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

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Nicholas Ballard[a] and Stefan A. F. Bon[b]*

The synthesis of poly(methacryloyl hydrazide) microgels via dispersion polymerization for the controlled release of carbonyl containing compounds is described. The kinetics of the dynamic reaction that occurs between the hydrazide containing colloidal particles and aldehyde compounds are explored for aqueous dispersions and it is shown that the aldehyde release profiles at conditions of physiological significance indicate a dynamic balance between the reaction components providing a route to sustained release of functional molecules.

Introduction

The use of polymers as delivery vehicles for controlled release has been studied extensively for a wide array of end applications.[1–5] In the majority of cases the desired effect of the polymer in question is to prolong the release time of some active ingredient and increase its efficacy. This may be achieved by slow degradation or stimuli induced contraction of a polymer matrix in which the compound is encapsulated[6–10] or alternatively, cleavage of a covalent bond to release the active compound.[11–14] In both cases the release rates can often be tuned by the physiological conditions under which it is employed (pH, temperature, light etc.).

Although drug delivery is the most frequently cited end application there are numerous alternative uses for controlled release systems. For example, the controlled release of fragrance has similar fundamental challenges as drug delivery and control over the release profile is key to improving product efficiency. Fragrance release by evaporation can be approximated as a first order process with respect to the volatile compound and therefore the release profile decay exponentially resulting in a burst of fragrance followed by a slow stream.[15] Fragrance formulations are often complex, consisting of many scents. Traditionally control of releases of fragrances is achieved by mixing components of variable vapour pressure. This gives a perfume its characteristic time release palette.

To a certain extent a specific vapour pressure can be controlled by altering the molecular weight of the fragrance compound but doing so is costly, control is limited and the fragrance can be negatively altered. Pro-fragrances, that is compounds that degrade to yield the desired compound, are a feasible alternative that allow for fragrance release to be controlled by external stimuli, such as light and pH, but still only limited control over the general release profile is obtained.[16,17] In order to maximize control over the release the pro-fragrance must be sensitive to relative concentration such that only when the detection of fragrance is limited, more is released. Here we report a versatile dynamic strategy to control the release of fragrance compounds and antibiotics by fabricating and making use of polymers, in the form of microgel dispersions in water, which are capable of covalently binding the active molecules in a reversible fashion.

We took our inspiration from the work on dynamic combinatorial libraries and dynamic polymers (dynamers), with the reversibility of a covalent bond being key to the dynamics. Lehn *et al.* showed the potential of dynamic systems in their seminal work on dynamic combinatorial libraries in which a pool of interchanging compounds in the presence of a molecular target was used to discover which combinations of formed compounds had a significant binding affinity.[18,19] Lehn *et al.* later undertook groundbreaking work in applying these reversible type reactions to polymer chemistry. Dynamic polymers, or 'dynamers', exhibit the ability to undergo component exchange thus providing configurational diversity.[20–22] One particularly desirable property of this type of dynamic system is that the equilibrium and exchange process can be controlled by physical conditions such as pH and temperature.[23] The stimuli responsive properties that make these materials so inherently interesting can be derived from a

variety of functional groups such as heat activated alkoxyamines,[24,25] light responsive coumarin polyurethanes,[26] Diels-Alder chemistry,[27] transesterification reactions,[28] and through boranate ester linkages.[29] Many of these highly efficient, dynamic reactions can be seen as reversible "click" chemistry.[30,31]

One of the most widely used functionalities for covalent assemblies, and the group upon which this research is based, is the imine group and its closely related compounds.[32–34] The hydrazide/hydrazone group is of particular interest in this class of compounds due to its ability to readily conjugate biomolecules and its pH sensitive reversibility.[35–39] Polyacylhydrazones, for example, contain a reversible, covalent hydrazone linkage between monomer units. This presents the possibility of dynamic exchange with other dialdehydes/dihydrazides into the polymer backbone and subsequent alteration of the materials physicochemical properties.[40,41]

On the molecular scale the hydrazone linkage has previously been used to control the release profile of aromatic aldehydes[17,42] and some anti-cancer anthracyclines.[11] In the latter example the hydrazide moiety was not in the polymer backbone but as a pendant group that allowed for subsequent functionalization. The ease of reversibility and the pH dependence of the exchange reaction makes the hydrazone link especially desirable for drug delivery where pH can act as a trigger for release.

However, despite the well known reactivity of the hydrazide functional group and its 'clickability' its use in polymer chemistry has been limited due to the associated problems in synthesis and stability of the methacryloyl hydrazide polymer. The homopolymer of methacryloyl hydrazide has usually been produced by boiling poly(methyl methacrylate) in hydrazine.[43,44] This reaction is almost the antithesis of click chemistry with low yields, harsh reaction conditions and complex side reactions occurring. The alternative of synthesizing the monomer and subsequently polymerizing it has been explored although to a lesser extent due to long term stability of the polymer.[45,46] The stability of the homopolymer of methacryloyl hydrazide in solution is hindered by the rapid transamidation that occurs in both air and water and causes gel formation preventing its application in commercial systems.[47,48] This problem of gelation due to transamidation can be negated in a microgel-type system shown here where the polymer network is already covalently crosslinked and allows the potential of the hydrazide functional group to be fully exploited. In addition problems with solubility after formation of the hydrophobic hydrazone are avoided because the particles are colloidally stable.

