Poly (*N*-Isopropylacrylamide) Microgel Based Assemblies for Organic Dye Removal from Water: Microgel Diameter Effects

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Abstract

Poly (*N*-isopropylacrylamide)-*co*-acrylic acid (pNIPAm-*co*-AAc) microgel based assemblies (aggregates) were synthesized from microgels of various diameters via polymerization of the crosslinker *N,N'*-methylenebisacrylamide (BIS) in the presence of microgels in solution. We investigated the ability of the respective aggregates to remove the organic, azo dye molecule 4-(2-Hydroxy-1-napthylazo) benzenesulfonic acid sodium salt (Orange II) from water at both room and elevated temperatures. The results from the microgel aggregates made from 1.1 μm diameter[1] microgels were compared to aggregates synthesized from 321 nm and 1.43 μm diameter microgels. Aggregates made from the same size microgels showed increased uptake efficiency as the concentration of BIS in the aggregates was increased, while for a given BIS concentration, the uptake efficiency increased with increasing microgel size in the aggregate. We attribute this to the "nature" of the aggregates; aggregates have void space between the microgels that can serve as reservoirs for Orange II uptake – the void spaces are hypothesized to increase with larger diameter microgels. By exploiting the thermoresponsive nature of the microgels, and microgel based aggregates, 85.3 % removal efficiencies can be achieved. Finally, all uptake

trends for the aggregates, at room temperature, were fit with a Langmuir sorption isotherm model.

Keywords: Water remediation and contamination, poly (*N*-isopropylacrylamide)-*co*-acrylic acid microgel aggregates, thermo responsive polymeric materials, azo dyes, pNIPAm

Introduction

Wastewater containing synthetic dye molecules has been a major concern for many years due to the hazardous effects on both human and aquatic life.[1,3] Effluents from industries like leather tanning, textile, paper production, and food technology contain large quantities of synthetic dyes.[2,4-8] For example, the textile industry alone uses several different dyes of which 30% are considered to be reactive. Reactive dyes are those that have functional groups such as: azo, anthraquinone and oxazine. These functional groups react with the fibres of the material to be dyed. [9] Azo dyes constitute nearly 60% of all the reactive dyes, most of which are used in the textile industry for their color. The danger with using azo dyes is they are known to transform into aromatic amines in the environment, which are carcinogenic in nature and can be hazardous to humans and animals. [3]

Among the major techniques for treating drinking water, coagulation and flocculation involve addition of coagulants to the wastewater, followed by sedimentation. [10] Specifically, this method has been employed to remove azo dyes and other reactive dyes, e.g., C.I. Reactive Black 5, C.I. Vat Yellow 4 from industrial waste water. [11-20] A major disadvantage of this approach is that it could lead to secondary pollution from the excessive use of coagulant compounds.[19] Electrocoagulation is another technique for water treatment that involves generation of a coagulant at an anode and several dyes like Samaron Yellow and Eriochrome Black T have been

removed from water using this approach. [19, 20] Another approach to treat water is absorption using activated carbon. Contaminants like polycyclic aromatic hydrocarbons (PAHs), pharmaceuticals, pesticides, including synthetic dyes have been removed from water using this approach. [23-26] Among other techniques, many physico-chemical and biological treatments have been employed for removal of organic dyes from industrial effluents, but the drawback of these techniques are they are often not easy to implement/use and are expensive.[25-32]

In the current study we use responsive polymers to remove contaminants from water. Responsive polymers are a class of polymers that "react" to external stimuli including: temperature, pH, ionic strength, light, force and analyte concentration, by changing their physical and/or chemical state. [33-40] Poly (*N*-isopropylacrylamide) (pNIPAm) is one of the most thoroughly studied responsive polymers. [41-49] PNIPAm is fully water soluble, and thermoresponsive, i.e. it exists as a random coil at T < ~32 ˚C and transforms to a globule conformation by expelling much of its solvating water at T > ~32 ˚C. The temperature that this occurs at is called the lower critical solution temperature (LCST). [42,43,46,47]

Synthesis of colloidal particles from NIPAm has been reported previously in numerous publications. [42,43,45,47-53,59] These particles, often referred to as microgels, are highly porous, water soluble and are thermoresponsive as detailed above. By simply adding different comonomers to the microgels during synthesis, they can be made to respond to other stimuli. [45,48-51,54,55] The most common comonomer employed for this purpose is acrylic acid (AAc), which has a pK_a ~ 4.25. Therefore, at pH > pK_a, the microgels swell due to Coulombic repulsion in the microgel network. This property also hinders the thermoresponsivity of pNIPAm-*co*-AAc microgels. [45,56-59]

PNIPAm based hydrogels and microgels have been used for removal of heavy metal ions like Pb (II) and Cu (II) and dyes like Nile red, brilliant green, brilliant cresyl blue. [60-63] Of relevance to this work is the azo dye 4-(2-Hyrodxy-1-napthylazo) benzenesulfonic acid sodium salt (Orange II), which has been removed from various industrial effluents and treated using physical and chemical methods. [64-66] In other reports, pNIPAm based hydrogels have been used to determine the partition coefficient of Orange II and methylene blue in the system at different temperatures. At T > 32˚C Orange II was reported to have more permeability through the hydrogels. [67,68] Recently, we reported on the use of ~ 1μm diameter pNIPAm based microgels and microgel based aggregates for the removal of Orange II, Figure 1, from water.[1,69] The microgels removed a maximum of 56.6% of the dye from water at elevated temperature and retained 74.4 % of the Orange II, while aggregates formed from these microgels removed 73.1% at elevated temperature and retained 75.6% of the dye. [1]

