Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Dynamic uptake and release from poly(methacryloyl hydrazide) microgel particles through reversible hydrazide-aldehyde chemistry

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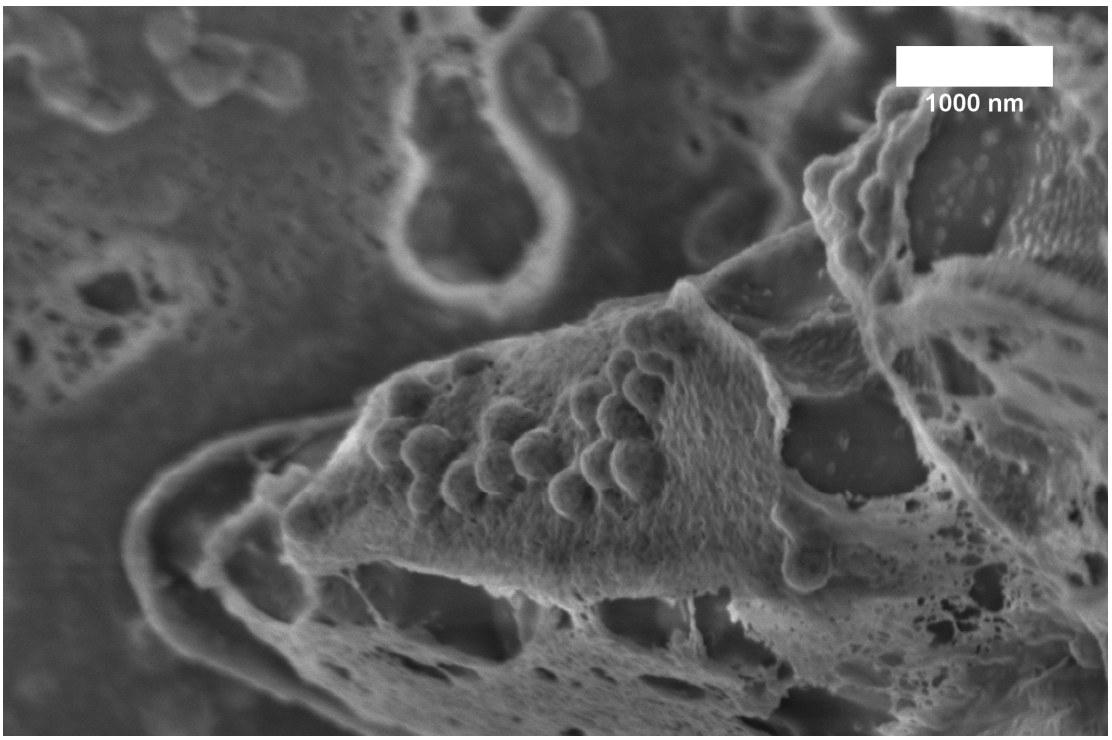


Figure S1 Cryo SEM image of microgel particles synthesized by dispersion polymerization.

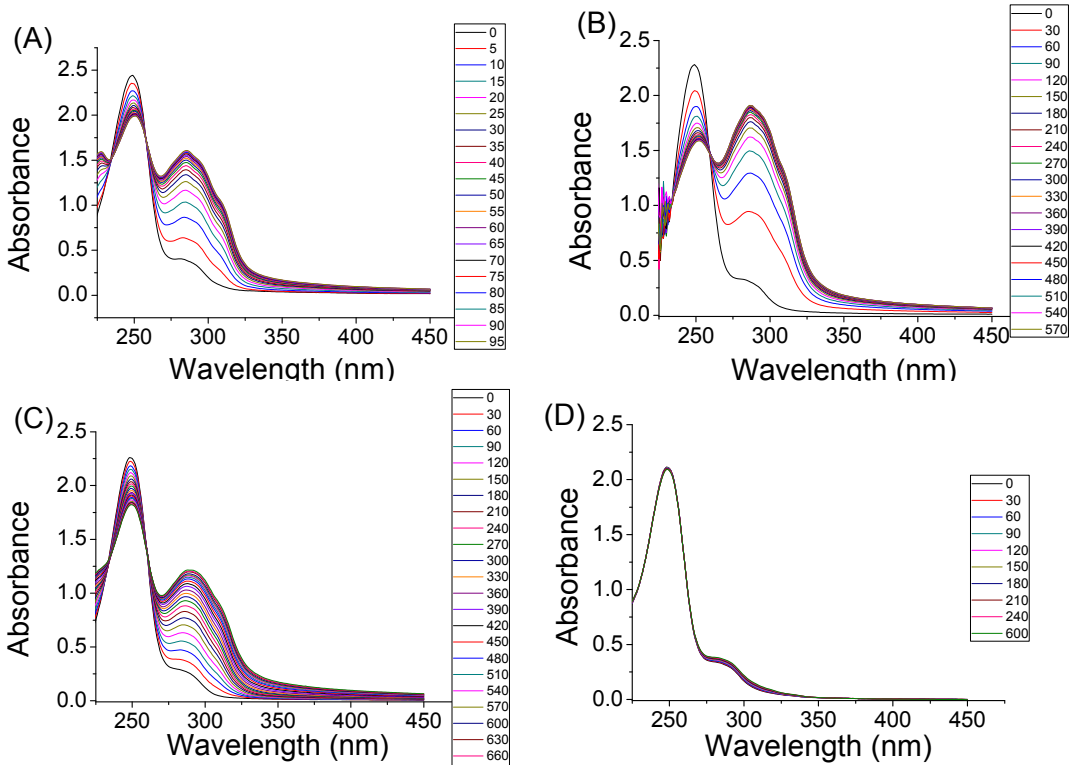


Figure S2 UV/visible spectra of equimolar amounts of methacryloyl hydrazide and benzaldehyde at (A) pH 1.89 (B) pH 3.86 (C) pH 5.01 (D) pH 7.03. The figure legends denote the corresponding time in minutes.

