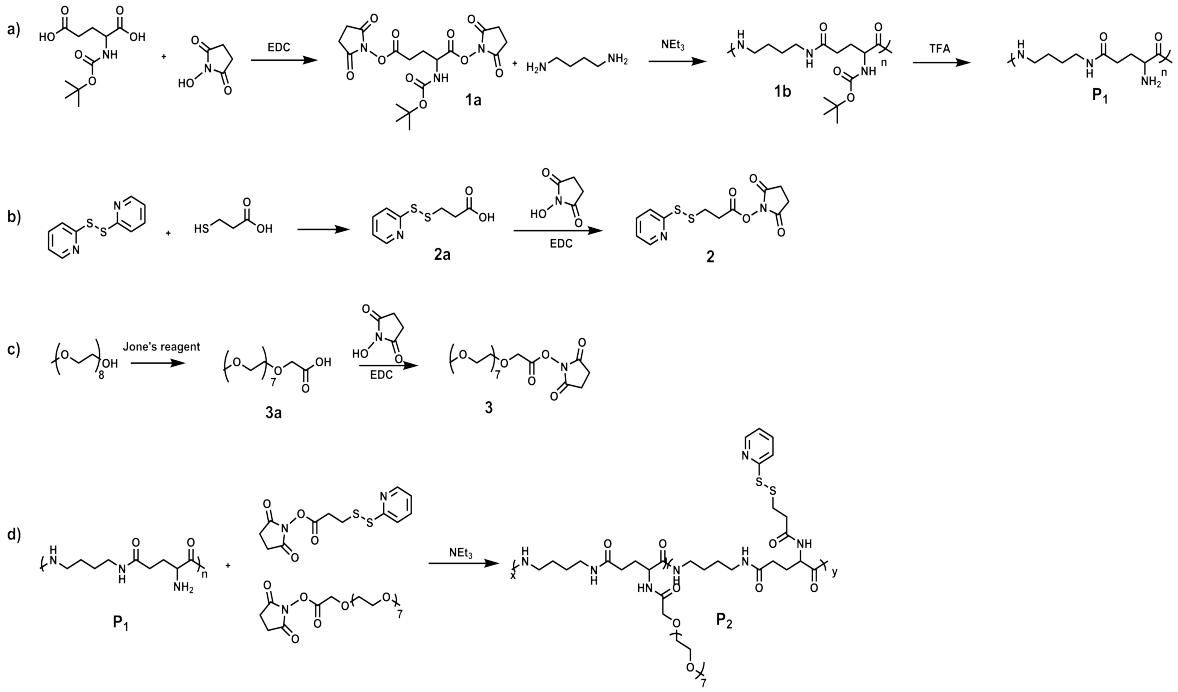
Supporting Information for:

Polyamide Nanogels from GRAS Components and Their Toxicity in Mouse Pre-implantation Embryos

Priyaa Prasad, [1] *Mijanur Rahaman Molla,* [1] *Wei Cui,* [2] *Mine Canakci,* [3] *Barbara Osborne,* [2,3] *Jesse Mager,* [2,3]* *and S. Thayumanavan,* [1,3]*

[1]Department of Chemistry, [2]Department of Veterinary and Animal Science and [3]Molecular and Cellular Biology Program, University of Massachusetts, Amherst, MA 01003 (USA).

Synthetic schemes:



Scheme S1. Synthesis of the a) polymer precursor P1, b) PDS-NHS ester 2, c) PEG-NHS ester 3, d) nanogel precursor polymer P2

Synthesis of *N*-boc-L-glutamic acid-succinimide ester (1a):[1] 500 mg (4.04 mmol) of *N*-boc-*L*-glutamic acid and 575 mg (9.99 mmol) of *N*-hydroxysuccinimide were added in a round bottom flask and dissolved in DMF followed by the addition of 1.917 g (10 mmol) of *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC.HCl). The reaction mixture was stirred overnight under argon atmosphere at ambient temperature. After concentration *in vacuo*, water was added to the mixture and the product was extracted with ethyl acetate (x3). The combined organic phase was washed with 0.1N HCl (x2), brine, saturated $NaHCO_3$ (x2) and dried over $MgSO_4$. Concentration under reduced pressure afforded the corresponding di-activated ester. The product was obtained as a white solid powder in 40% yield.