In this paper we present the one step synthesis of a hydrazide functional methacrylate monomer in high yield and the subsequent synthesis of the corresponding polymer microgel via dispersion polymerization. It is our aim to illustrate that by confining the reactive hydrazide functional group to colloidal particles we can obtain reactive microgels that will dynamically uptake and release functional aldehyde compounds according to external stimuli and local concentration (see **Figure 1**). By adding external conditions that effect the local concentration (such as evaporation of binding molecule or dilution) the position of equilibrium can be shifted triggering release of active compounds. Such effects should allow for effective mediation of release based on the local equilibrium established by competitive binding of the carbonyl containing molecules and the functional microgels.

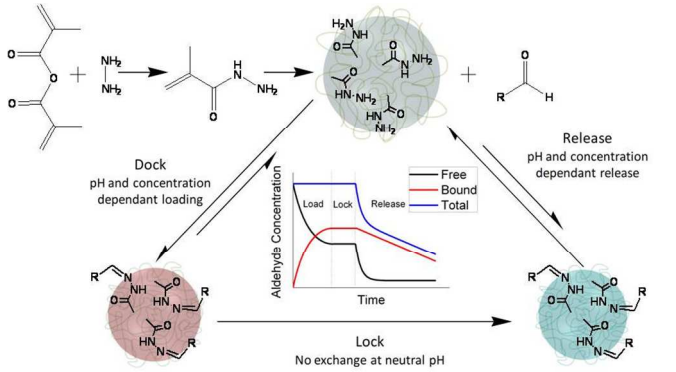


Figure 1. Overall reaction scheme showing formation and release of aldehyde compounds from poly(methacryloyl hydrazide) microgel particles

Experimental Section

Materials

Methacrylic anhydride (92%), hydrazine monohydrate (98%), polyvinylpyrrolidone (PVP, K-30 Mw 40,000 g.mol^{-1}), benzaldehyde (≥99.5%), streptomycin sulphate salt (M_w 728.69 g.mol-1) and N, N'-methylene bisacrylamide (99%) were purchased from Sigma Aldrich and were used without further purification. Azobisisobutyronitrile was purchased from Wako and used without further purification. All solvents were of reagent grade purity and were purchased from fisher scientific.

Characterization

Particle size was determined by dynamic light scattering performed on a Malvern Zetasizer ZS using a scattering angle of 173° at a standard temperature of 25 °C unless otherwise stated. NMR measurements were performed on a Bruker DPX-400 400MHz spectrometer and the spectrum was analysed with Mestrec v2.3a. UV spectra were recorded using a Perkin Elmer Lambda-45 UV-vis spectrometer. Melting points were recorded using a Stuart SMP3 melting point apparatus at a rate of 2°C per minute. Microanalysis was performed by Warwick Analytical Services and high resolution mass spectrometry was performed at the Mass Spectroscopy Facility at the University of Warwick.

Synthesis of methacryloyl hydrazide

A solution of methacrylic anhydride (51.75 g, 0.34 mol) in chloroform (250 ml) was added dropwise to a stirred solution of hydrazine monohydrate (70 ml, 1.44 mol) at 0°C. After addition the mixture was left to stir at room temperature for 10 minutes. The organic layer was removed and the aqueous layer washed

three times with chloroform. The volatiles were removed from the organic fraction by rotary evaporation to yield a white crystalline solid. This was recrystallized from a mixture of 10:1 toluene:dichloromethane to yield fine needle like crystals (26.86 g, 79%). Melting point range: 83-84°C; IR (υ_{max}/cm-1, neat) 3204, 3021, 1661, 1606, 1520, 1344, 1238, 1135, 1022, 922; ^{1}H NMR (400.03 MHz, $CDCl_3$, 298 K): δ = 1.90 (s, 3H), δ = 3.91(s, 2H), δ = 5.30(s, 1H), δ = 5.68 (s,1H), δ = 7.69(s, 1H); ^{13}C NMR (100.59 MHz, $CDCl_3$, 298 K): δ = 16.4, 120.4, 138.1, 169.4; High resolution MS-ES calc. for $C_4H_8NaN_2O$ $[M + Na]^+$: 123.0534 ; found: 123.0531; Anal. Cald. For $C_4H_8N_2O$: C, 47.99; H, 8.05; N, 27.98; Found: C, 47.19; H, 7.95; N, 26.96

Synthesis of poly(methacryloyl hydrazide) microgel particles

In a typical experiment methacryloyl hydrazide (3 g), PVP-K30 (3 g), N', N'methylenebisacrylamide (300 mg) and AIBN (60 mg) were dissolved in a mixture of propan-2-ol (40 ml) and water (10 ml). The mixture was purged of oxygen by bubbling with nitrogen then sealed with a slight overpressure of nitrogen and heated to 60°C overnight. The resulting milky suspension was centrifuged and redispersed in ethanol 3 times to remove excess PVP then freeze-dried and suspended in water at 5wt%.

