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Thermosensitive nanoparticles AO34 (Poly(n-vinylcaprolactam-co-methyl methacrylate) (P(NVCL-co-MMA)) loaded with Coumarin-6

Purpose

Determine the formation method of poorly drug soluble coumarin-6 loaded nanoparticles in P(NVCL-co-MMA) and potential for thermal sensitivity.

Background

It is well known that N-vinylcaprolactam (NVCL) based polymers display thermoreverse sensitivity [1] as well as copolymers of this with methyl methacrylate [2]. Unlike polymers with higher concentrations of NVCL, AO34 is not directly water soluble at cold temperature and as such can be used to make water-insoluble nanoparticles which also display a phase transition.

Method

Polymer-Drug Solution

The polymer was dissolved in ethanol (ETOH) at a concentration of 5% w/v. Separately 2.5% w/v Coumarin-6 C6 (hydrophobic dye) was dissolved in DCM and added as 0.1 ml of C6 solution into 1ml of polymer solution. This renders a solution such that the final ratio of dye /polymer was 5% w/w dye to polymer.

Nanoparticles

Subsequently nanoparticles were generated by adding 100 ul of the polymer-ethanol solution into 10ml of distilled water in a 15ml centrifuge tube agitated using an IKA Euro-Turrax T20b at 27000RPM equipped with a S25N-10G tip. After formation the nanoparticle suspension was filtered by passing through a 450nm PVDF syringe filter. In order to assay temperature sensitivity, the dispersion was split into 3 parts and placed (1ml each) into 3 separate semi-micro disposable cuvettes. One was placed in 37C incubator, another left at room temperature (20-25C) and a third placed in the refrigerator (5C) overnight.



Figure 1. Homogenizer for generating nanoparticles.

The next day the cuvettes were tested directly for absorbance at 469nm (maximum absorbance for C6) on a Genesys20 UV/Vis spectrophotometer relative to DI water blank.

Results

The change in absorption and nanoparticle behavior with temperature was visually obvious as shown in Figure 2. The nanoparticles held at 37C overnight aggregated due to high temperature and solid (orange in color due to coumarin-6) was observed on the bottom of the cuvette. Room temperature nanoparticles exhibited no change from day prior, while the particles stored at 5C displayed a higher degree of absorption due to preferred water solubility of the NVCL portion at lower temperature.

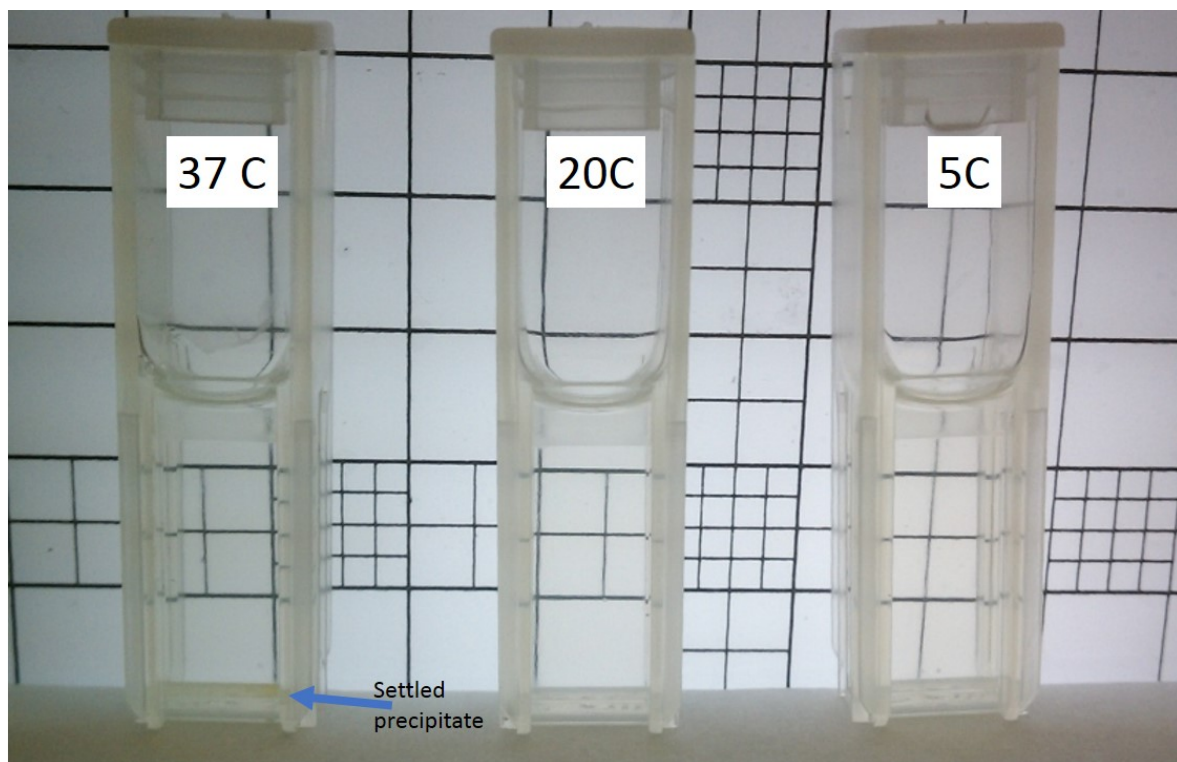


Figure 2. Comparison of AO34 nanoparticle suspensions at different temperatures.

The results of UV-Vis correlated to this as follows.

Condition	Absorbance 469nm
Overnight 37C	0.145
Overnight 5C	0.183
Overnight 20C	0.097

Based on measurement of the absorbance of the room-temperature stored nanoparticles the concentration of C6 in the solution was 3 ug/ml. Since coumarin-6 has extremely poor water solubility (<0.3 ug/ml) the presence of coumarin-6 in these indicates the formation of nanoparticles with hydrophobic regions capable of solubilizing C6 under these conditions 10 times its normal solubility in water.

Conclusion

Poly(N-vinylcaprolactam-co-methyl methacrylate) (90:10) (AO34) is an ethanol soluble polymer which can be utilized to form nanoparticle suspensions and these suspensions display a sensitivity towards temperature changes.

References

[1] Meeussen, Frank, et al. "Phase behaviour of poly (*N*-vinyl caprolactam) in water." *Polymer* 41.24 (2000): 8597-8602.

[2] Shah, Sunil, et al. "Synthesis and characterization of thermo-responsive copolymeric nanoparticles of poly (methyl methacrylate-co-*N*-vinylcaprolactam)." *European Polymer Journal* 46.5 (2010): 958-967.