^{1}H-NMR (400 MHz, $(CD_3)_2CO$, TMS): δ (ppm) = 6.8 (m, 1H), 4.7 (m, 1H), 2.9 (m, 2H), 2.8 (s, 8H), 2.4 (m, 1H), 2.3 (m, 1H) 1.37 (s, 9H).

Synthesis of polymer 1b: 100 mg (0.226 mmol) of **1a** was added in a round bottom flask with DMF. While stirring, 52.5 μL (0.376 mmol) of triethyl amine was then added to the solution followed by 22.7 μL (0.226 mmol) of 1,4-diaminobutane. The reaction was stirred overnight and quenched by cooling it using an ice bath. The final product was purified by precipitating it in diethylether followed by dialysis using a cut-off membrane of M_n 3.5 kDa in methanol. The product was isolated as a sticky, pale white solid.

GPC (DMF, PMMA standard) Mn: 8.3 kDa; PD: 1.4. ^{1}H-NMR (400 MHz, CD_3OD): δ 3.98 (s, 1H), 3.17 (m, 4H), 2.25 (m, 2H), 2.01 (m, 1H), 1.85 (m, 1H), 1.47 (s, 4H), 1.46 (s, 9H).

Synthesis of polymer P1: In a typical procedure, polymer **1b** was taken in a round bottom flask followed by the addition of TFA/DCM mixture. TFA was taken in 5 times excess. The reaction mixture was left for stirring for 4 hours at ambient temperature. Excess solvent and TFA were removed by evaporation under reduced pressure.

^{1}H-NMR (400 MHz, CD_3OD): δ 3.88(m, 1H), 3.19 (m, 4H), 2.37 (m, 2H), 2.09 (m, 2H), 1.55(s, 4H).

Synthesis of pyridyldisulfide-propionic acid (PDS-acid) (2a): 1.87 g (8.50 mmol) of aldrithiol was added in a round bottom flask with 15 mL ethanol and kept for stirring in an ice bath. A catalytic amount of acetic acid was added to this solution. Then, 0.45 g (4.25 mmol) mercaptopropionic acid in 2.5 mL ethanol was then added to the reaction mixture dropwise. The reaction mixture was stirred overnight. The product was concentrated in vacuo and purified by column chromatography using silica gel as stationary phase and hexane / ethyl acetate (9:1) as eluent. The product was isolated as a light yellow solid in 80% yield.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.48 (m, 1H), 7.65 (m, 2H), 7.14 (m, 1H), 3.07 (t, 2H), 2.79 (t, 2H).

Synthesis of pyridyldisulfidepropionic acid-*N*-succinimidyl ester (PDS-NHS) (2): 620 mg (2.88 mmol) of PDS-acid **2a** and 365 mg (3.17 mmol) of *N*-hydroxysuccinimide were dissolved in dichloromethane (DCM) in a round bottom flask and cooled in an ice bath. 607 mg (3.17 mmol) of EDC.HCl in DCM was then added to the reaction mixture. This was stirred overnight. The product was concentrated in vacuo and redissolved in ethyl acetate and washed with water, $NaHCO_3$ and brine followed by drying it over $MgSO_4$. The product was concentrated in vacuo and purified by flash chromatography using silica gel as stationary phase and DCM/Methanol (98:2) as eluent. The product was separated as a light yellow solid in 37% yield.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.48 (m, 1H), 7.65 (m, 2H), 7.14 (m, 1H), 3.12 (m, 4H), 2.84 (s, 4H).

Synthesis of methoxypoly(ethyleneglycol) acid (3a): A reported procedure was followed for the synthesis[1] : ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.15 (s, 2H), 3.55-3.76 (m, 24H), 3.37 (s, 3H)

Synthesis of methoxypoly(ethylene glycol)succinimidyl ester (MePEG-NHS) (3): A reported procedure was followed for the synthesis[1]

^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.5 (s, 2H), 3.55-3.76 (m, 24H), 3.37 (s, 3H), 2.84 (s, 4H)