This investigation builds on our previous work by determining how the size of the microgels that make up aggregates affects Orange II removal efficiency. PNIPAm-*co*-AAc microgels with hydrodynamic diameter (D_H), measured from dynamic light scattering, of ~ 321 nm (pNIPAm-*co*-AAc-1, "small"), D_H ~ 1.1 μm (pNIPAm-*co*-AAc-2, "medium"), and diameter (measured from light microscopy) of ~1.43 μm (pNIPAm-*co*-AAc-3, "big") were synthesized and aggregates of the microgels made via polymerization of the crosslinker *N,N'*-methylenebisacrylamide (BIS) in the presence of the respective microgels. We show that, for a given microgel diameter, the uptake efficiency increases with increasing concentration of BIS in the aggregates. We go on to show that for any concentration of BIS in the aggregates, the uptake efficiency increases as the size of the microgels in the aggregates increases. That is, the pNIPAm-*co*-AAc-1 microgel aggregates were found to remove a maximum of 70.6%, in contrast

to 85.3% removal efficiency achieved by pNIPAm-*co*-AAc-3, at elevated temperatures. It should be noted here that the uptake studies for pNIPAm-*co*-AAc-2 aggregates were reported previously, and exhibited 73.1%.% uptake at elevated temperature. [1] We also determined the overall retention efficiency of all the microgel based aggregates by monitoring the amount of Orange II that leaked from them following uptake. The pNIPAm-*co*-AAc-1 microgel aggregates retained 67.0% of the dye that was initially removed from the solution, while the retention efficiency of the aggregates with pNIPAm-*co*-AAc-3 microgels was 76.9%. This is compared to the 75.6% retention for the medium microgel aggregates.[1] The uptake from the "big" and "small" microgel aggregates were also fit by a Langmuir sorption isotherm.

This study represents a simple and straightforward technique to remove high amounts of the organic dye Orange II from water in a short amount of time – the observed behavior can be expected for other organic dyes/molecules. The observed behavior is a manifestation of how the structure of the aggregates affects their function. Future work on these systems will focus on investigating the reusability of the aggregates by developing extraction techniques to remove the organic molecule taken up by the aggregates, separating the aggregates, and re-exposing them to contaminated water. This will be a significant advance over currently available technology. In addition, the ability of these systems to specifically remove contaminants like metals, PAHs, PCBs, naphthenic acids, etc. from water will be investigated.

Materials and Methods

Materials: *N*-isopropylacrylamide (monomer) was purchased from TCI (Portland, Oregon) and purified by recrystallization from hexanes (ACS reagent grade, EMD, Gibbstown, NJ). *N,N'*-methylenebisacrylamide (BIS) (~99%), acrylic acid (AAc) (~99%), and ammonium persulfate

(APS) (~98%) were obtained from Sigma-Aldrich (Oakville, Ontario) and were used as received. Orange II was obtained from Eastman Organic Chemicals (Rochester, NY). All the phosphate salts used for preparing buffer solutions of pH 7 (ionic strength of 0.235 M) were obtained from EMD and were used as received. Deionized (DI) water with a resistivity of 18.2 MΩ·cm was obtained from a Milli-Q Plus system from Millipore (Billerica, MA), and filtered through a 0.2 μm filter, prior to use. Microgel samples were lyophilized using a VirTis benchtop K-manifold freeze dryer (Stone Ridge, NY).

Synthesis of Microgels:

a) Synthesis of pNIPAm-*co*-AAc-1 microgels (D_H ~321 nm): These were prepared using a previously used protocol. [48] The overall monomer concentration was 65.2 mM (13.05 mmol), and 85% *N*-Isopropylacrylamide (NIPAm, 11.1 mmol), BIS (0.652 mmol), and sodium dodecyl sulfate (SDS, 0.2 mmol) were added to 190 mL deionized water, previously filtered through a 0.2 μm filter. This was then transferred into a 3-neck round bottom flask that was fitted with a reflux condenser, nitrogen inlet, and a thermometer. The solution was purged with N_2 and allowed to heat to 70 °C for ~1 hour. To this 10% AAc (1.30 mmol) was added to the reaction mixture in one aliquot immediately prior to initiation. 0.3 mmol of APS in 10 mL of DI water was added to the monomer solution for initiation. The reaction was allowed to proceed at 70 °C for 4 hours under a nitrogen atmosphere. The resulting suspension was allowed to cool overnight, and then it was filtered through a type 1 Whatman filter paper to remove any large aggregates. Approximately half of the microgel solution was then distributed into rehydrated dialysis tubing (12-14k nominal MWCO, 25 mm flat width, Fisherbrand Regenerated Cellulose, Nepan, ON) for purification. The tubes were placed into two 2 L beakers with deionized water and a stir bar for two weeks and the water was replaced twice daily. Dialysis was used to remove

unreacted monomers and crosslinker, and small molecular weight linear polymers, from the microgels. The hydrodynamic diameter (D_H) of these microgels was determined by dynamic light scattering studies using a ALV/CGS-3 Compact Goniometer System (Hesse, Germany). In brief, a dilute solution of microgels in DI water, maintained at ~23 ˚C, was irradiated by a 22 mW HeNe laser, and scattered light collected. . Data was analyzed using ALV-5000/EPP correlator software, from which the hydrodynamic diameter was determined. Three sets of samples were analyzed using this protocol and an average D_H of 321 nm ± 8.83 nm was obtained.