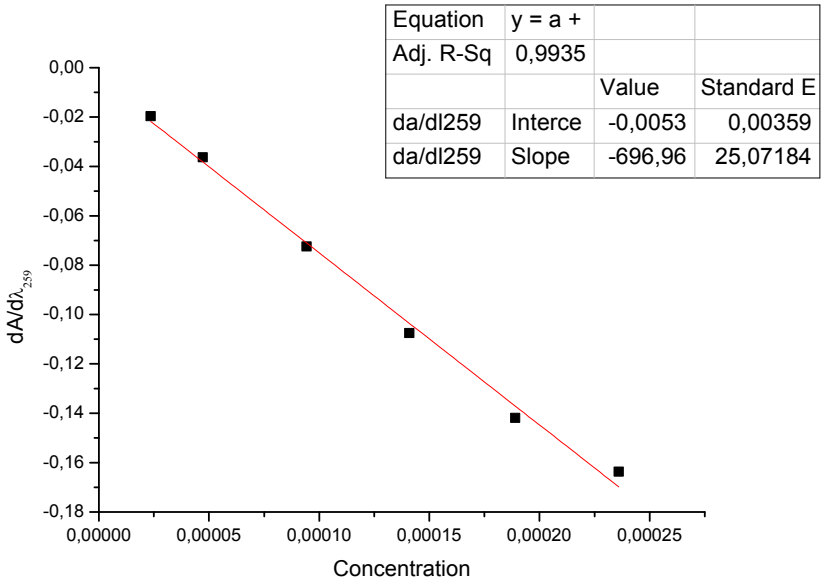
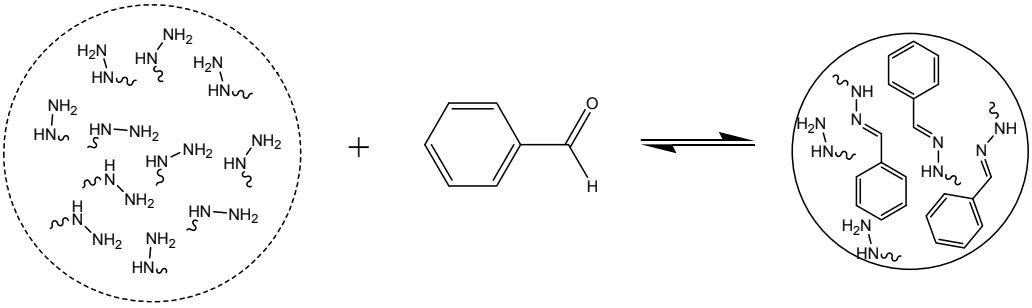


Figure S3 Calibration curve for first derivative photospectrophotometry (dA/dλ) versus concentration of benzaldehyde.

Kinetic equation for evaluating rate constants of formation and hydrolysis of hydrazones

The rate equation for the reversible second order reaction can be obtained analytically by following a similar method to that of Dirksen *et al.*[2]



The rate constant for hydrazone formation, k_1, and hydrolysis, k_2, can be calculated assuming a reversible reaction that follows second order kinetics. The rate equation for the change in concentration of benzaldehyde **2** over time is given by:

$$\frac{d[\mathbf{2}]}{dt} = -k_1[\mathbf{1}][\mathbf{2}] + k_2[\mathbf{3}] \qquad \textbf{Equation 1}$$

Where $[\mathbf{3}]$ is the concentration of hydrazone functional groups and $[\mathbf{1}]$ is the concentration of hydrazide functional groups. Both **3** and **1** are a function of the concentration of benzaldehyde **2** over time such that

$$[\mathbf{3}] = [\mathbf{2}]_\mathbf{0} - [\mathbf{2}] \qquad \textbf{Equation 2}$$

$$[\mathbf{1}] = [\mathbf{1}]_\mathbf{0} - [\mathbf{2}]_\mathbf{0} + [\mathbf{2}] \qquad \textbf{Equation 3}$$

Assuming that the initial concentration of [3] is 0 and where the subscript 0 indicates the concentration at time 0. Substituting this into equation 1 gives

$$\frac{d[\mathbf{2}]}{dt} = -k_1[\mathbf{2}]([\mathbf{1}]_\mathbf{0} - [\mathbf{2}]_\mathbf{0} + [\mathbf{2}]) + k_2([\mathbf{2}]_\mathbf{0} - [\mathbf{2}]) \qquad \textbf{Equation 4}$$