Polymer aldehyde binding kinetics

To phosphate buffer (2 ml, pH 1.89, 0.15 M) containing 0.5wt% CTAB was added 200 µl of a suspension of poly(methacryloyl hydrazide) microgel (0.025wt%, 2.5×10^{-3} M) and varying amounts of a benzaldehyde solution (80-200 µl, 2.36×10^{-3} M). The UV spectrum was recorded every 5 minutes for 100 minutes. Alternatively a citric acid buffer (pH 3.86, 0.15 M), an acetic acid/sodium acetate buffer (pH 5.01, 0.15 M) or a phosphate buffer (pH 7, 0.15 M) were used and the time interval was 30 minutes until equilibrium was established. The concentration of aldehyde bound to the microgels at any given time was determined by first-derivative spectrophotometry (comparing dA/dL at 259 nm to a set of benzaldehyde standards (see **Figure S3** for calibration)), from the initial aldehyde concentration.

Dynamic release of aldehyde compounds under air flow

25ml of a phosphate buffered benzaldehyde solution (0.25wt% benzaldehyde, pH 1.89, 0.5wt% CTAB) was placed in a Schlenk tube. Compressed air at a pressure of 20 psi was blown over the sample. At selected time intervals 50 µl of the solution was removed and made up to 5 ml with the buffer solution and the UV/Vis spectrum was recorded. Alternatively a suspension of poly(methacryloyl hydrazide) microgel particles (1.25 ml, 5wt%) was added to 25 ml of the benzaldehyde solution and left overnight to ensure equilibrium is reached. The suspension was then placed in a Schlenk tube and subjected to the same air flow as before. Samples were diluted by 100 times and the concentrations of bound and free aldehyde were calculated by first derivative spectrophotometry at a wavelength of 317 and 259 nm respectively by comparison to standards. At these wavelengths there is little interference from the other compound (see **Figure S4)**. The amount of benzaldehyde released was calculated by

$$Release = 1 - \left(\left([R-CHO]_{bound} + [R-CHO]_{free}\right)/[R-CHO]_{t0}\right)$$

Where [RCHO] is the concentration of benzaldehyde and the subscripts refer to the amount of bound benzaldehyde at time t, the amount of free benzaldehyde at time t and the concentration of benzaldehyde initially added to the sample at t=0.

Dynamic release under concentration gradients

5 ml of a suspension of microgel particles at varying concentrations (0-2 molar equivalents of streptomycin sulphate) in buffer was placed inside a dialysis membrane. The dialysis membrane was submerged in a 5 ml solution of streptomycin sulphate (10 $mg.ml^{-1}$, 0.013 $mol.L^{-1}$) and left for 24 hours under light stirring at room temperature to achieve equilibrium. At twenty minute intervals 2.5ml of the reaction mixture on the outside of the dialysis membrane was removed and 2.5 ml of the appropriate buffer was added to replace it. The concentration of streptomycin sulphate in the removed sample was determined by a colorimetric method. To 2.5 ml of the test solution 0.5 ml of NaOH (2 M) was added. This was heated in a test tube in a water bath at 95°C for 3 minutes then removed and cooled in water for 3 minutes. 2 ml of iron ammonium sulphate solution (1 wt% in 0.75 M HCl) was added and the colour was allowed to develop for 10 minutes before the UV absorption at 540 nm was measured. The concentration was determined from a calibration curve of known streptomycin concentrations (see **Figure S7**). The release was calculated from the known initial streptomycin concentration and the cumulative amount of measured streptomycin removed.

Results

Synthesis of methacryloyl hydrazide and poly(methacryloyl hydrazide) microgel particles

A water soluble hydrazide functional monomer, methacryloyl hydrazide (MH), was synthesized by the reaction of methacrylic anhydride and hydrazine monohydrate in chloroform according to a modified method of Okawara *et al.*.[49] The product was purified by recrystallization and obtained in 79% yield. Previous syntheses of this molecule had described the use of equimolar amounts of hydrazine monohydrate and methacrylic anhydride but the yield in this case is low, typically less than 20%, with a large amount of the divinyl species being formed. The formation of this is due to the high reactivity of the target molecule, methacryloyl hydrazide, which can undergo a second addition to methacrylic anhydride. In order to minimize the occurrence of this a large excess of hydrazine, typically 5 molar equivalents, is used. Under these conditions the monomer was formed in high yield. Although a use of a large excess is undesirable the excess hydrazine can be easily removed after reaction due to its high polarity and can in principle be reused, although due to its low cost this would not be cost effective on a lab scale.