b) Synthesis of pNIPAm-*co*-AAc-2 microgels (~ 1.1 μm): These microgels were prepared by a surfactant free, free radical precipitation polymerization as reported before.[45] The total monomer concentration was maintained to be 140 mM. Of this, 85% was *N*-isopropylacrylamide (NIPAm), 5% was *N,N'*-methylenebisacrylamide (BIS) crosslinker and 10% was acrylic acid (AAc) . To a clean beaker, NIPAm (11.9 mmol) and the crosslinker, BIS (0.700 mmol), were added and dissolved in deionized water (75 mL) in a beaker with stirring. A 20 mL syringe affixed with a 0.2 μm filter was used to filter the mixture into a clean 250 mL, 3-neck round bottom flask fitted with a condenser, thermometer, stir bar and a N_2 inlet. The beaker was rinsed with 24 mL of deionized water, which was again filtered and transferred to the mixture in the round bottom flask. The temperature was set to 65˚C with N_2 bubbling through the solution for ~ 1 h, after which AAc (1.4 mmol) was added to the mixture and stirred for a few minutes. To this, 0.197 mmol APS in 1 mL DI water was added. The mixture was allowed to stir for 4 h, under N_2 atmosphere. The solution was allowed to cool, while stirring overnight.

Following stirring overnight, the microgels were filtered through a type 1 Whatman filter paper, which was then rinsed with deionized water. The microgels were then cleaned via centrifugation to remove unreacted monomer and crosslinker, as well as linear polymer from the microgels. To do this, the microgel solution was separated into 15 mL centrifuge tubes obtained from Corning Incorporated (Corning, NY) (~ 12 mL microgel solution/tube) and centrifuged at a speed of ~8400 relative centrifugal force (rcf) in a Baxter, biofuge 17R (Mount Holly, NJ) at 23 °C, for 30 min. Centrifugation yielded a pellet of microgels at the bottom of the centrifuge tube, and the supernatant was removed. ~12 mL of fresh DI water was added and the microgel pellet was redispersed using a Fisher Vortex, Genie 2 vortexer (Pittsburgh, PA). This cleaning protocol was repeated six times. The D_H of these microgels was determined by dynamic light scattering to be 1.10 μm ±14.5 nm. [69]

c) Synthesis of pNIPAm-*co*-AAc-3 microgels (Diameter ~1.43 μm) : These were synthesized following a previously published procedure. [70] The total monomer concentration was 153.8 mM (20 mmol) and 85% *N*-Isopropylacrylamide (NIPAm, 17.0 mmol), 5% *N*, *N'*-methylenebisacrylamide (BIS, 1.00 mmol) were added to 100 mL of deionized water in a small beaker and stirred. Once dissolved, the solution was filtered through a 0.2 μm filter into a 3-neck round bottom flask. The beaker was rinsed with 25 mL of deionized water and filtered into the flask. The flask was fit with a condenser, a nitrogen inlet, and a temperature probe to provide heating via a feedback-loop controlled hotplate (Torrey Pines Scientific, Carlsbad, CA). The flask was heated in an oil bath to 45 °C while the solution was allowed to stir and purge with N_2 gas for ~1.5 hours. Once the solution reached the set temperature, acrylic acid (AAc, 2.00 mmol, 10% of overall monomer concentration) was added followed by initiation of the reaction by addition of 0.078 M aqueous solution of ammonium persulfate in 5 mL DI water (overall

solution volume resulted in 130 mL). After initiation, the reaction solution was then heated at a rate of 30 °C/hour to 65 °C and the reaction was allowed to proceed overnight under nitrogen atmosphere. The resulting suspension was allowed to cool to room temperature, followed by filtration through a plug of glass wool to remove any coagulum formed during the reaction. The microgels were then cleaned via the same protocol as mentioned in the previous section. To determine the diameter of these microgels, an Olympus IX71 inverted microscope (Markham, Ontario) fitted with a 100 X oil immersion objective, differential interference contrast (DIC) optics and an Andor Technology iXon+ camera (Belfast, Ireland) was used. One drop of a very dilute solution (~100 X dilution) of the cleaned microgels was placed on a microscope cover slip (25 mm x 25 mm, Fischer Scientific, Ottawa) and a number of photographs were obtained at random areas. The photographs were then analyzed using Andor Solis imaging software, to determine microgel diameter. The length scale was determined by photographing a micrometer scale of 50 divisions, each 2 μm in length (Edmund Optics, NJ), at the same magnification that the microgels were photographed at. The apparent diameters of 20 different microgels were measured, and an average diameter of 1.43 μm ± 5.35 nm was determined.

Synthesis of Microgel Aggregates: Microgel aggregates were synthesized using three different concentrations of BIS- 100 mg (2 mg BIS/mL of total reaction solution), 500 mg (10 mg BIS/mL of total reaction solution) and 750 mg (15 mg BIS/mL of total reaction solution). The aggregates with 100 mg BIS were prepared by directly adding 10 mL of cleaned microgels from the above syntheses to a filtered solution (filtered through 0.2 μm filter affixed to a 20 mL syringe) of 100 mg of BIS in 39 mL of deionized water, to a beaker and stirred. This solution was transferred into a 250 mL 3-necked round bottom flask that was fit with a condenser, thermometer, stir bar and a N_2 inlet. The temperature was set to 65˚C and N_2 was bubbled through the solution for ~ 1

h. After 1 h, a 1 mL aqueous solution containing 0.0175 mmol of APS was added to this mixture and left to stir for 4 h, under N_2 atmosphere. The solution was allowed to cool with stirring overnight. This procedure was followed for the synthesis of the 10 mg/mL, and 15 mg/mL BIS aggregate samples, by adding 500 mg and 750 mg BIS to the reaction mixture, respectively. All microgel aggregates were cleaned using the same centrifugation procedure followed for cleaning microgels, but without filtering. Representative DIC microscopy images of "small" "medium" and "big" microgel aggregates with 500 mg BIS are provided (see Supporting Information).