Which can be rearranged to give

$$\frac{d[\mathbf{2}]}{dt} = -k_1[\mathbf{2}]^2 - (k_1[\mathbf{1}]_0 - k_1[\mathbf{2}]_0 + k_2)[\mathbf{2}] + k_2[\mathbf{2}]_\mathbf{0} \qquad \textbf{Equation 5}$$

The right hand side is a quadratic equation that has two roots given by

$$a_+ = \frac{-(k_1[\mathbf{1}]_0 - k_1[\mathbf{2}]_0 + k_2) + \sqrt{(k_1[\mathbf{1}]_0 - k_1[\mathbf{2}]_0 + k_2)^2 - 4(-k_1)(k_2[\mathbf{2}]_\mathbf{0})}}{2k_1} \quad \textbf{Equation 6}$$

$$a_- = \frac{-(k_1[\mathbf{1}]_0 - k_1[\mathbf{2}]_0 + k_2) - \sqrt{(k_1[\mathbf{1}]_0 - k_1[\mathbf{2}]_0 + k_2)^2 - 4(-k_1)(k_2[\mathbf{2}]_\mathbf{0})}}{2k_1} \quad \textbf{Equation 7}$$

Because k_1, k_2, $[\mathbf{2}]_0$ and $[\mathbf{1}]_0$ are real both solutions must also be real. Note that $a_+ < 0$ and $a_+ > 0$. By the method of separation of variables for equation 4 we can obtain

$$\int_{[2]_0}^{[2]} \frac{1}{-k_1[\mathbf{2}]^2 - (k_1[\mathbf{1}]_0 - k_1[\mathbf{2}]_0 + k_2)[\mathbf{2}] + k_2[\mathbf{2}]_\mathbf{0}} d[\mathbf{2}] = \int_0^{\mathrm{t}} 1 d\mathrm{t} = t \quad \textbf{Equation 8}$$

Multiplying by $-k_1$ on both sides gives

$$\int_{[2]_0}^{[2]} \frac{1}{[\mathbf{2}]^2 - \frac{(k_1[\mathbf{1}]_0 - k_1[\mathbf{2}]_0 + k_2)}{-k_1}[\mathbf{2}] - \frac{k_2}{k_1}[\mathbf{2}]_\mathbf{0}} d[\mathbf{2}] = -k_1 t \quad \textbf{Equation 9}$$

This can now be factorized to give

$$\int_{[2]_0}^{[2]} \frac{1}{([\mathbf{2}] - a_+)([\mathbf{2}] - a_-)} d[\mathbf{2}] = -k_1 t \quad \textbf{Equation 10}$$

Since

$$\frac{1}{[\mathbf{2}] - a_+} - \frac{1}{[\mathbf{2}] - a_-} = \frac{([\mathbf{2}] - a_-) - ([\mathbf{2}] - a_+)}{([\mathbf{2}] - a_+)([\mathbf{2}] - a_-)} = \frac{(a_+ - a_-)}{([\mathbf{2}] - a_+)([\mathbf{2}] - a_-)} \quad \textbf{Equation 11}$$

Equation 10 can be rearranged to give

$$\frac{1}{(a_+ - a_-)} \int_{[2]_0}^{[2]} \frac{1}{[\mathbf{2}] - a_+} - \frac{1}{[\mathbf{2}] - a_-} d[\mathbf{2}] = -k_1 t \quad \textbf{Equation 12}$$

Which can be integrated to give

$$\frac{1}{(a_+ - a_-)} (\ln|[\mathbf{2}] - a_+| - \ln|[\mathbf{2}]_0 - a_+| - \ln|[\mathbf{2}] - a_-| + \ln|[\mathbf{2}]_0 - a_-|) = -k_1 t \quad \textbf{Equation 13}$$

Taking the exponential gives

$$\frac{|[\mathbf{2}] - a_+||[\mathbf{2}]_0 - a_-|}{|[\mathbf{2}] - a_-||[\mathbf{2}]_0 - a_+|} = \mathrm{e}^{-k_1(a_+ - a_-)t} \quad \textbf{Equation 14}$$

There are three separate cases that are now possible in order to solve the equation. $[\mathbf{2}]_0 > a_+, a_+ > [\mathbf{2}]_0 > a_-$ and $[\mathbf{2}]_0 < a_-$. Since a_- is negative then the last case can be dismissed as $[\mathbf{2}]_0 > 0$. The first case is of prime interest here since the

concentration of aldehyde is decreasing over time. In this case the absolute signs in equation 6 can be dropped and the solution is given as

$$[\mathbf{2}] = \frac{a_+([\mathbf{2}]_0 - a_-) - a_-([\mathbf{2}]_0 - a_+)e^{-k_1(a_+ - a_-)t}}{([\mathbf{2}]_0 - a_-) - ([\mathbf{2}]_0 - a_+)e^{-k_1(a_+ - a_-)t}} \qquad \textbf{Equation 15}$$

Hence at infinite time the equilibrium concentration of [**2**] is given by a_+. The rate constants can be obtained by a fit of the change in with time of [**2**] by standard non-linear methods.

