Application of pH-Responsive Nanogels for Targeted Drug Delivery

[redacted]

Introduction

Non-selective drugs result in harm to benign cells, increased administrations and concentrations, and adverse side effects. Drug delivery systems can alleviate these negative affects by selectively delivering drugs to the treatment site, preventing whole body dispersion. **pH gradients are common of cancer, kidney disease, and diabetes** where the inability for cells to metabolize correctly leads to the production of acidic byproducts, creating a localized acidic environment around the disease site (also known as metabolic acidosis) (Pizzorno, 2015). **The pH gradient of certain disorders can be exploited by nanotechnology to provide a drug delivery system** that specifically releases drugs in the low pH environment of these disorders and will withhold drugs in high pH media (ex. Blood). **pH-responsive nanogels (pNGs) are polymer particles around 1-1000 nm that expand/shrink in response to pH-changes**. **This behavior of pNGs could let pNGs carry drugs and selectively release drugs at disease sites with a low pH by expanding.**

Generally **extracellular pH in normal tissue (ie. brain) is 7.2-7.5 while tumor tissue is generally more acidic at 6.5-7.0** with maximum proliferation at pH of 6.8-7.3 (Hao, Xu, Li, 2018; Zhang, Lin, Gillies, 2010). **Metabolic acidosis in kidney disease and diabetes can result in blood pH of 7.1.**

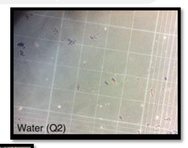
Methods

Use NIPAm, DEAEM, MBA, and SDS, and APS to create nanogels.

Isolate nanogel pellet. Then, resuspend pellets in water.

Test nanogel solution in pH solutions to measure the absorbance vs. pH for each synthesis.

Dry nanogels and place nanogels in two test tubes. Add 2 mL water to one and CP to the other.



Isolate loaded nanogels and apply water nanogels, CP nanogels, and CP solution to cells. Take cell viability.

Abstract and Data Analysis

Abstract: Drug delivery devices specifically transport drugs to help reduce drug toxicity to healthy cells and repetitive drug administrations. This study analyzes the use of pH-responsive nanogels, polymer nanoparticles that can expand and shrink in response to pH-changes, as a drug delivery device. These nanogels have the potential to specifically target diseases characterized by a lower pH environment, such as cancer and kidney disease, by releasing drugs in a low pH and withholding drugs inside in a high pH. Poly(NIPAm-co-DEAEM) nanogels were synthesized using dispersion polymerization of N-isopropylacrylamide and 2-(Diethylamino)ethyl methacrylate. Optimization of nanogel response was done through modification of surfactant (SDS) and crosslinker concentrations. The optimal nanogel solution was chosen on the criteria that nanogel expansion occurred at pH < 6.0-7.0 and shrinkage occurred at pH > 6.0-7.0. Absorbance versus pH data on the optimal nanogel supported nanogel expansion from pH 1.0-6.0 and shrinkage from pH 6.0-9.0 (R2 = 0.95). Drug loading into the optimal nanogel was modeled using cyclophosphamide and showed successful retention of cyclophosphamide with positive colorimetric observations. The control (non-loaded) nanogels were biocompatible with neurons with an 90.34% viability. Cyclophosphamide was shown to be cytotoxic with a 28.75% viability ($p<0.01$). Cyclophosphamide loaded nanogels had an insignificant 88.55% viability compared to the control ($p>0.01$) which shows that cyclophosphamide was strongly withheld in the nanogel, reducing any neuronal cytotoxic effect. The data supports the effective use of Poly(NIPAm-co-DEAEM) nanogels for targeted drug delivery by effectively withholding cytotoxic drugs in higher pH media common of benign cells.

Table 1: Matrix of synthesis name and the corresponding reactant mass or volume

Synthesis	NIPAm (g)	DEAEM (mL)	SDS (g)	APS (g)	MBA (g)
A	1.004	1.000	0.536	0.300	0.100
B	1.052	1.000	0.530	0.317	0.020
C	1.030	1.000	0.542	0.304	0.028
D	1.024	1.000	0.219	0.247	0.023
E	1.090	1.000	0.469	0.318	0.023
B-2	1.001	1.000	0.531	0.343	0.020

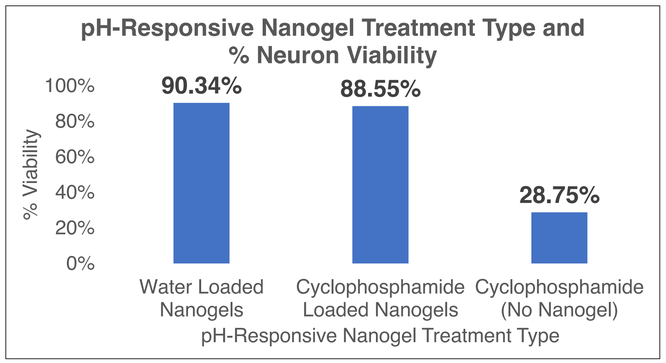


Figure 1: This graph depicts the cell viabilities for each nanogel treatment and cyclophosphamide treatment calculated from a trypan blue & hemocytometer test.