Poly(methacryloyl hydrazide) microgels were synthesized by dispersion polymerization in 80/20 vol/vol isopropanol water mixture using AIBN as initiator, PVP as stabilizer and methylene bisacrylamide as crosslinker forming a milky white suspension. Following polymerization at 60°C for 24 hours the reaction mixture was centrifuged and redispersed in water 3 times then freeze dried and resuspended as a 5 wt% solution in water. The particles were 320 nm with a dispersity of 0.10 measured by dynamic light scattering and this was confirmed by cryogenic SEM (see **Figure S1**). We proceeded to investigate the use of these microgels as dynamic storage and delivery agents for aldehydes, that is benzaldehyde as a fragrance compound as well as streptomycin sulphate salt as a model antibiotic.

Kinetics of hydrazone formation

The rate and extent to which the hydrazone is formed from the respective aldehyde and hydrazide is of prime importance to the end application. We therefore looked into the kinetics of hydrazone formation using benzaldehyde as a model compound. The effect of pH on the rate and equilibrium position of hydrazone formation was examined by mixing the microgel particles and benzaldehyde at an equimolar ratio in buffered solution and monitoring the extent of reaction over time by UV/vis spectroscopy. The presence of the aldehyde caused a decrease in the swelling which could be observed by an increase in turbidity in comparison to the highly swollen initial hydrophilic microgel. The effect of scattering from the microgels was negligible at the low concentration used (see **Figure S2** that shows that the change in absorbance due to scattering is negligible compared with the change in absorbance due to the conversion of aldehyde to hydrazone) but was accounted for in quantitative measurements by taking the first derivative of absorption since the scattering did not vary significantly with wavelength. The kinetic constants for both the forward and back reaction were calculated by fitting the data to the rate equation for the reversible second order reaction of an aldehyde with a hydrazide (see **Figure 2**) (for derivation of kinetic equations see supporting information).[50]

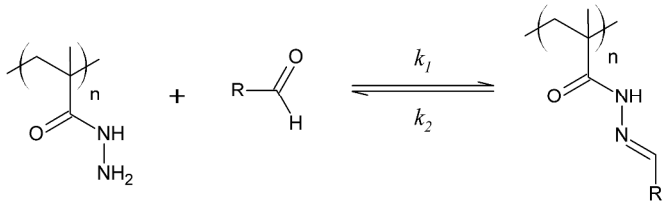


Figure 2 Reversible reaction between microgel containing hydrazide and aldehyde compounds

Figure 3a demonstrates that the reaction kinetics and the position of equilibrium varied drastically with pH. Due to the multi-step mechanism of imine formation that the rate determining and yield limiting steps are subject to change depending on the pH, as has previously been shown for hydrazone formation, resulting in a complex relation of the reaction with pH.[51] At low pH the time taken for the equilibrium to be established was much faster (k_1=2.45 $M^{-1}s^{-1}$, k_2=8.33×10^{-5} s^{-1} at pH 1.89) but the position of equilibrium was only slightly in favour of the hydrazone formation ($K_{eq}=k_1/k_2$=2.9×10^4 M^{-1}). As the pH was increased the reaction slowed down significantly changing by a factor of ~2 upon raising the pH to 3.87 and then by ~10 times by increasing to pH 5 (k_1=1.36 $M^{-1}s^{-1}$, k_2=6.01×10^{-6} s^{-1} at pH 3.86 and k_1=0.16 $M^{-1}s^{-1}$, k_2=8.45×10^{-6} s^{-1} at pH 5). The equilibrium constant at pH 3.86 was strongly in favour of formation of the hydrazone ($K_{eq}= k_1/k_2$=2.2×10^5 M^{-1}) but shifted back to moderate values at pH 5 ($K_{eq}=k_1/k_2$=1.83×10^4 M^{-1}). At neutral pH the rate of hydrolysis and formation are so slow that essentially no reaction occurred. This is of notable interest because it allows for the loading of particles at acidic pH and subsequent locking of the equilibrium at pH 7. It should be noted that due to the position of equilibrium lying almost entirely on the side of the free carbonyl no experiments with ketone compounds were conducted.

The effect of relative concentration on the rate and equilibrium position of hydrazone formation was examined by mixing the microgel particles and varying quantities of benzaldehyde in pH 1.87 buffered solution and monitoring the extent of reaction over time by UV/vis spectroscopy. **Figure 3b** shows the fit of the data to the rate equation derived for varying initial benzaldehyde concentrations at constant values of k_1 and k_2 (k_1=2.45 $M^{-1}s^{-1}$, k_2=8.33×10^{-5} s^{-1}) and indicates a reasonable fit in all instances allowing us to predict and control the concentration of free aldehyde in solution by control of pH and reagent concentration.