The second case where the concentration of aldehyde [**2**] is lower than the equilibrium value is given by

$$[\mathbf{2}] = \frac{a_+([\mathbf{2}]_0 - a_-) + a_-(a_+ - [\mathbf{2}]_0)e^{-k_1(a_+ - a_-)t}}{([\mathbf{2}]_0 - a_-) + (a_+ - [\mathbf{2}]_0)e^{-k_1(a_+ - a_-)t}} \qquad \textbf{Equation 16}$$

Release of benzaldehyde from aqueous solution in the presence of microgels

The theoretical release profile can be obtained by solving the following differential equations.

$$\frac{d[A]_{(aq)}}{dt} = k_2[Hn] - \left(k_1[A]_{(aq)}[Hd] + k_{evap}[A]_{(aq)}\right)$$

$$-\frac{d[Hn]}{dt} = \frac{d[Hd]}{dt} = k_2[Hn] - k_1[A]_{(aq)}[Hd]$$

$[A]_{aq}$ is the aqueous phase concentration of aldehyde, [Hd] is the concentration of hydrazide functional groups and [Hn] is the concentration of hydrazone functional groups in the system. In order to relate to experimental results the initial concentrations of species were calculated assuming the system was at equilibrium from the experimental concentrations used and the equilibrium concentration of the aldehyde from Equation 16 with t=∞. The rate constants k_1 and k_2 are obtained from

UV/vis experiments and the value for k_{evap} was obtained from the experimental data obtained in the absence of hydrazide particles such that the concentration of aldehyde with time is given by

$$[A] = [A]_0 e^{-k_{evap}t}$$

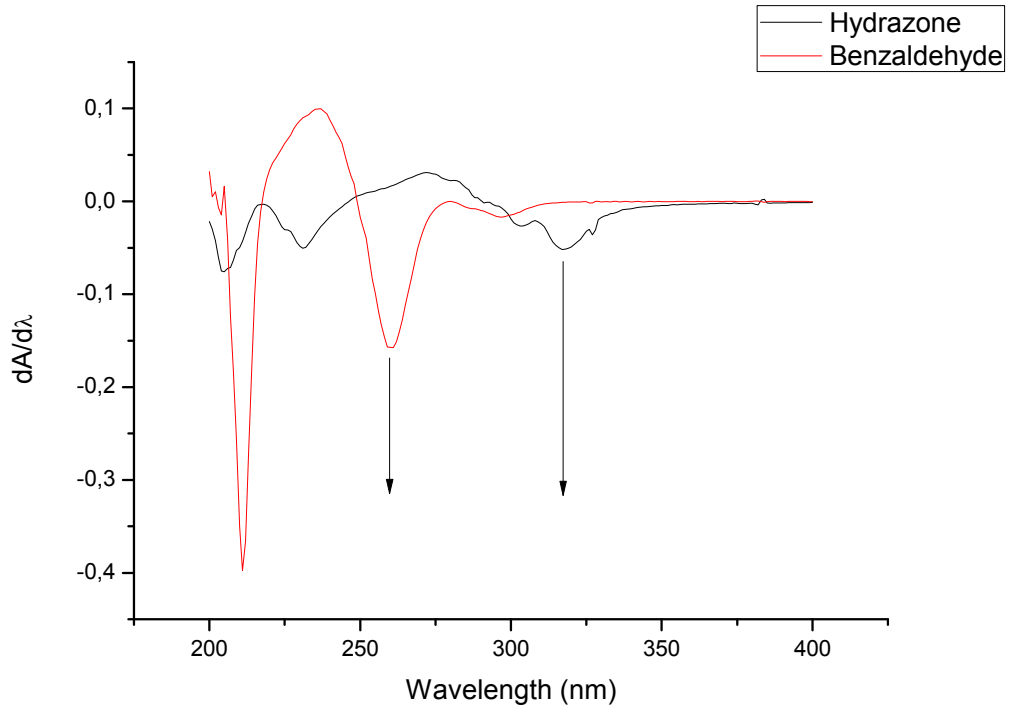


Figure S4 Derivative of absorption versus wavelength for benzaldehyde and conjugated benzaldehyde to microgel particles (hydrazone). The arrows show the points from which the values of dA/dλ were used for quantitative concentration measurements.

