Simultaneous Pharmaceutical Encasement in PNIPAm Hydrogels

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Background

Hydrogels are a network of polymeric chains that are extensively swollen with water, and which have become an immense area of interest in pharmaceutics (drug delivery systems research). We have focused on hydrogel delivery systems for drugs--particularly PNIPAm hydrogels. These hydrogels are unique because they possess a lower critical solution temperature (LCST)--the critical temperature below which components of a mixture become miscible at all compositions (Atkins 2006, White 1999). The pure LCST has been reported around 33°C (slightly under normal body temperature) (Okano et al., 1990). The LCST of PNIPAm-based hydrogels allows them to contract and release the stored water and aqueous components once the LCST temperature is reached.

PNIPAm-based hydrogels are prepared with a monomer and cross-linking agent as the primary components of the hydrogel. The monomer is N-isopropylacrylamide (NIPAM) and the cross-linking agent is N,N-methylenebisacrylamide (MBAM). The components are dissolved in water and then an aqueous solution of ammonium persulfate (a radical initiator) and N,N,N,N-tetramethylethylenediamine (TEMED)--a radical initiation enhancer--are used when making the polymer below the LCST (Zhang et al).

Studies by Massingill et al. demonstrated that once the bulk processing of these hydrogel complexes is understood, then it becomes possible to initiate these reactions in a microfluidic environment—a process completed in a special reaction and extrusion instrument where these polymers can be formed in very small spaces under specific temperature and pressure conditions to yield polymer-based nanoparticles containing drug molecules (Massingill et al, 2008).

The project communicated here has been aimed toward understanding the functionality and feasibilities of the bulk process, so that adjustments can be made in the microfluidic reaction chamber to prevent fouling of the instrument (e.g. which drugs work with in-situ incorporation (incorporation as the gel forms) as opposed to diffusional methods).

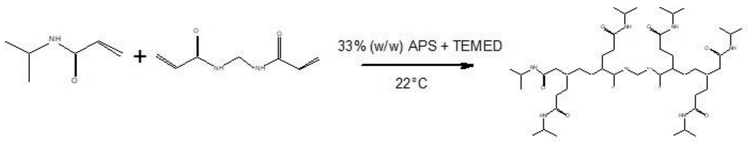
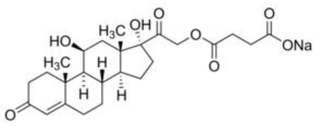
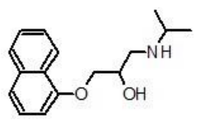


Figure 1 - General Reaction Scheme for PNIPAm Hydrogel
ChemSketch by ACD Labs - Casey J. McCormick, B.Sc.

The above figure is a representative drawing of the structure of a PNIPAm hydrogel, but is representative of only one small segment of the gel matrix. All bonds are not shown as the structure is vast and complicated.



Hydrocortisone Hemisuccinate (HCHS-21)



Propranolol (PRPL)

Figure 2 - Drugs Tested in these Studies
ChemSketch by ACD Labs - Casey J. McCormick, B.Sc.

Methods

Phase 1 (Control)	100 mg NIPAM 5 mg MBAM 950 µL ASTM Type I Water 20 µL TEMED 20 µL 33% (w/w) APS	APS solution was prepared in Type I Water prior to mixture. Monomer/Crosslinking Agent were dissolved in the water. TEMED was added, then APS. Left to react for 24 hours in fume hood in a closed container.	Gels washed over a 4-hour period with two 25 mL aliquots of Type I Water. Wash water was retained for UV-VIS analysis and gel was stored in refrigerator until LCST assays could be performed.
Phase 2 (Drug Gels)	200 mg NIPAM 10 mg MBAM 4.162 µmol Drug 1.90 mL ASTM Type I Water 40 µL TEMED 40 µL 33% (w/w) APS	Phase 1 (above procedure) is followed with drug dissolved alongside monomer and crosslinker prior to adding TEMED and APS. Amounts were increased to give the gel more volume.	Wash and storage procedures remained the same as above.
LCST Assays	Washed gels were placed in a 0.5-inch diameter test tube of 9-inch length, then covered with parafilm.	Gel test tubes were placed in a Fisher IsoTemp 300 water bath and maintained at 37°C for one hour. Water was extracted with a 9-inch Pasteur pipette.	The gels were bathed for one additional treatment at 60 minutes at the same temperature to retrieve any additional water. The water collected from each gel was stored in an individual sample vial for UV-VIS analysis.