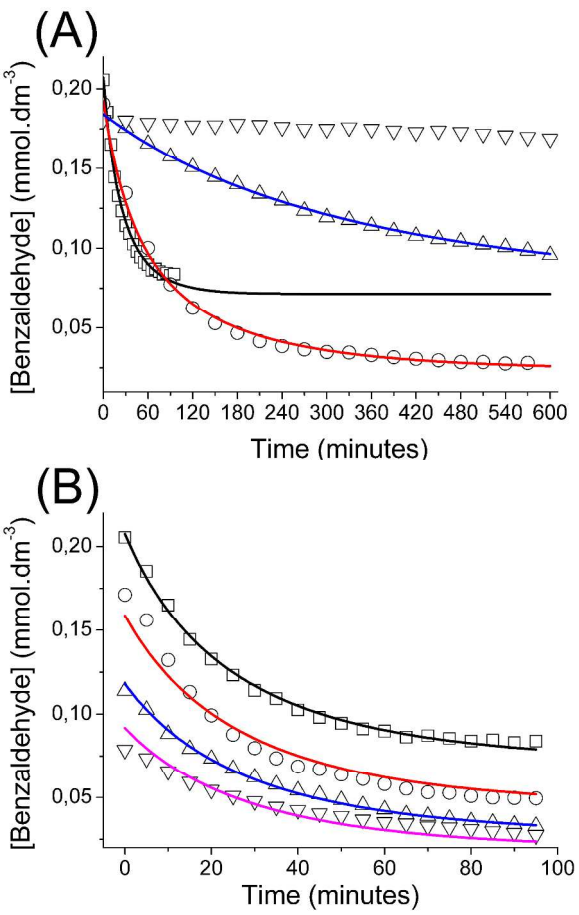


Figure 3 Decrease in benzaldehyde concentration over time under different conditions. (A) At equimolar concentration of benzaldehyde to hydrazide functional groups at pH 1.89 (squares), pH 3.87(circles), pH 5.01(upward pointing triangles) and pH 7(downward pointing triangles). (B) At constant hydrazide concentration (~2×10^{-4}M) but varying the concentration of initial benzaldehyde concentration 2×10^{-4} (squares), 1.6×10^{-4} (circles), 1.2×10^{-4} (upward pointing triangles) and 0.8×10^{-4} mol.dm^{-3}(downward pointing triangles). The lines represent the fit of the data to the kinetic rate law.

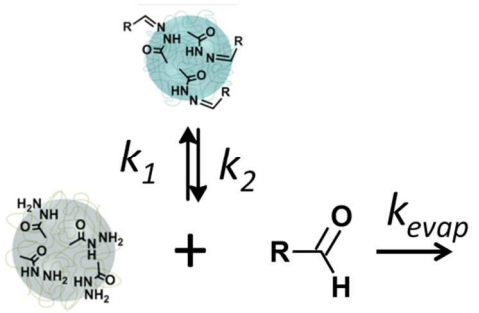
Controlled release from poly(methacryloyl hydrazide) microgels

Having studied the kinetics of the reaction of microgel particles with aldehydes we then investigated the potential of the microgel particles as mediators for the controlled release of our two active substances. This offered the opportunity to explore the dynamic behaviour of the particles when a concentration gradient of the free aldehyde was introduced. Because of the dynamic nature of the hydrazone bond we can vastly alter the release profile of functional aldehydes by tuning the pH and microgel particles concentration.

As an initial investigation into the dynamic activity of the microgel particles we looked at the evaporation of benzaldehyde in the presence of the microgel particles. Lehn *et al.*[17,42] have previously illustrated that molecular hydrazones can be deposited onto cotton surfaces and slowly release the fragrance but this required alcoholic solvents due to the relative insolubility of the hydrazones in water. In our case the microgels are colloidally stable after addition of the hydrophobic aldehydes with moderate amounts of surfactant. 25ml of a phosphate buffered benzaldehyde solution (0.25wt% benzaldehyde, pH 1.89, 0.5wt% cetrimonium bromide (CTAB)) was placed in a Schlenk tube. In one experiment a suspension of poly(methacryloyl hydrazide) microgel particles (1.25 ml, 5wt%) was added to the buffered benzaldehyde solution and left to equilibrate overnight, while in the other no microgel particles were added.Compressed air at a constant pressure was blown over the samples and at selected time intervals the UV spectrum of the mixture was recorded to measure concentration.

The concentrations of bound and free aldehyde were calculated by first derivative spectrophotometry at a wavelength of 317 and 259 nm respectively compared to standards (see **Figure S4** and **S5**). At these wavelengths there is negligible interference from other compounds in the system and scattering from the microgel suspension does not affect the first derivative plot hence the total concentration of aldehyde in the system, and its variation with time, can be accurately calculated.

