convert the acetals to aldehydes[2,3,6].



Synthesis of hydrophobically-modified nanogels: Nanogel self-assembly was conducted using a rapid, thermally-driven process that produces monodisperse, covalently cross-linked, degradable nanogels while simultaneously encapsulating the drug of interest all in a single step (20 mins.)



Polymer Characterization

Table 1. *Linear precursor polymer properties. Hydrazide content was confirmed by conductometric titration. Molecular weight and polydispersity index was measured using a DMF GPC calibrated with PEG standards.*

Polymer	$M(EO)_2MA$:$OEGMA_{475}$	Functional group mol %	M_n (10^3 g/mol)	Đ
$PO_{30}H_{30}$	70:30	Hydrazide 30%	24	1.88
$PO_{30}H_{30}$-$OLAMA_4$	70:30	Hydrazide 30%	26	1.61
$PO_{30}H_{30}$-$OLAMA_8$	70:30	Hydrazide 30%	35	1.30
$PO_{10}A_{30}$	90:10	Aldehyde 30%	21	1.82

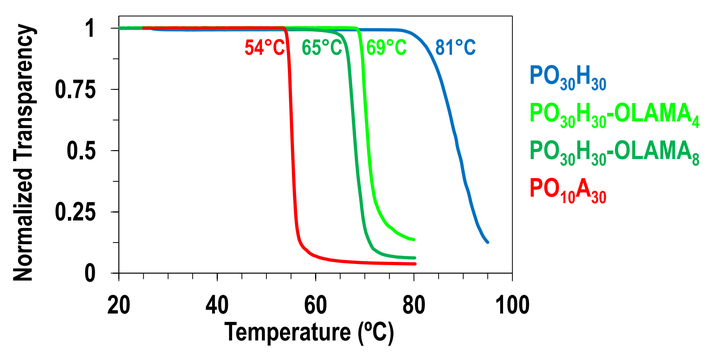


Figure 1. *The lower critical solution temperature (LCST) as measured using UV-Vis spectrophotometry of polymer solutions in water. The LCST value reported is defined as the temperature where the solution is 95% transparent.*

- Increasing the quantity of $OEGMA_{475}$ increases the polymer hydrophilicity and thus the LCST.
- Increasing the OLAMA chain length increases the polymer hydrophobicity and lowers the LCST.

Nanogel Characterization

Table 2. *Nanogel size and population properties were analyzed using dynamic light scattering. The nanogel electrophoretic mobility is measured using phase analysis light scattering. All measurements are carried out in 10 mM PBS.*

Core Polymer	Diameter (nm)	Polydispersity	Mobility (μ/s)/(V/cm)
$PO_{30}H_{30}$	319 ± 23	0.21 ± 0.02	-0.18 ± 0.42
$PO_{30}H_{30}$-$OLAMA_4$	216 ± 6	0.11 ± 0.04	-0.38 ± 0.39
$PO_{30}H_{30}$-$OLAMA_8$	186 ± 5	0.13 ± 0.03	-0.47 ± 0.25

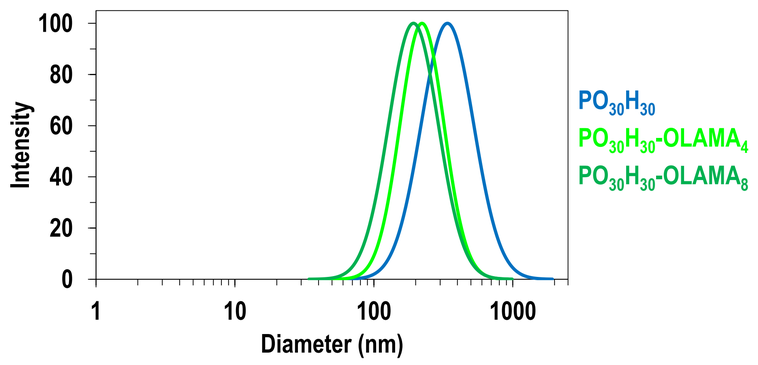


Figure 2. *Particle size distribution measured by dynamic light scattering.*

- Increasing the quantity of OLAMA in the nanogels decreases the hydrodynamic radius of the nanogels.
  - Increased hydrophobicity = reduced nanogel swelling
- Nanogels with up to 20 mol% OLAMA monomer exhibit narrower polydispersity and near-zero surface charge amenable to long-term circulation

Drug Loading

Nanogel drug uptake was investigated using olanzapine, an atypical thienobenzodiazepine APD, used for the treatment of schizophrenia and bipolar disorder, and quantified using the definitions below.