Orange II uptake: All aggregates were lyophilized and stock solutions were made from each sample to contain of 5.2 mg aggregates/mL solution. This was done by redispersing 52.1 mg of each in 10 mL of pH 7 buffer solution of 0.235 M ionic strength in a volumetric flask. A stock solution of Orange II (0.023 M) in deionized water was prepared. Using a micropipette, 300 μL of the aggregate solution and 15 μL of Orange II were transferred into a 15 mL centrifuge tube (Corning Incorporated (Corning, NY)). A buffer solution of pH 7 (ionic strength 0.235 M) was used to bring the volume of the solution up to 3 mL yielding 114 μM Orange II and final concentration of aggregates to be 521 μg/ml of the reaction solution. This sequence of adding buffer after exposing the dye to the aggregates was maintained for all the experiments. After five minutes of exposure, this solution was centrifuged for 30 minutes, at ~8400 rcf. This centrifugation time was used to ensure that all the dispersed aggregates were removed from solution (as confirmed from differential interference contrast microscopy, data not shown). The supernatant was carefully removed from the tube without disturbing the pellet of aggregates at the bottom of the tube and transferred to a quartz cuvette. The absorbance was measured using a HP8452A UV-Vis spectrophotometer with a diode array detector (previously Agilent Technologies, Inc., Santa Clara, CA). The initial concentration of Orange II for all the uptake

studies was maintained at 114 μM and before very experiment, the initial absorbance of Orange II was measured in the absence of the aggregates. Also, it was observed that centrifuge tube did not have any effect on the initial absorbance of Orange II, as a function of time the solution stayed in the tube.

To study the uptake of Orange II as a function of temperature, the solution of Orange II and aggregates was held at 50 ˚C (microgels deswell), for different intervals of time and then cooled down to room temperature (microgels reswell). The solutions were then centrifuged, and the supernatant was then analyzed by UV-Vis to evaluate the percent uptake of the dye.

Orange II leaking studies:

To evaluate and compare the ability of the aggregates to retain Orange II, 900 μL of microgel aggregates were exposed to 114 μM Orange II in a total volume of 9 mL using the same buffer that was used for the uptake studies. The concentrations and volumes were scaled up three times in order to get a detectable absorbance signal from the solutions after leaking. The aggregates were exposed to the dye for five minutes and centrifuged for 30 minutes. The supernatant solution was then carefully removed without disturbing the aggregates packed at the bottom. To this tube, 9.0 mL of fresh buffer solution (the same buffer that was used for the uptake studies) was added and the aggregates were redispersed by vortexing. This solution was immediately divided into nine, one mL samples in 1.5 mL Eppendorf tubes obtained from Fisher Scientific, (Ottawa, ON) and the solutions were allowed to incubate for various intervals of time. For example, immediately following splitting up the original solution, one tube was centrifuged for 30 min and the supernatant solution removed and UV-Vis performed on the supernatant solution, this was considered t = 0. Other tubes were incubated for 10, 20, 30, 40, 50, 60, 70 and 80 minutes respectively, before each was centrifuged.

Counting of Aggregates:

Solutions containing 41.6 μg aggregates/mL of the respective aggregates (microgels of all diameters containing 100 mg, 500 mg and 750 mg BIS) were prepared. These solutions were prepared from the same stock solution as above. A drop of each solution was placed on a microscope cover slip (25 mm x 25 mm, Fischer Scientific, Ottawa) and pictures of 10 random areas of the sample were obtained using a Olympus IX71 inverted microscope (Markham, Ontario) fitted with a 100 X oil immersion objective, differential interference contrast (DIC) optics and an Andor Technology iXon+ camera (Belfast, Ireland). There were different sizes of aggregates in these samples that were designated as "big", "medium" and "small". When making the designation of size (detailed in Tables SI1, SI2 and SI3) we measured the apparent diameter of the aggregates in approximately two orthogonal directions. A micrometer scale of 50 divisions, each 2 μm in length (Edmund Optics, NJ) was used to calibrate the microscope, and subsequently used to measure the sizes of these aggregates.

Results and Discussion

Previously, we illustrated that pNIPAm based microgels can be used for removing the organic dye molecule Orange II from aqueous solutions.[1] We went on to show that aggregates of microgels were significantly more effective at removing Orange II from solution as the same mass of unaggregated microgels. We concluded that it was the structure or nature of the aggregated microgels that resulted in the enhanced uptake.[1] Here, we investigate how the uptake depends on the diameter of the microgels that make up the aggregate.

Effect of BIS concentration on removal efficiency:

Initially, the effect of varying concentrations of BIS in the various aggregates was investigated. Microgel aggregates were prepared using 100 mg BIS, 500 mg BIS and 750 mg BIS. To conduct

this experiment, the initial absorbance of 3 mL of a 114 μM Orange II solution in the absence of microgel aggregates was recorded. This was compared to the absorbance of the supernatant solution after addition of 100, 200, 300 and 400 μL (final concentrations of 173, 347, 521 and 693 μg aggregates/mL of the reaction solution) of the standard aggregate solutions at all BIS concentrations, for all microgel diameters. For all experiments, the initial concentration of Orange II was 114 μM and the total volume of solution was held constant at 3 mL from run to run. A calibration curve (see Supporting Information) was used to calculate the number of moles of Orange II in solution before and after addition of aggregates. Figure 2 shows the uptake data for the 100 mg , 500 mg and 750 mg BIS aggregates made from all sets of microgels. It is evident that for a given microgel diameter, the maximum removal efficiency increases with increasing BIS concentration. For example, at room temperature, the removal efficiency for the pNIPAm-*co*-AAc-1 based aggregates increased from 30.4% to 40.1% as the BIS concentration increased from 100 to 500 mg. A similar trend was observed for the pNIPAm-*co*-AAc-3 microgel aggregates, where the uptake increased from 49.1% to 53.0% when the concentrations of BIS increased from 100 mg to 500 mg, at room temperature. In previous work we reported a similar trend for the pNIPAm-*co*-AAc-2 microgel aggregates ,[1] where the removal efficiency increased from 40.8% to 44.1% when the concentrations of BIS increased from 100 to 500 mg. It should be noted that for all the sets of aggregates there was only a minimal increase in the uptake percent as the concentration of BIS was increased to 750 mg , therefore we did not increase the BIS concentration further.