The release of benzaldehyde from an aqueous solution against time in the presence and absence of the poly(methacryloyl hydrazide) microgels is plotted in **Figure 4** along with a schematic of the reaction set up. The experimental data for the evaporation in the absence of the particles can be described by a single rate constant, k_{evap}, and using this and the rate constants derived from UV/vis data above a good agreement between the theoretical release profile obtained by solution of the differential equations that describe the system (see supporting information) and experimental data is achieved. It can be seen that the addition of the microgel particles induces a more linear release profile as well as reducing the rate of evaporation substantially. Addition of the microgel particles significantly lowers the concentration of free aldehyde in water and hence the evaporation rate according to **Scheme 1**. Subsequently, as the aldehyde in the aqueous phase evaporates the microgel releases aldehyde in order to maintain equilibrium. This has the effect of 'topping up' the free aldehyde concentration and allows for the decay in concentration to occur in a more linear fashion thus providing a more steady flow of the evaporating substance. This dynamic balance allows fragrance to be released from the microgels only when the fragrance is diminished and can also be controlled by pH. In fact, based on the solution to the differential equations that describe the system in the absence of the hydrazide containing microgels complete release (>99.5%) of the aldehyde is achieved within 1 day whereas in the presence of the equimolar concentration of hydrazide groups in the microgel particles at pH 1.86 it would take 1 year and at pH 3.86 it would take 6 years to achieve the equivalent release percentage (see **Figure S6**).



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

Scheme 1 Kinetics of release of aldehyde in the presence of hydrazide microgels. $[A]_{aq}$ 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.

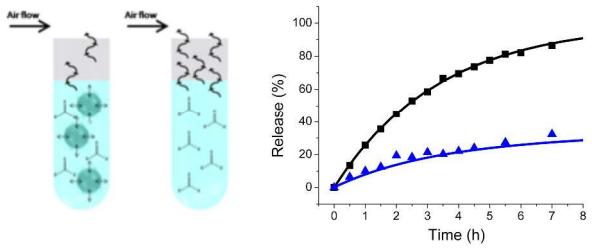
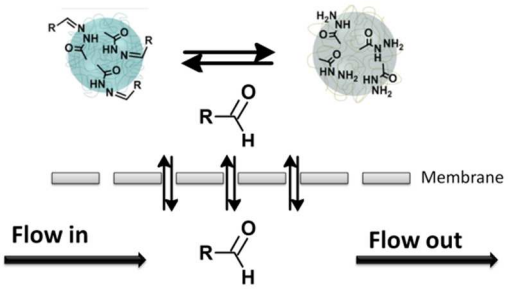


Figure 4 Schematic of reaction setup and experimental results of release over time of benzaldehyde in the absence (black squares) and presence (blue triangles) of hydrazide containing particles. Lines represent the theoretical release profiles obtained from the solution to the differential equations that represent the system (see supporting information) and rate constants of k_1=2.45 $M^{-1}s^{-1}$, k_2=8.33×10^{-5} s^{-1} and k_{evap}=8.02×10^{-5} s^{-1}.

Following this we looked into the possibility of using the microgel particles as a drug delivery vehicle. We used, as a model compound, streptomycin sulphate, a hydrophilic, aldehyde containing antibiotic. It should be noted that ketone containing compounds can be easily loaded into the microgel suspensions in non-aqueous media via the hydrazide moiety and subsequently transferred to water and released by a pH trigger but such studies are outside the scope of this investigation which focuses on the *dynamic* equilibrium involving simultaneous formation and hydrolysis of the hydrazone bond.

The microgel suspension was placed inside a dialysis membrane and submerged in a streptomycin sulphate solution in buffer and left for 24 hours to equilibrate (see **Scheme 2**). At selected time intervals an aliquot of the exterior solution was removed and the concentration of streptomycin was determined by the chemical assay developed by Leghorn *et al*.[52] The reaction mixture was topped up with buffer solution having the effect of diluting the streptomycin solution, similar to what one would expect to occur in the body as the antibiotic is used up. It should be noted that the release profile for the experimental system described is nearly identical to one of continual dilution (see Supporting Information and **Figure S8**).



Scheme 2 Schematic of reaction set up illustrating multiple kinetic processes in controlled release of streptomycin sulphate.

Figure 5 shows the decrease in concentration of total streptomycin concentration over time when different amounts of the microgel particles were used. As expected at higher concentrations of microgel particles the release rate was significantly slower due to the increase in the bound streptomycin. Any free aldehyde is rapidly removed from the system and subsequent release follows a steady profile that is related to the rate of hydrolysis of the hydrazone bond, itself a function of the relative aldehyde and microgel particle concentrations. The net effect of this is to slow down release when the concentration of free aldehyde is high and speed up release when aldehyde concentration is low allowing for dynamic, concentration dependant release of the aldehyde compound. The kinetics could be modelled by considering the complex equilibria between bound and free aldehyde within the dialysis membrane and transport from the interior of the dialysis membrane to the outside induced by the dilution of the solution and given mathematically by the rate constant for transport across the membrane, k_{tr}, and the difference in concentration between the interior and exterior of the membrane (see Supporting Information).