Synthesis of polymer P2: 75 mg (0.3483 mmol) of **P1** and 97 μL (0.6966 mmol) of triethyl amine was dissolved in DMF in a round bottom flask. 113 mg (0.2786 mmol) of **3** and 87 mg (0.2786 mmol) of **2** were then added to the reaction mixture and stirred overnight. The product was then dialysed in methanol with a 3.5 kDa cut-off membrane. GPC (DMF, PMMA standard) Mn: 9.0 kDa. PD: 1.6. ^{1}H-NMR (400 MHz, MeOD, TMS): δ (ppm) = 8.4 (m,1H), 8 (m,1H), 7.93 (m, 2H), 7.58 (m, 2H), 7.3 (m,1H), 7.28(m, 1H), 4.38 (m, 2H), 4.04 (m, 2H), 3.6-3.7 (m, 25H), 3.52-3.54 (m, 2H), 3.35 (s, 3H), 3.12-3.21 (m, 8H), 2.26 (m, 4H), 2.08 (m, 2H), 1.93 (m, 2H), 1.51 (s, 8H). The molar ratio between two blocks was determined by integrating the methoxy proton in the polyethylene glycol unit (3.3 ppm) and the aromatic proton in the pyridine (8.4 ppm).

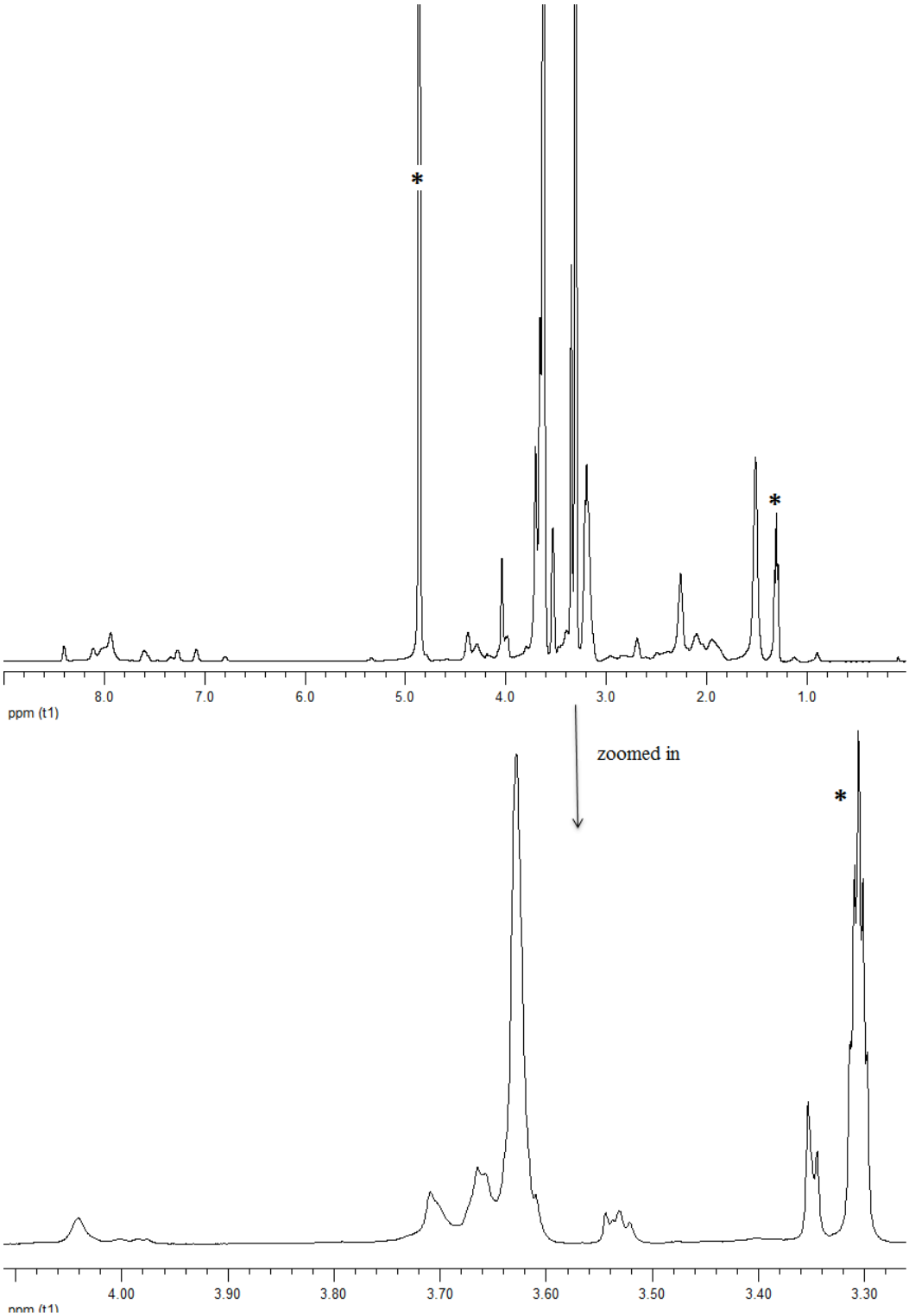


Figure S1. ^{1}H NMR of polymer **P2**

Procedure for nanogel formation: The precursor polymer was converted to the corresponding nanogel using a reported procedure using water as the self-assembling solvent.[2]

Crosslink density:

In order to determine the crosslinking density, UV-vis measurements were performed with samples of the solution reacted with DTT. Once this was measured, we calculated the amount of pyridothione based on its known molar extinction coefficient (8.08 x 10^3 M^{-1} cm^{-1} at 343 nm). The absorbance shown is normalized as the polymer backbone shows an absorbance at the same wavelength. The percentage of crosslinking was calculated by assuming that formation of a single, crosslinking disulfide bond would require cleavage of two PDS units and produce two pyridothione molecules.