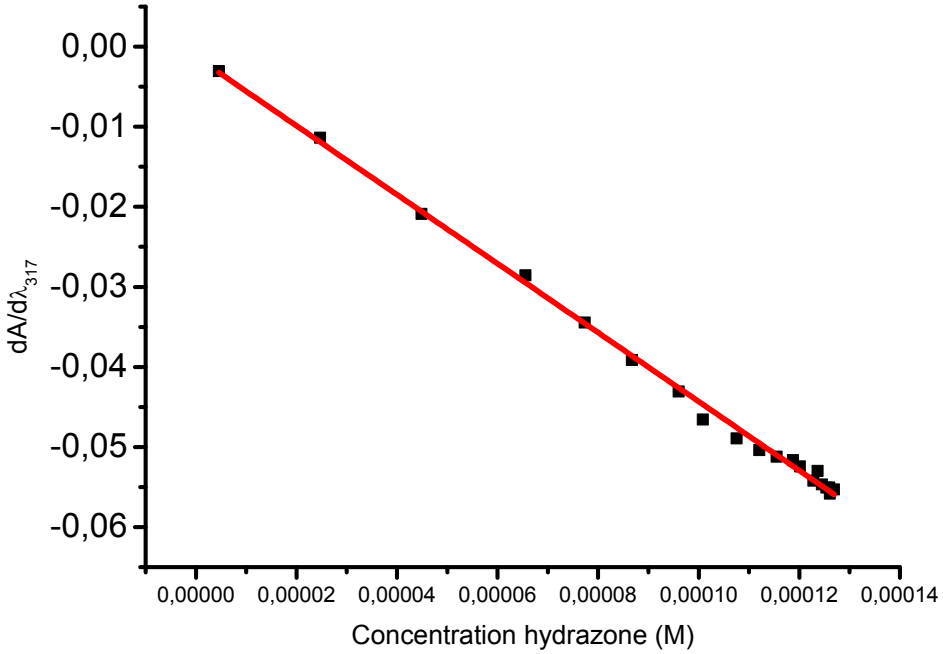


Figure S5 Calibration curve for concentration of hydrazone based on first derivative spectrophotometry at a wavelength of 317 nm. The points were obtained from the UV/vis spectra of mixtures of microgels and known concentrations of benzaldehyde. The starting benzaldehyde (known) and final benzaldehyde concentrations were calculated from the calibration curve in figure S3 and the hydrazone concentration was calculated from the difference of the two.

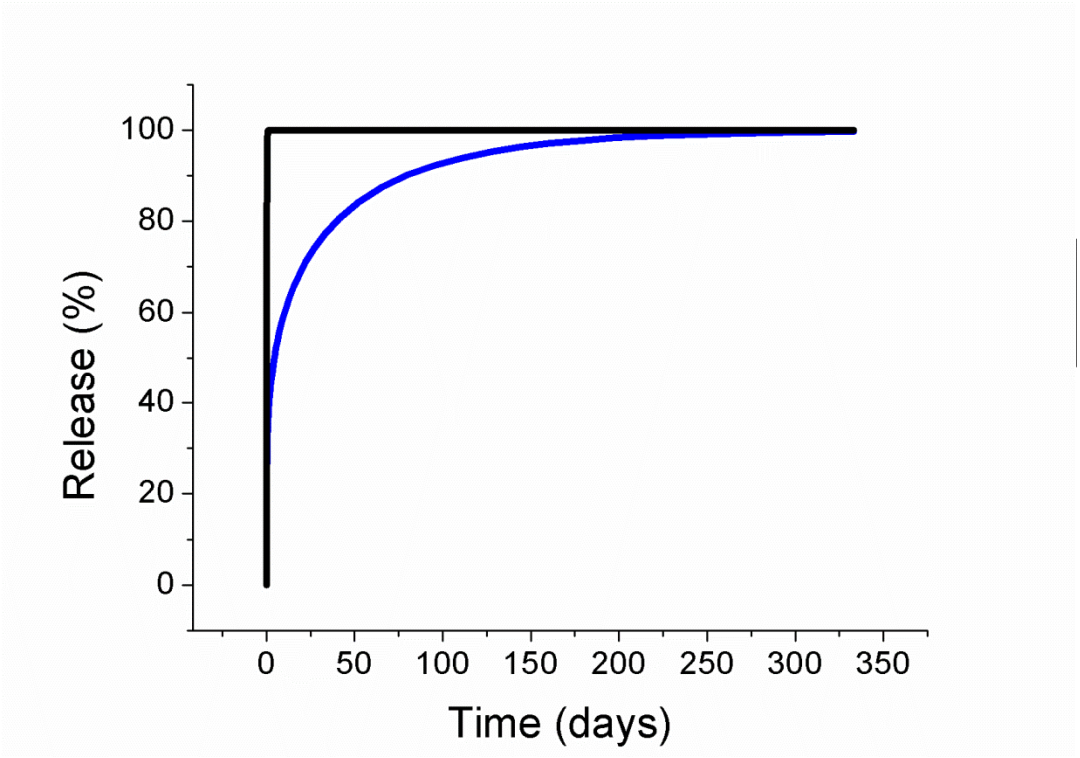


Figure S6 Theoretical release profiles obtained by solution of differential equations using rate constants $k_{evap} = 8.33 \times 10^{-5}$ s^{-1} (obtained from fit of data to exponential curve in absence of hydrazide

containing microgels), and the rate constants obtained from UV/vis experiments for hydrazide/aldehyde binding kinetics in the absence (black line) and presence(blue line) of microgel particles at equimolar ratio to initial aldehyde concentration and at pH 1.86.