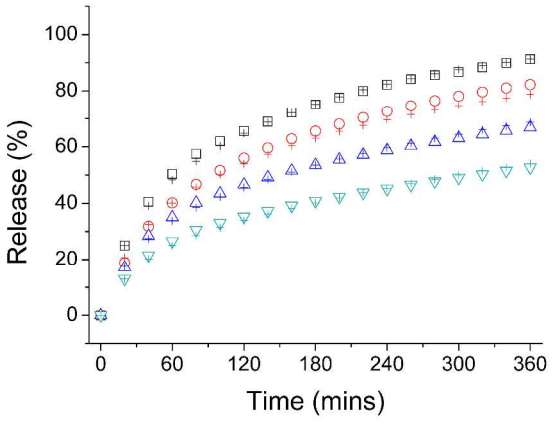


Figure 5. Release profile of streptomycin sulphate in the presence of varying concentration of poly(methacryloyl hydrazide) microgel particles at a pH of 3.86

with 0(black squares), 0.5(red circles), 1 (blue upward pointing triangles) and 2 (cyan downwards pointing triangles) molar equivalents of hydrazide functional groups to aldehyde. The crosses are the theoretical release plots based on the solution to the series of differential equations that represent the system using k_{tr} = *1.22* × 10^{-4} s^{-1} K=90 M^{-1} and k_1=*0.005* $M^{-1}s^{-1}$.

Having demonstrated that the microgel particles can be effective mediators in drug delivery we looked to investigate the ability of the particles to offer a pH sensitive delivery profile. **Figure 6** illustrates that the release profile is heavily dependent on the pH of the reaction mixture. At pH 5 the equilibrium lies less to the side of the hydrazone formation so the concentration of free streptomycin is significantly higher than the analogous situation at pH 3.86 and thus the rate of release is faster. At low pH, contrary to the benzaldehyde system, the equilibrium constant is significantly higher that at pH 3.86 and thus the rate of release is further decreased. This highlights the highly pH sensitive nature of the formation and hydrolysis of the hydrazone bond. The general trend of increasing rate of reaction, as given by the estimated parameters with decreasing pH was observed to be similar to that of the benzaldehyde reaction.

To test the ability of the particles to respond dynamically to pH changes we first loaded the microgel particles with streptomycin at pH 3.86 and then froze the system by altering the pH to 7 (see **Figure 6**). At pH 7, where there is no exchange, the concentration of free streptomycin decreases rapidly and then tails off as the bound streptomycin cannot be released. Reverting the pH back to 3.86 where exchange can occur we saw an increase in the rate of release as bound streptomycin can once again be hydrolyzed. This result indicates the remarkable activity of the microgel particles on the release profile of carbonyl containing compounds, namely that the release profile is sensitive to both the concentration of the released species and pH which prevents overloading the concentration of the compounds in one area. We believe that the ability of these microgel particles to react and respond to changes in pH under conditions of physiological significance could have an immense impact on the use of dynamic polymers in biological and chemical applications.

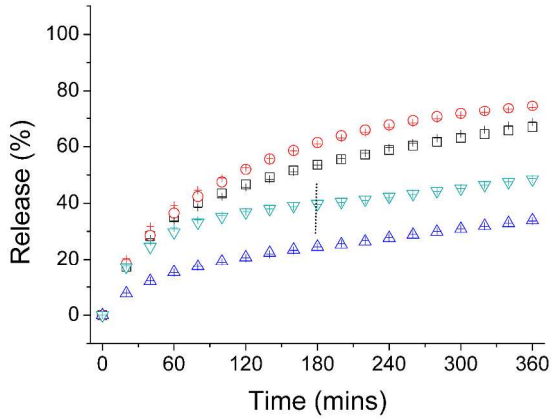


Figure 6. Release profile of streptomycin sulphate in the presence of equimolar concentration of poly(methacryloyl hydrazide) microgel particles pH of 3.86(black squares), 5(red circles), 1.86 (blue upward pointing triangles) and loading at pH 3.86 followed by release at pH 7 with subsequent change at the marked point back to pH 3.86 (cyan downwards pointing triangles). The crosses are the theoretical release plots based on the solution to the series of differential equations that represent the system using k_{tr} = *1.22* × 10^{-4} s^{-1}, K=90 M^{-1} and k_1=*0.005* $M^{-1}s^{-1}$ (pH 3.86), K=66 M^{-1} and k_1=*6.5*× 10^{-4} $M^{-1}s^{-1}$ (pH 5), K=1022 M^{-1} and k_1=0.035 $M^{-1}s^{-1}$ (pH 1.86) .