Calculation of crosslink density:

- Sample concentration: 0.5 mg/mL of polymer.
- The molar ratio of each unit: PDS:PEG= x mol : y mol=30:70 (from NMR)
- PDS unit molecular weight = 414 g/mol, PEG unit molecular weight = 581 g/mol

So, x mol * 414 g/mol + y mol * 581 g/mol= 0.5 *10^{-3}g ; x mol = 30/70 y mol

Therefore, x mole (PDS) is 2.8 * 10^{-8} mol in this solution

NG1 Absorbance is 0.125 at 343 nm.

By Beer's law, A=εbc

0.125=8.08*10^3 M^{-1} cm^{-1} * 1 cm * c

c = 1.5 * 10^{-5} M

Therefore, 1 mL of resulting nanogel solution contains 1.5 *10^{-8} mol pyridothione (byproduct). It is 53 mol% of total PDS unit (2.8*10^{-8} mol). We assume that two pyridothione are from one disulfide formation and PDS unit is 30 mol% of total polymer.

Therefore, 53 % /2 *0.3 = 7.95 % crosslinking density. Therefore, the crosslink density of this nanogel is considered to be 8%.

NG2 Absorbance is 0.23 at 343 nm.

By Beer's law, A=εbc

$0.23= 8.08*10^{3}\ M^{-1}\ cm^{-1} * 1\ cm * c$

$c = 2.8 * 10^{-5}\ M$

Therefore, 1mL of resulting nanogel solution contains $2.8*10^{-8}$ mol pyridothione (byproduct). It is 100 mol% of total PDS unit ($2.8*10^{-8}$ mol). We assume that two pyridothione are from one disulfide formation and PDS unit is 30 mol% of total polymer. Therefore, 100 % /2 *0.3 = 15% crosslink density.

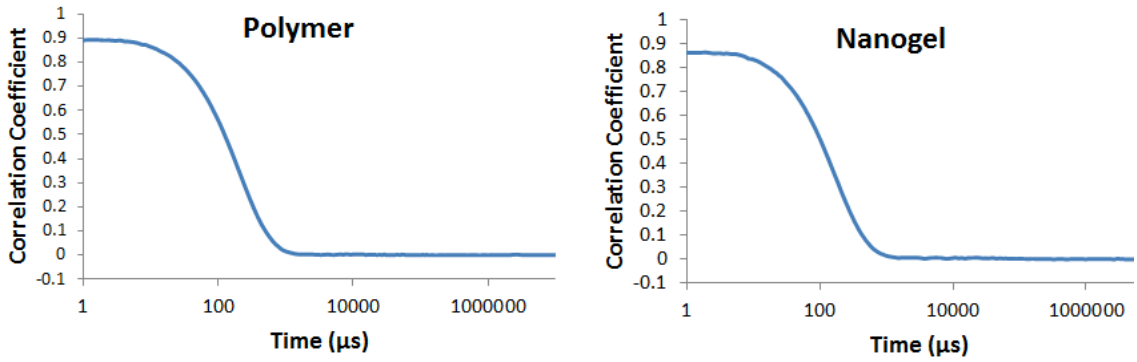


Figure S2. Autocorrelation function for the dynamic light scattering data shown in Figure 1 and 2 for the polymer and nanogel respectively