Results

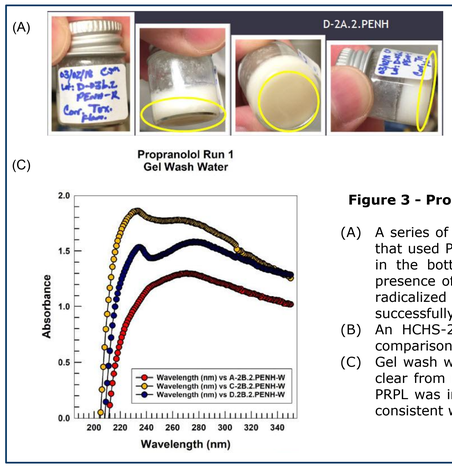
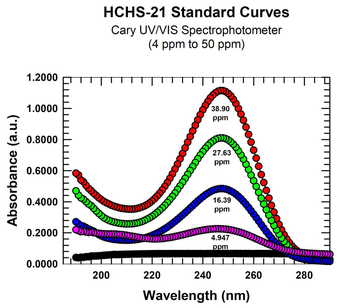
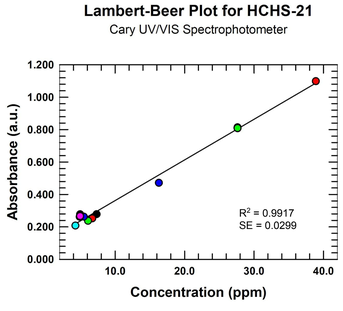


Figure 3 - Propranolol in-Situ Results

(A) A series of pictures reflective of all lot numbers from the Phase 2 set of reactions that used PRPL as the drug of choice. It was observed that a brown layer formed in the bottom of each gel upon addition of the radical initiator and with the presence of the PRPL. It is believed that the APS in conjunction with the TEMED radicalized the PRPL and caused an undesired reaction--making it fail to successfully incorporate in the desired manner.
(B) An HCHS-21 gel from the same set of experiments in Phase 2 is shown for comparison--it's clear there is not discolored bottom layer in the reaction vessel.
(C) Gel wash water from the propranolol gels was run on the Cary 300 UV-VIS. It is clear from relative minima (inflection point) in the graph that a larger amount of PRPL was in the wash water than the gel. The dip in this graph near 248 nm is consistent with the measured point for PRPL in UV-VIS studies.

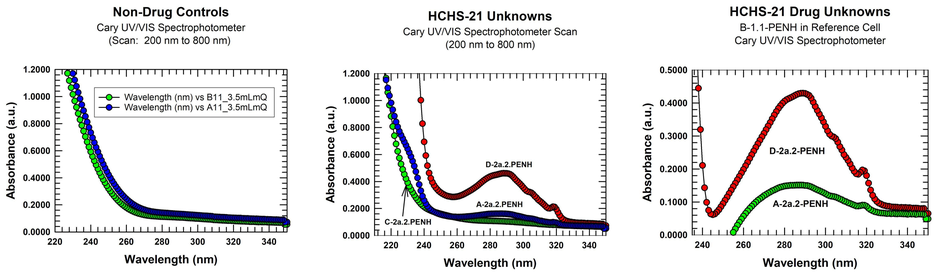




Left: Curve at left is a standard curve made for HCHS-21 in the concentration range of 5 ppm to 50 ppm. It was found that this range worked well for most substances the group was testing because other ranges were outside of the dynamic range of the instrument. Initially, all reactants were tested on a range of 1 ppm to 1000 ppm, and the majority of those lost linearity in the instrument. Some of the curves from those were distinctive enough that they could be ruled out by viewing the new outputs from gels.