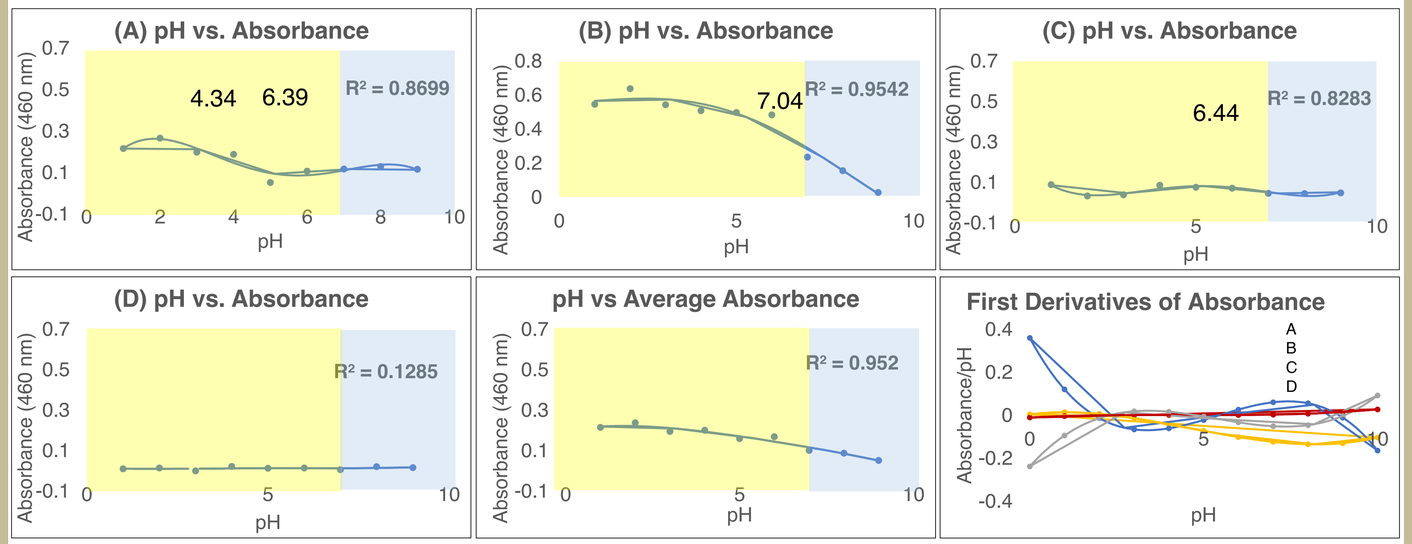


Figure 2: These line graph depict absorbance vs. pH for each nanogel synthesis. This shows the magnitude of nanogel expansion as high absorbance equates to high nanogel radius. The yellow regions depict pH < 7 and purple regions depict pH > 7. The black dashed lines indicate the central point of nanogel pH – response where the nanogels shift from expansion to shrinkage (vice versa). Synthesis D does not have a central point. The lower right graph depicts the rate of change of absorbance for each nanogels.

Results

Absorbance correlates to the degree of nanogel expansion as the larger the nanogel, the more light is absorbed and the smaller the nanogel, the less light is absorbed.

Synthesis A:
The nanogel solution was polydisperse and the nanogels were slightly aggregated.

Synthesis B:
Produced promising absorbance versus pH data as it had **significantly higher absorbance values in the more acidic pH range of 1 - 7.04 compared to the low absorbance values in the more basic pH range of 7.04 - 9.0.** Indicated that the **nanogels successfully expanded** to a larger particle size at a lower pH and shrunk to a smaller particle size at a higher pH.

Synthesis C and D:
Nanogels had trivial changes in absorbance.

Biocompatibility Testing:
The p-value for the water loaded nanogel (control) and cyclophosphamide is 0.00039, which supports our claim that the **cyclophosphamide is cytotoxic to healthy neuron cells**. The p-value for the control and the cyclophosphamide loaded nanogel is 0.225, which supports our claim that **the cyclophosphamide loaded nanogel does not impact the health of neurons.**

Conclusions

Synthesis B produced nanogels that fit the pH-responsive criteria as the nanogel had a pH-response within the accepted range 6.5 - 7.1 with expansion below the **central pH of 7.04**. The **hypothesis for the cell biocompatibility testing was supported** as the cyclophosphamide loaded nanogels had little effect on the neuron's health while the cyclophosphamide itself had a large effect. **The effective pH-response of the nanogels from 6.5 - 7.1 supported the potential release mechanisms of drugs from inside the nanogel in acidic media.**

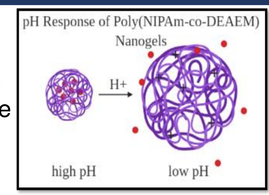


Figure 3: Mechanisms of expansion/shrinkage of the nanogel and its drug release.

Acknowledgements

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