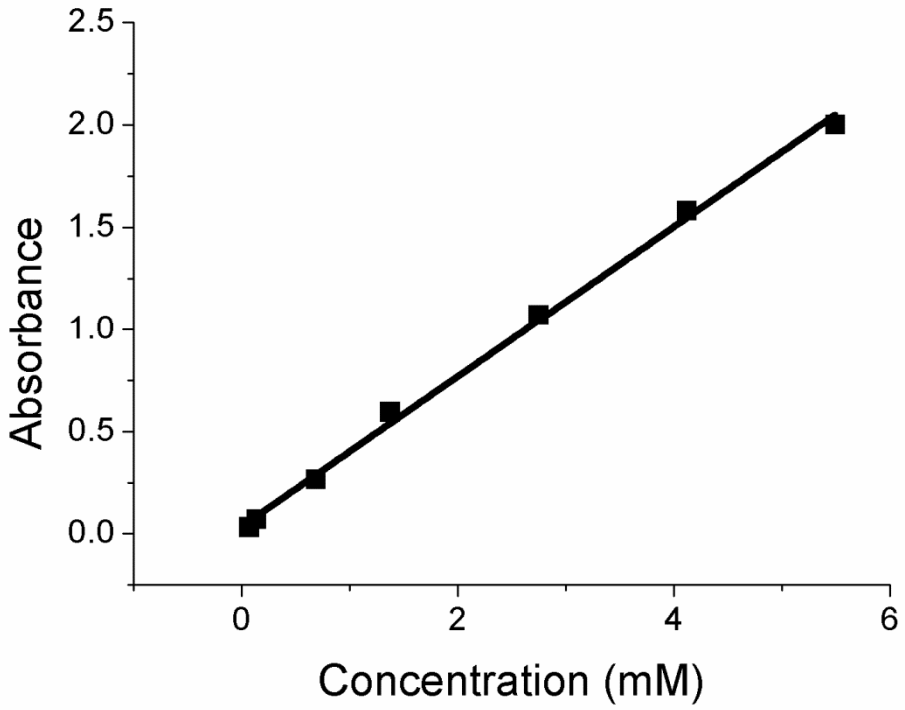


Figure S7 Calibration curve for colorimetric determination of streptomycin at wavelength of 540 nm following the assay of Leghorn *et al.*[1]

Release of streptomycin sulphate from aqueous solution in the presence of microgels

The theoretical release profile can be obtained by solving the following differential equations.

$$\frac{d[A]_{(out)}}{dt} = k_{tr}\left([A]_{(in)} - [A]_{(out)}\right)$$

$$\frac{d[A]_{(in)}}{dt} = k_2[Hn] - \left(k_1[A]_{(aq)}[Hd] + k_{tr}\left([A]_{(in)} - [A]_{(out)}\right)\right)$$

$$-\frac{d[Hn]}{dt} = \frac{d[Hd]}{dt} = \frac{d[A]_{(in)}}{dt} = k_2[Hn] - k_1[A]_{(aq)}[Hd]$$

$[A]_{in}$ and $[A]_{out}$ are the aqueous phase concentrations of aldehyde inside and outside the dialysis membrane respectively, [Hd] is the concentration of hydrazide functional

groups within the dialysis membrane and [Hn] is the concentration of hydrazone functional groups. The rate of transport of the aldehyde across the dialysis membrane was described by the rate constant, k_{tr}, and the difference in concentration.

In order to relate to experimental results the initial concentrations of species were calculated assuming the system was at equilibrium from the experimental concentrations used and the equilibrium concentration of the aldehyde from Equation 16 with t=∞. In accordance with the experimental system the differential equations were solved in twenty minute intervals and the concentration of the aldehyde was reduced in accordance with the experimental protocol before continuing the simulation.

The rate constant for k_{tr} was obtained by a parameter estimation based on the solution to the above in the absence of any hydrazide. The equilibrium constants were calculated from the known concentration of aldehyde added and the initial measurement of aldehyde concentration at time 0 that represents the equilibrium concentration according to

$$K = \frac{[Hn]_{eq}}{[A]_{eq}[Hd]_{eq}} = \frac{[A]_0 - [A]_{eq}}{[A]_{eq}([Hd]_0 - ([A]_0 - [A]_{eq}))}$$

The individual rate constant k_1 was obtained by parameter estimation based on the minimization of the sum of squares of the difference between experimental and theoretical points.

In order to confirm that the experimental dilution method (that was used in order to obtain enough sample to accurately calculated the streptomycin concentration) accurately represents a similar process under continuous dilution a comparison between the two methods was conducted by solving the differential equations for a continually diluted system at an equivalent rate of dilution to that of above.

$$\frac{d[A]_{(out)}}{dt} = k_{tr}\left([A]_{(in)} - [A]_{(out)}\right) - \frac{[A]_{(out)}}{\mathrm{V}}\mathrm{Q}$$

$$\frac{d[A]_{(in)}}{dt} = k_2[Hn] - \left(k_1[A]_{(aq)}[Hd] + k_{tr}\left([A]_{(in)} - [A]_{(out)}\right)\right)$$

$$-\frac{d[Hn]}{dt} = \frac{d[Hd]}{dt} = \frac{d[A]_{(in)}}{dt} = k_2[Hn] - k_1[A]_{(aq)}[Hd]$$

Where Q is the volumetric flow rate and V is the volume of liquid outside of the dialysis membrane. **Figure S8** shows that whether continuous or batch based dilution are used a near identical release profile is obtained.