$$\textit{Drug Loading Capacity (DLC)} \quad \frac{Mass\ of\ drug\ encapsulated}{Mass\ of\ nanogel} * 100\%$$

$$\textit{Encapsulation Efficiency (EE)} \quad \frac{Mass\ of\ drug\ encapsulated}{Initial\ mass\ of\ drug\ loaded} * 100\%$$

Table 3. *Olanzapine uptake in various nanogel formulations.*

Core Polymer	DLC (%)	EE (%)
$PO_{30}H_{30}$	3.3	42
$PO_{30}H_{30}$-$OLAMA_4$	3.4	43
$PO_{30}H_{30}$-$OLAMA_8$	3.6	46

- Increased OLAMA content results in similar DLC/EE but smaller and more uniform nanogel populations → hydrophobicity vs. volume trade-off

Cytocompatibility

The cytocompatibility of the nanogels was assessed using SH-SY5Y cells, a human cell line derived from a neuroblastoma in the bone marrow. SH-SY5Y cells were treated with olanzapine-loaded nanogels, blank nanogels and the linear precursor polymers for 24 hours. A MTT assay was used to assess the metabolic activity of the cells after the treatment period.

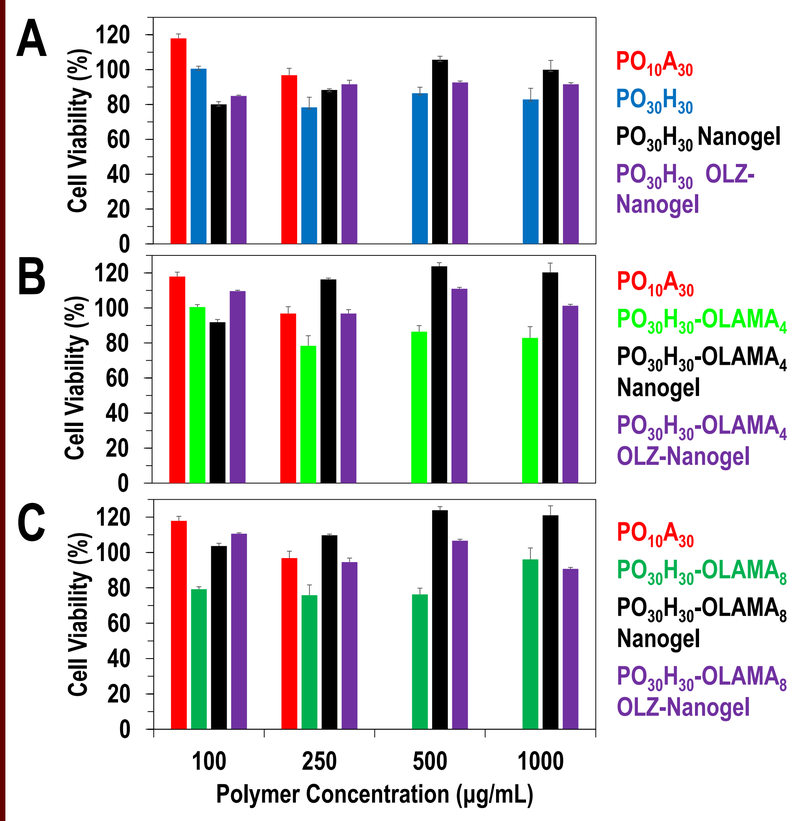


Figure 3. *SH-SY5Y cell viability after 24 hours of treatment with the polymer precursors and nanogels. A) $PO_{30}H_{30}$ series, B) $PO_{30}H_{30}$-$OLAMA_4$ series and C) $PO_{30}H_{30}$-$OLAMA_8$ series.*

- No significant loss of cell viability in the presence of the nanogels and their precursor polymers (→ degradation products)

Conclusions

- Hydrophobically-modified POEGMA nanogels can by synthesized using a rapid, simple, aqueous self-assembly method.
- These nanogels are covalently cross-linked via acid-labile hydrazone linkages that can degrade, enabling clearance of the nanogel after drug delivery.
- These nanogels demonstrate high drug loading capacity, high monodispersity, and good encapsulation efficiency of olanzapine, a hydrophobic APD, demonstrating their potential utility for the delivery hydrophobic drugs.
- The linear precursor polymer, the nanogels and olanzapine-loaded nanogels show minimal cytotoxicity indicating that these formulations have potential for *in vivo* drug delivery applications.

References

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Acknowledgements

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