Right: For each of the standard solutions of HCHS-21 there were 20 measurements taken as a rapid read at 247 nm--allowing for error propagation of absorbances. All measurements taken were plotted together to take a linear regression.

Bottom Left: 83.5 µL of the water extruded from control gels A-1.1-PENH and B-1.1-PENH were admixed with 3.42 mL of ASTM Type I Water and analyzed on the Cary 300 UV-VIS Spectrophotometer. **Bottom Middle**: Unknowns for HCHS-21 that extruded water were treated as with the samples in bottom left, and then analyzed in the similar fashion. **Bottom Right**: The gels from bottom middle had their samples re-analyzed using B-1.1-PENH as the blank solution to clarify common noise in the spectra between these samples.



Determining the Concentrations

$$A = \varepsilon b C + A_{bl}$$

$$A = (0.0251 \pm 0.0002)[ppm] + (0.1657 \pm 0.0061)$$

$$\varepsilon = 0.00251 \pm 0.00002 \frac{(a.u.)(kg)}{(mg)(mm)}$$

$$[ppm]_{drug} = \frac{A - 0.1657 \pm 0.0061}{0.0251 \pm 0.0002}$$

Conclusions

Lot Number	Corrected Absorbance (from baseline)	Concentration	% Weight HCHS-21
A-2a.2-PENH	0.1898 ± 0.0002	High: 49.98 ± 0.47 ppm Low: 30.25 ± 0.35 ppm	High: 0.004998 ± 0.000047 Low: 0.003025 ± 0.000035
D-2a.2-PENH	0.4772 ± 0.0001	High: 526.2 ± 4.2 ppm Low: 514.1 ± 4.1 ppm	High: 0.05262 ± 0.000420 Low: 0.05141 ± 0.000411

Percent Recovery

Based on the data above, it was determined that A-2a.2-PENH had a percent recovery of 3.3 % - 5.5 %; however, D-2a.2-PENH had a percent recovery of 56.4 % - 57.8 %. These percent recoveries are based on the theoretical percent compositions arrived at from the methods--not the specific measurements of each initial lot. They are meant to provide a rough estimate.

Brief Discussion

It is clear from reviewing the spectra that although HCHS-21 is indeed successfully incorporated into the hydrogel matrix in-situ, there is a chemical alteration to the drug which has taken place. The UV-VIS spectrum jumped wavelengths by a magnitude of ~40 nm. The shape of the curve was extremely similar to that expected for HCHS-21. While the amplitude should be trustable for concentration--it does not explain the change. It is believed that the succinate moiety of the 17□-hydroxyl group of the steroid ring has enough degrees of freedom that during the radical initiated reaction linkage occurs between the drug and freely formed oligomers of poly-NIPAM that may or may not also have cross-linkages with MBAM. These are soluble enough that they dissolve in water, and therefore are excreted due to binding the drug.

These results must be further elucidated through kinetics studies and spectroscopic analyses to determine the extent and nature of these changes and understand them. Alterations to the drug in this manner would likely render it unabsorbable and therefore ineffective.

Acknowledgements

We extend sincere thanks to:

1. Hajer Taheri for sharing the cross-linking agent, TEMED, and APS used for the project which is also something needed in her research.
2. Parker Lusk et al for the use of HPLC-grade water in our initial standard preparations.
3. Department of Chemical Engineering for financing the monomer and HCHS-21.
4. Dr. Andrew Callender, Mr. Gene Mullins, Dr. Jeff Boles, and the Department of Chemistry for allowing use of instruments in Foster Hall and troubleshooting some processes.

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