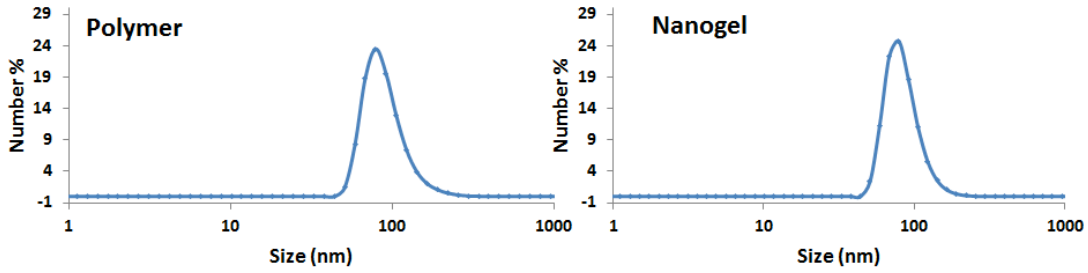


Figure S3. Size measurement of the polymer and nanogel by dynamic light scattering (DLS)

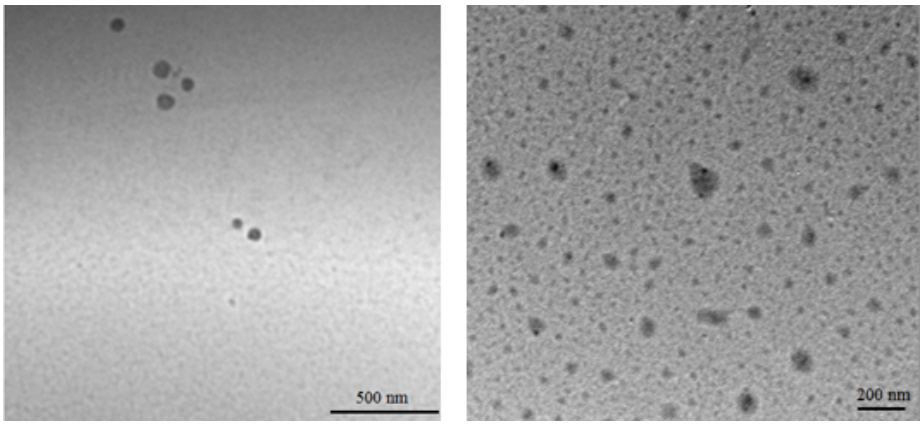


Figure S4. TEM images of *left*: polymer and *right*: nanogel

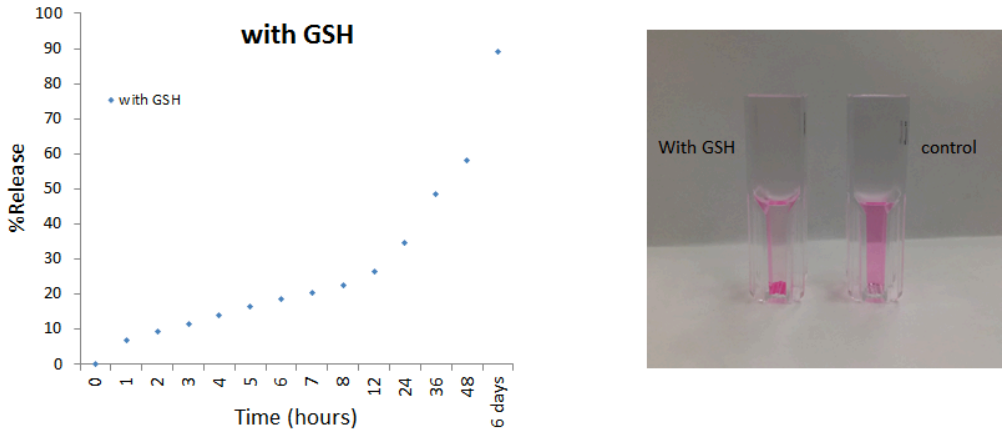


Figure S5. *Left*: % release of nanogel incubated with GSH for 6 days; *Right*: image of nanogel encapsulated with DiI incubated with GSH showing precipitate after 6 days.