Although the case reported here has focused on hydrazide-aldehyde chemistry the underlying principle of dynamic controlled release may be applied to any given reversible reaction including disulfide formation, oxime formation, boronic ester formation and thermally induced radical crossover reactions. With this in mind it would be of interest to understand more generally the relation between the process equilibria and conditions, and release rates. The reactions conducted here can be generalized to a second order reversible reaction between a reagent bound to a polymeric microgel (the hydrazide functional group in this case) and the active compound to be released. The release of the active compound is assumed to proceed via an additional reaction that is first order with respect to the active compound concentration such as the evaporation example for the benzaldehyde-hydrazide reaction shown here (see **Scheme 1**). In this instance the release rate is proportional to the concentration of free active compound which is determined by initial concentrations of reactants (both relative and absolute) and the rate constants of all processes. With higher equilibrium constant, *K*, the concentration of the free active compound is reduced and thus the rate of release is slowed down (see **Figure S11)**. The effect of the individual rate constants k_1 and k_2 is irrelevant if they are high enough to maintain equilibrium where the free active compound concentration is determined by the equilibrium constant K, but if they are low, or if the rate of removal of the active compound, k_3, is high, then the dynamic exchange may be out of equilibrium and the concentration of free active compound will be determined by the rate of the release of the active compound from the bound form (see **Figure S12**). The initial concentrations of the reactants make a large difference to the release rates since the forward and backward reactions are second and first order respectively hence changing concentrations alters the position of equilibrium. With high concentrations of the active compound and/or the bound polymeric reactant the bound species is favoured and hence the lower relative concentration of the free active compound results in a slower release rate (see **Figure S13** and **S14**). The above is summarised in **Table 1**. Through application of these general results to the growing array of dynamic covalent systems it should be possible to finely tune and tailor release rates and release profiles according to specific environments and applications by appropriate choice of the dynamic covalent bond employed.

Table 1 Effect of reaction conditions on release rate

Effect	Release Rate
Increasing equilibrium constant	Decreased Rate
Increasing k_1,k_2	Increased Rate
Increasing concentration of reactants	Increased Rate

Conclusions

We have described the synthesis of hydrazide functionalized microgel particles and demonstrated their dynamic equilibrium with carbonyl compounds in aqueous suspensions. The results of kinetic experiments showed a complex relationship between bound and free aldehyde that is affected heavily by pH and concentration. We have shown potential application of the particulate materials in release of fragrances and other small molecules and demonstrated the ability of the particles to react in a dynamic fashion to their chemical environment. The use of colloidally stable microgel particles allowed for loading and release of both hydrophobic and hydrophilic compounds in aqueous media in the complete absence of organic solvent. The present results indicate that the dynamic microgels can provide an efficient means of slow, controlled release of organic compounds, the release profile being determined by both pH and concentration. Furthermore we have demonstrated that by knowledge of a few accessible rate constants the release profile can be accurately predicted thus making it possible to pre-programme the release of an active compound tailored to specific applications. We believe that this concept of using dynamic microgels to control storage and release of active compounds can be applied to, and will contribute to, advances in a wide range of scientific fields.

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Notes and references

[a] POLYMAT and Grupo de Ingeniería Química, Dpto. de Química Aplicada, University of the Basque Country UPV/EHU, Joxe Mari Korta Zentroa, Tolosa Etorbidea 72, 20018, Donostia/San Sebastían, Spain.
[b] Department of Chemistry, University of Warwick, Coventry, UK, CV4 7AL;E-mail: S.Bon@warwick.ac.uk; Web:www.bonlab.info
Electronic Supplementary Information (ESI) available: Electronic Supplementary Information (ESI) available: Full experimental details, mathematical derivation of rate equations and complete details of mathematical models employed. See DOI: 10.1039/b000000x/See DOI: 10.1039/b000000x/

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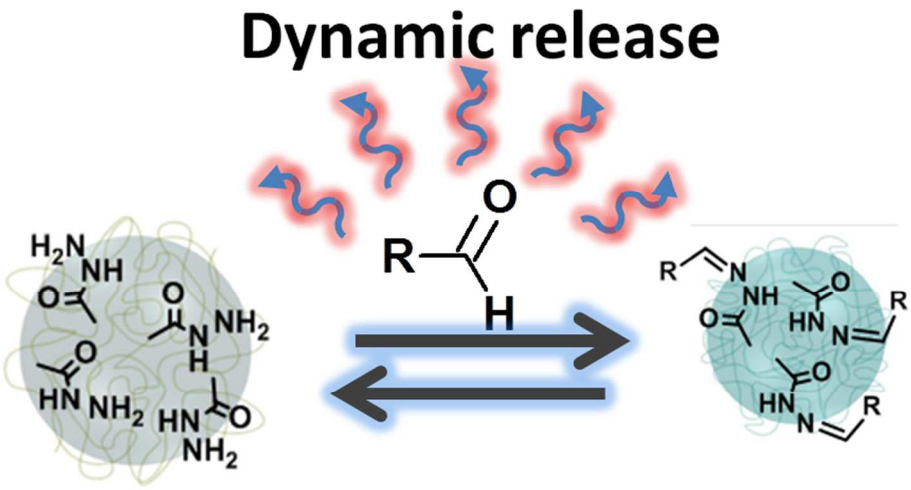

The uptake and release time-profiles of aldehydes from aqueous formulations can be finetuned using hydrazide functional microgels
79x39mm (300 x 300 DPI)