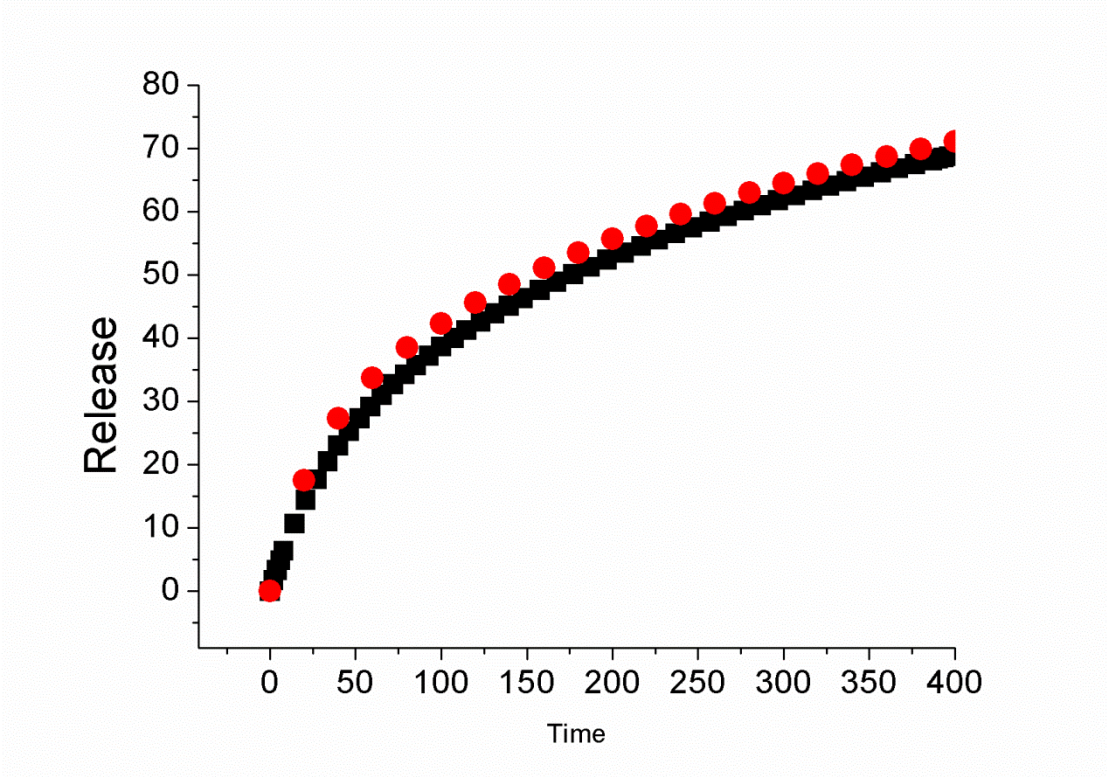


Figure S8 Difference in release profile depending on method of dilution for streptomycin experiments either by venting (red circles, as performed experiments) or by continuous dilution of species (black squares). It can be observed that a negligible difference between the two is seen.

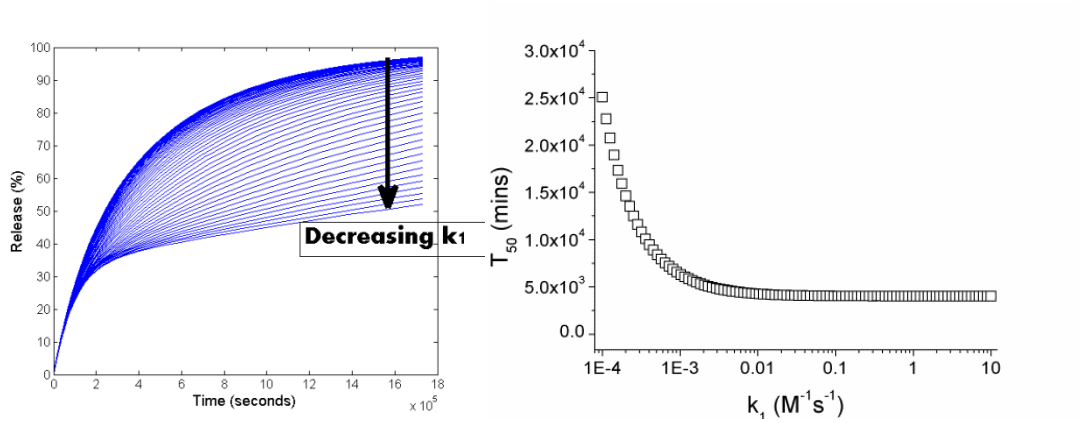


Figure S9 Effect of rate of reaction on release of active compound. K=500 M^{-1}, k_3 = 1 × 10^{-5} s^{-1}, $[1]_0$=$[2]_0$=0.01 M. (Left) Variation of release with time upon varying k_1 by 5 orders of magnitude. (Right) Effect of k_1 on time T_{50} the time to release of 50% of active compound.