We hypothesize that the trend of increased uptake efficiency with increasing concentration of BIS in the aggregates could be due to: 1) an increase in the number or size of aggregates in solution; and/or 2) a change in the hydrophobicity of the aggregates with BIS concentration used

to form the aggregates. Similar to our previous report, an aggregate counting experiment was performed to explain the observed trend of higher removal efficiencies for higher concentrations of BIS in the aggregates. In short, solutions containing 41.6 μg aggregates/mL solution of the 100 mg, 500 mg and 750 mg BIS aggregates, for all the various microgel diameters, were prepared and the number and size of the aggregates in the given volume were determined (see Supporting Information). We observed that for the pNIPAm-*co*AAc-1 aggregates, the aggregate number increased from 33 ± 7.8 to 49 ± 9.6 as the amount of BIS increased from 100 mg to 750 mg. For this same change in BIS concentration, the number of pNIPAm-*co*-AAc-2 microgel aggregates increased from 52 ± 10.0 to 76 ± 15.3, while the numbers increased from 67 ± 10.5 to 92 ± 13.6 for the pNIPAm-*co*-AAc-3 microgel aggregates. From these results, it is evident that the aggregate number increased with BIS concentration for a given microgel diameter. It can also be observed that pNIPAm-*co*-AAc-3 microgel aggregates have the highest overall aggregates number (92 ± 13.6) compared to 49 ± 9.6 for the pNIPAm-*co*-AAc-1 microgel aggregates. Furthermore, the pNIPAm-*co*-AAc-3 microgel aggregates were significantly larger than aggregates generated from the smaller diameter microgels.

To understand what factor is most important to affect removal efficiency -- aggregate size or number -- we look to the high aggregate concentration regime in Figure 2 (a, b, c). In this regime, aggregate concentration (i.e., aggregate number) has no impact on uptake efficiency, therefore the differences in uptake efficiency must be due to the nature of the aggregates. In this region, for all BIS concentrations, the uptake efficiency increases with microgel diameter; it appears that aggregate size and/or aggregate hydrophobicity is the most important factor leading to enhanced uptake efficiency. However, since unaggregated “big” microgels remove more

organic dye than a given mass of "small" unaggregated microgels, this can also be playing a role. In the lower concentration regime in each of the panels in Figure 2, there could be multiple reasons for the observed trend in the uptake efficiencies. These include either or all of the arguments above, which are:-an increase in aggregate number, and/or increase in the aggregate size, and/or hydrophobicity of the aggregates.

As a control, we aggregated 500 mg BIS alone in 40 mL DI water using the protocol mentioned in the Experimental, but in the absence of microgels. After the aggregation of BIS, either 10 mL of pNIPAm-*co*-AAc- 1 or pNIPAm-*co*-AAc- 3 microgels were added to the mixture; this solution has the same amount of BIS and microgels as the standard aggregated sample. Uptake studies were performed by monitoring the removal efficiency as a function of the aggregate concentration from these syntheses. The data in Figure 2 (b) shows that the control aggregates from the pNIPAm-*co*-AAc- 1 and 3 removed only 7.8% and 8.5% of the dye from the solution, respectively, as opposed to 40.1% and 53.0% from their aggregated microgel counterparts, at room temperature. This reveals that, like our previous report,[1] it is important to have microgels in their aggregated form to result in enhanced removal efficiency, i.e., the presence of aggregated BIS does not lead to enhanced uptake.

Effect of Particle Size in the Aggregates on the Removal Efficiency:

In our previous reports we evaluated the ability of microgels and microgel aggregates to remove Orange II from water as a function of their concentrations.[1, 69] We reported a maximum uptake of 29.5% for the microgels of ~1.1 μm at room temperature, which increased to 45.1% upon aggregation of these microgels with 500 mg BIS. Here, we investigate the effect of microgel diameter in the aggregates on the removal efficiency. Figure 2 (b) shows the percent uptake as a

function of concentration of the aggregates with pNIPAm-*co*-AAc-1, pNIPAm-*co*-AAc-2 (data from our previous report) [1] and pNIPAm-*co*-AAc-3 microgels. The figure reveals the maximum removal efficiencies to be a function of the diameter of the microgels used to synthesize the aggregates; a maximum of 40.1% uptake was achieved for pNIPAm-*co*-AAc-1, 45.1% was achieved for pNIPAm-*co*-AAc-2, while 53.0% uptake was achieved for pNIPAm-*co*-AAc-3. From aggregate counting/sizing experiments it was evident that the dimensions of the 500 mg BIS, pNIPAm-*co*-AAc-3 microgel aggregates were much larger than those of pNIPAm-*co*-AAc-1 microgel aggregates. A similar trend of increased uptake efficiency with microgel diameter in the aggregates was also observed at the different BIS concentrations, Figure 2 (a, c). This trend is observed at all aggregate concentrations, most importantly, in the high concentration regime. Since, for a given BIS concentration, the big microgel aggregates are larger than the small microgel aggregates, we attribute the enhanced uptake to the size of the aggregate. Larger aggregates have more volume, and therefore more free volume between the aggregated microgels, which can act to remove more dye from the aqueous solution. We hypothesize that the size of microgels within the aggregates affects the size of the aggregates that are formed. In other words, the big particles form bigger aggregates, which in turn achieve higher removal efficiencies. We also studied the uptake efficiency of the unaggregated microgels (with the exception of the pNIPAm-*co*-AAc-1 sample, which were too small to centrifuge) and observed the unaggregated pNIPAm-*co*-AAc-3 microgels to remove 38.0% of Orange II, as opposed to 29.5% for the pNIPAm-*co*-AAc-2 the microgels (see Supporting Information). This fact can also lead to the enhanced uptake for the pNIPAm-*co*-AAc-3 microgel aggregates in the high concentration regime.