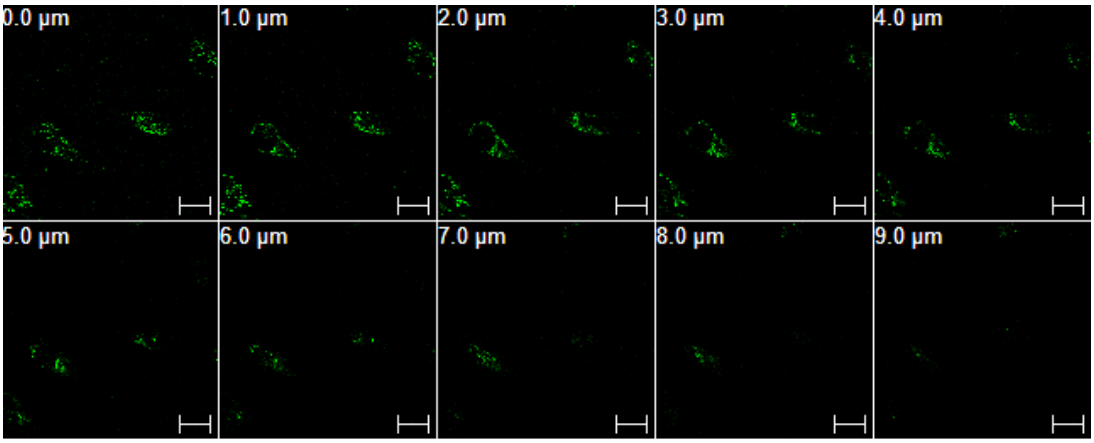


Figure S6. Confocal microscopy images of HeLa cells incubated with DiO encapsulated nanogels for 6 hours showing cellular uptake as seen by z-stack images with each slice at a depth of 1 µm (scale bar: 20 µm).

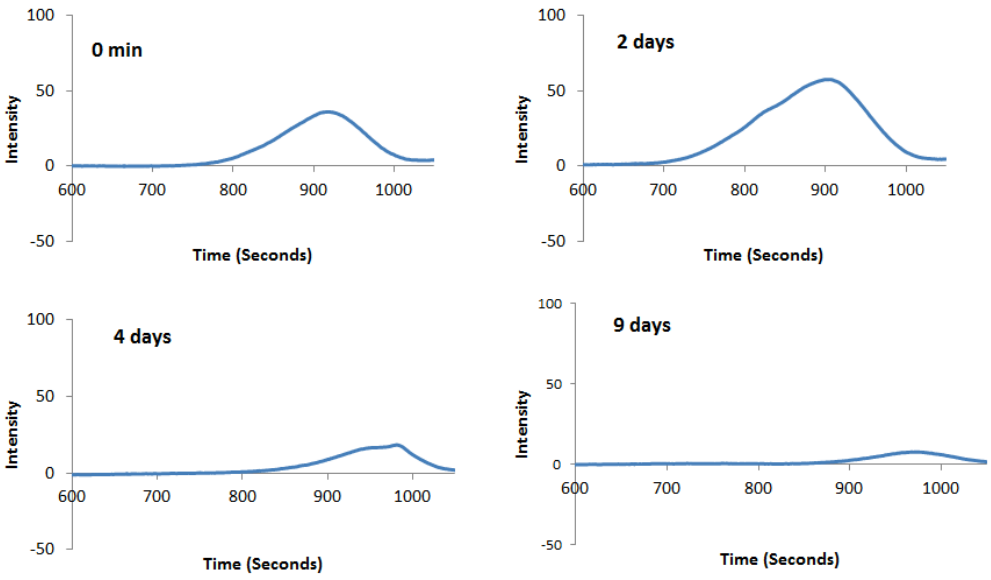


Figure S7. Nanogel degradation in serum over a period of 6 days as seen by GPC chromatogram in DMF by the gradual increase in elution time indicating decrease in molecular weight.

REFERENCES

1. Pilloni, M.; Nicolas, J.; Marsaud, V.; Bouchemal, K.; Fronfia, F.; Scano, A.; Ennas, G.; Dubernet, C. *Int. J. Pharm.* **2010**, *401*, 103–112.

2. Ryu, J.-H.; Jiwpanich, S.; Chacko, R.; Bickerton, S.; Babu, R. P.; Thayumanavan, S. *J. Am. Chem. Soc.* **2010**, *132*, 17227-17235.