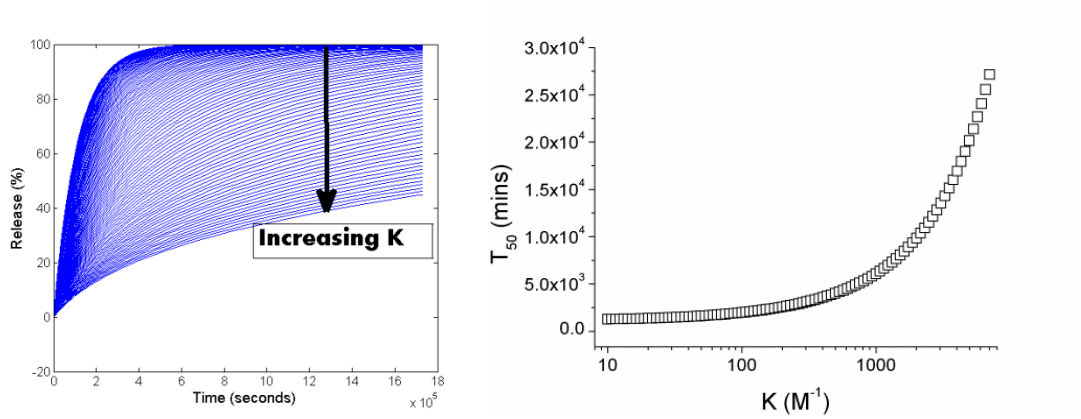


Figure S10 Effect of equilibrium constant on release of active compound. k_1=1 $M^{-1}s^{-1}$, k_3 = 1 × 10^{-5} s^{-1}, $[1]_0$=$[2]_0$=0.01 M. (Left) Variation of release with time upon varying *K* by 3 orders of magnitude. (Right) Effect of *K*on time T_{50} the time to release of 50% of active compound.

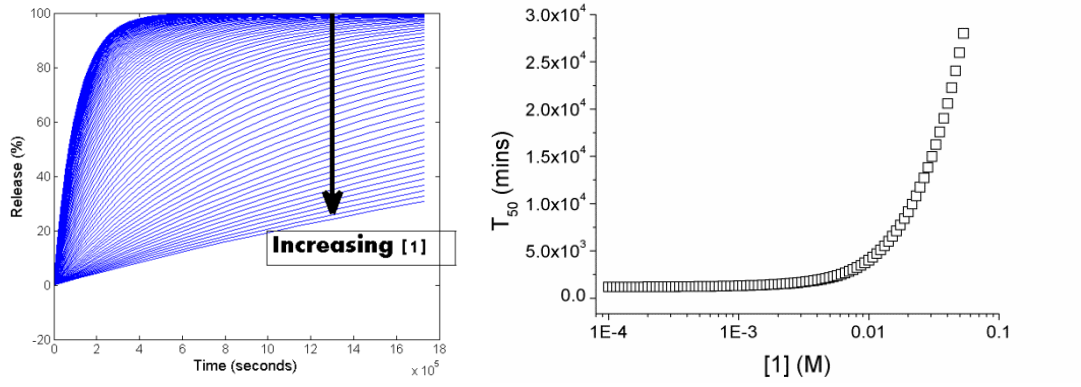


Figure S11 Effect of concentration of polymer bound reactant on release of active compound. k_1=1 $M^{-1}s^{-1}$, K=500 M^{-1}, k_3 = 1 × 10^{-5} s^{-1}, $[2]_0$=0.01 M. (Left) Variation of release with time upon varying [1] by 3 orders of magnitude. (Right) Effect of [1] on time T_{50} the time to release of 50% of active compound.

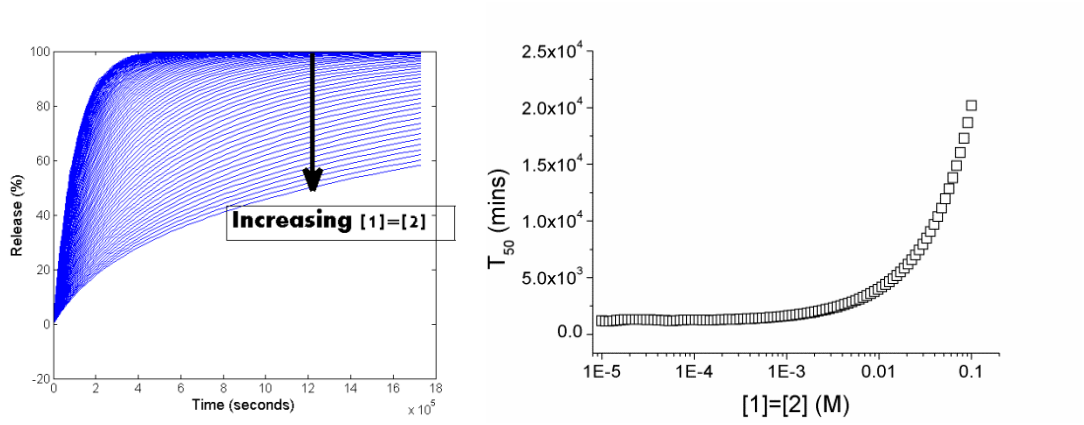


Figure S12 Effect of concentration of reactants on release of active compound. k_1=1 $M^{-1}s^{-1}$, K=500 M^{-1}, $k_3 = 1 \times 10^{-5}$ s^{-1}. (Left) Variation of release with time upon varying [1]=[2] by 4 orders of magnitude. (Right) Effect of [1]=[2] on time T_{50} the time to release of 50% of active compound.

References

1. G. E. Boxer, V. C. Jelinek, and P. M. Leghorn, *J. Biol. Chem.*, 1947, **169**, 153.

2. A. Dirksen, S. Dirksen, T. M. Hackeng, and P. E. Dawson, *J. Am. Chem. Soc.*, 2006, **128**, 15602–3.