It should be pointed out here that even though the unaggregated microgels have more active component (microgels) than the same mass of aggregated microgels, they still remove significantly less Orange II compared to the aggregates. For example, unaggregated pNIPAm-co-AAc-3 microgels remove 38.0% of dye from water compared to 53.0% removal efficiency for the same mass of aggregated pNIPAm-co-AAc-3 microgels. To account for this lack of active component in the aggregates, we adjusted the mass of the aggregates, such that they had the same number of microgels as the unaggregated samples. The data shows that a maximum of 72.8% removal efficiency was achieved at room temperature for the 500 mg BIS pNIPAm-*co*-AAc-3 microgel aggregates (see Supporting Information).

Effect of temperature cycle on removal efficiency:

To monitor the effect of single temperature cycle, we added 300 μL of the 500 mg BIS aggregates composed of pNIPAm-*co*-AAc-1 and 3 microgels to 15 μl of Orange II in pH 7 (0.235M ionic strength) in a 3 mL total volume. The solution was heated above 32 ˚C for different intervals of time and cooled down to room temperature. Specifically, we exposed the microgel aggregates to the dye solution for five minutes and heated to 50 ˚C for 20, 40, 60, 90 and 120 minutes, the solution was then cooled down to room temperature (~23 ˚C) for 30 minutes and centrifuged immediately. Figure 3(a) shows how the percent uptake depends on heating time for the pNIPAm-*co*-AAc-1 and 3 microgels aggregates. The percent uptake at time 0 corresponds to the uptake of the microgel aggregates at room temperature (39.6% and 52.1% for pNIPAm-*co*-AAc-1 and 3 microgel aggregates). It was observed that the uptake of the dye increased significantly from 44.1% to 61.4% and 69.8% to 83.7% for the pNIPAm-*co*-AAc-1 and 3 microgel aggregates, respectively, as the heating time increased from 20 minutes to 60

minutes. Also, there was minimal increase in the uptake when the solution was heated for 90 minutes (~63.1% and 84.6% for the "small" and "big" microgel aggregates respectively). The dye removal by the pNIPAm-*co*-AAc-3 microgel aggregates was very significant (maximum of ~85%) as can be seen visually in Figure 4; the solution color for the heated sample is significantly reduced compared to the solution of Orange II in the absence of aggregates, at room temperature. A control experiment confirmed that high temperature alone did not affect the absorbance of Orange II (see Supporting Information). Hence the reduction in Orange II intensity was due to increased uptake due to the aggregate's thermoresponsive nature.

The effect of multiple heating and cooling cycles on the percent uptake of the dye was also investigated. This was done by exposing the 500 mg BIS pNIPAm-*co*-AAc-1 and 3 microgel aggregates (aggregate concentration of 521 μg/mL of reaction solution) to 15 μL Orange II in the pH 7.0 buffer solution used above. This solution was heated to 50 ˚C and cooled down to room temperature in two cycles, keeping the overall heating and cooling time the same as with the single heating cycle. For example, the solution of aggregates was exposed to the dye and heated to 50 ˚C for 10 minutes and cooled down to room temperature for 15 minutes and heated again to 50 ˚C for another 10 minutes and finally cooled for 15 minutes. So overall the microgels were heated for 20 minutes and cooled for 30 minutes, but over two cycles. This protocol was repeated for all 40, 60, 90 and 120 minutes heating periods. Figure 3(b) shows that the maximum percent uptake observed for the aggregates of pNIPAm-*co*-AAc-1 microgels increased to 70.6%, compared to the single heating cycle where the maximum uptake observed was 63.1%. But there was minimal increase for the pNIPAm-*co*-AAc-3 microgel aggregates for an additional hot cycle; the maximum uptake achieved was 85.3% compared to 83.7% uptake for the single heating cycle (see Supporting Information). The overall percent uptake of the dye by the

pNIPAm-*co*-AAc-3 microgel aggregates was ~ 10% higher than similarly treated "medium" microgel aggregates, that yielded a maximum of 73.1% at elevated temperature [1] We also investigated the effect of cycling between high and room temperature three times for the aggregates of pNIPAm-*co*-AAc-1 microgels. There was no further improvement in the removal efficiency.

Leaking of Orange II from aggregates:

In our previous work we reported that pNIPAm-*co*-AAc-2 microgel aggregates retained 75.6% of the dye that was removed from the aqueous solution.[1] We studied the ability of the pNIPAm-*co*-AAc-1 and 3 microgel aggregates to retain Orange II as well. This was done by exposing 900 μL of the microgel aggregates to 114 μM Orange II in a total volume of 9 mL pH 7 buffer. This solution was allowed to incubate for five minutes and immediately centrifuged, similar to the other uptake experiments at room temperature. The supernatant solution was removed and the microgel aggregates packed at the bottom of the tube were redispersed in 9 mL fresh buffer (same buffer as above) using a vortexer. This solution was equally divided into 9 Eppendorf tubes and incubated for different intervals of time from 0 to 80 minutes and centrifuged. The supernatant solution from each of these tubes was evaluated for the amount of Orange II present in them and the percent leak of Orange II was determined. As an example, the "10 min" sample was incubated for a period of ten minutes, which was then immediately centrifuged and the supernatant removed. The solution absorbance was then analyzed to evaluate the percent leak. The "0 min" sample was immediately centrifuged without incubation. The supernatant from these samples was evaluated for the number of moles of the dye present in it and compared with the number of moles of Orange II originally present in the aggregates. Figure 5 represents the

percent leak of Orange II from the aggregates from both the microgels, as a function of leaking (incubation) time. The percent leak of the dye increased from 25.4% to 33.0% and 16.2% to 23.1% for the aggregates from pNIPAm-*co*-AAc-1 and 3 microgel aggregates, respectively, as the incubation time increased from 10 to 80 minutes. The Figure also shows a minimal increase in the percent leak of the dye after a period of 50 minutes. Overall, the maximum percent leak of the dye was calculated to be 33.0% (retention efficiency of 67.0%) and 23.1% (retention efficiency of 76.9%) for the pNIPAm-*co*-AAc-1 and 3 microgel aggregates, respectively. We also monitored if any more dye leaked out upon addition of fresh buffer. To do this, we added fresh buffer to each of these samples that underwent one leaking cycle. Each of these sample aggregates were redispersed in the buffer and incubated for 80 minutes, centrifuged, and the supernatant solution analyzed via UV-Vis. We observed that there was no additional leaking from the aggregates. Additionally, we investigated the possibility of the microgels leaking Orange II at elevated temperature due to deswelling of aggregates above their LCST. To do this, we redispersed all the aggregate samples that underwent one leaking cycle in their supernatant solutions and incubated them for an additional 80 minutes on a hot plate at 50˚C and was immediately centrifuged for 30 minutes at 50˚C by regulating the thermostat on the centrifuge. The supernatant was analyzed by UV-Vis and no more dye leaked out from the aggregates of both microgels.

Langmuir sorption isotherm for the removal of Orange II:

The removal efficiency for the pNIPAm-*co*-AAc 1 and 3 microgel aggregates as a function of the concentration of Orange II in solution was monitored. To do this, 5, 10, 15, 20, 25, 30 and 35 μL of Orange II solution was added to 300 μL of the 500 mg BIS from both the aggregates were

added. The total volume of this solution was made to be 3 mL using pH 7.0 buffer. This solution was incubated for five minutes similar to other uptake experiments. The solution was then centrifuged for 30 minutes and the supernatant was analyzed for the number of moles of the dye "sorbed" on the aggregates (as explained previously). A Langmuir isotherm model (Figures 6a and 7a) was used to fit the results from this experiment (equation 1), which gave a good fit with a R^2 of 0.9716 and 0.9934 for the aggregates from pNIPAm-*co*-AAc-1 and 3 microgels, respectively. A maximum sorption of 145.6 μmoles/g (0.051 g Orange II/g aggregates) was achieved from the aggregates from pNIPAm-*co*-AAc-1 microgels with a Langmuir coefficient of 0.0123 ± 0.00212 L/μmoles The aggregates from pNIPAm-*co*-AAc-3 microgels resulted in a maximum sorption of 171.3 μmoles/g (0.06 g Orange II/g aggregates), with a Langmuir coefficient of 0.03202 ± 0.00243 L/μmoles. Previously, for the aggregates from pNIPAm-*co*-AAc-2 microgels, we reported an R^2 of 0.9848 and a maximum sorption of Orange II of 152.8μmoles/g (0.054 g Orange/ g aggregates).[1] Upon comparison, it is evident that the aggregates from pNIPAm-*co*-AAc-3 resulted in a better fit using the Langmuir sorption and a better system for removal of Orange II/g aggregates.

We also plotted a Langmuir sorption isotherm by considering only the mass of the microgels in each of these aggregates (Figures 6b and 7b). The pNIPAm-*co*-AAc-1 system resulted in an R^2 value of 0.9714 and a maximum of 2720 μmoles/g (0.950 g Orange II/g microgels), with a Langmuir coefficient of 0.0123 ± 0.00212. The pNIPAm-*co*-AAc-3 system resulted in an R^2 value of 0.9923 and the maximum Orange II sorbed was calculated to be 3145 μmoles/g (1.10 g Orange II/g of microgels), with a Langmuir coefficient of 0.03217 ± 0.00229 L/μmoles. Whereas, in our previous work, we reported an R^2 value of 0.9853 and maximum of 2871 μmoles/g (1.006 g Orange II/g of microgels) sorbed, for a similar experiment. [1] These results

make it evident that the microgels in the aggregates are more efficient in removing Orange II/g than the unaggregated sample. Concheiro and coworkers investigated the uptake of naphthalene disulfonic acid (NS-2) by pNIPAm based systems, and fit their results with a Langmuir isotherm model. They report a maximum loading of 13 mmoles/L of gel, which translates to 3.747g NS-2/L of gel, the fit has a R^2 value of ~ 0.97. [71] If we approximate the density of a water swollen gel as 1.0 g/mL (assuming this density will give the maximum loading capacity), the maximum loading capacity is 0.0037 g NS-2/g of gel. In comparison, the system reported here is far more efficient at removing organic molecules from water.

$$C_{i,s} = C_{i,smax} * K_{ads} * C_{i,m}/(1 + K_{ads} * C_{i,m}) \quad (1)$$

$C_{i,s}$ is the concentration of Orange II in aggregates (sorbent)

$C_{i,m}$ is the concentration of Orange II in mobile phase (in buffer after centrifugation)

K_{ads} is the Langmuir coefficient

Conclusion

We report the synthesis of pNIPAm microgel aggregates from pNIPAm-*co*-AAc-1 (D_H ~321 nm), pNIPAm-co-AAc-2 (D_H ~1.1 µm) and pNIPAm-*co*-AAc-3 (diameter ~1.43 µm) microgels and compare their ability to remove the organic, azo dye molecule, Orange II from aqueous solutions. The results indicate that for the aggregates synthesized from "big" microgels, a maximum removal efficiency of 85.3% was achieved while only 70.6% was achieved by the aggregates from "small" microgels at elevated temperature. Also, for aggregates synthesized from the different microgel diameters, the percent uptake of the dye increased as the concentration of BIS was increased from 100 mg to 500 mg, with minimal enhancement as the concentration was increased to 750 mg BIS. The results suggest that the size of the microgels in the aggregates and the size of the aggregates themselves are important factors that affect the

uptake efficiencies. Also, by exploiting the thermoresponsive nature of these aggregates and by increasing the number of heating/cooling cycles the removal efficiency can be enhanced. Leaking studies were performed to determine the overall retention efficiencies of the aggregates which resulted in 67.0% and 76.9% for pNIPAm-*co*-AAc-1 and 3 microgel aggregates, respectively. A Langmuir sorption model was fit to our data, and the maximum Orange II sorbed on the aggregates was determined to be 0.051 g Orange II/g aggregates and 0.06 Orange II/g aggregates were achieved by the "small" and "big" microgel aggregates respectively. The Langmuir model was also plotted for the removal efficiencies for the samples by considering the mass of the microgels in the aggregates alone. A maximum of 0.95 g Orange II/g microgels were sorbed in the case of pNIPAm-*co*-AAc-1, with an R^2 value of 0.9714 . Whereas, an R^2 value of 0.9923 and the maximum Orange II sorbed on the microgels was determined to be 1.1 g Orange II/g microgel, in the case of pNIPAm-*co*-AAc-3. These studies combined with our previous[1,69] reports would serve as a basis for our future studies to employ these systems to remediate water contaminated with compounds like naphthenic acids, PAHs, PCBs, drug molecules, etc. that pose a threat to human and aquatic life.

Supporting Information Available: Calibration plot used for the calculation of removal efficiencies, uptake of Orange II as a function of unaggregated pNIPAm-*co*-AAc-1 microgels vs. unaggregated pNIPAm-*co*-AAc-2 microgels, uptake of Orange II as a function of concentration of aggregates with pNIPAm-*co*-AAc-1 in the particle number experiment, UV-Vis spectra for Orange II held at high temperature in the absence of aggregates, uptake studies of Orange II for multiple heating and cooling cycles for aggregates with pNIPAm-*co*-AAc-1 and 3, tables detailing the results from aggregate counting experiment and DIC microscopy images of aggregates.

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References

1. Parasuraman, D.; Serpe, M. J., *ACS Applied Materials & Interfaces* **2011,** Submitted.
2. Forgacs, E.; Cserhati, T.; Oros, G., *Environment International* **2004,** *30* (7), 953-971.
3. Chen, H. Z.; Xu, H. Y.; Heinze, T. M.; Cerniglia, C. E., *Journal of Industrial Microbiology & Biotechnology* **2009,** *36* (12), 1459-1466.
4. Gupta, G. S.; Shukla, S. P.; Prasad, G.; Singh, V. N., *Environmental Technology* **1992,** *13* (10), 925-936.
5. Kabdasli, I.; Tunay, O.; Orhon, D., *Water Science and Technology* **1999,** *40* (1), 261-267.
6. Ivanov, K.; Gruber, E.; Schempp, W.; Kirov, D., *Papier* **1996,** *50* (7-8), 456-&.
7. Bhat, R. V.; Mathur, P., *Current Science* **1998,** *74* (3), 198-202.
8. Cook, S. M. F.; Linden, D. R., *Journal of Environmental Quality* **1997,** *26* (5), 1438-1440.
9. Karadag, D.; Tok, S.; Akgul, E.; Ulucan, K.; Evden, H.; Kaya, M. A., *Industrial & Engineering Chemistry Research* **2006,** *45* (11), 3969-3973.
10. Hao, O. J.; Kim, H.; Chiang, P. C., *Critical Reviews in Environmental Science and Technology* **2000,** *30* (4), 449-505.
11. Gaydardzhiev, S.; Karthikeyan, J.; Ay, P., *Environmental Technology* **2006,** *27* (2), 193-199.
12. Holt, P. K.; Barton, G. W.; Wark, M.; Mitchell, C. A., *Colloids and Surfaces a-Physicochemical and Engineering Aspects* **2002,** *211* (2-3), 233-248.
13. Sarasa, J.; Roche, M. P.; Ormad, M. P.; Gimeno, E.; Puig, A.; Ovelleiro, J. L., *Water Research* **1998,** *32* (9), 2721-2727.
14. Lee, J. W.; Choi, S. P.; Thiruvenkatachari, R.; Shim, W. G.; Moon, H., *Dyes and Pigments* **2006,** *69* (3), 196-203.
15. Shi, B. Y.; Li, G. H.; Wang, D. S.; Feng, C. H.; Tang, H. X., *Journal of Hazardous Materials* **2007,** *143* (1-2), 567-574.
16. Zemaitaitiene, R. J.; Zliobaite, E.; Klimaviciute, R.; Zemaitaitis, A., *Colloids and Surfaces a-Physicochemical and Engineering Aspects* **2003,** *214* (1-3), 37-47.
17. Golob, V.; Vinder, A.; Simonic, M., *Dyes and Pigments* **2005,** *67* (2), 93-97.

18. Mishra, A.; Bajpai, M., *Journal of Hazardous Materials* **2005,** *118* (1-3), 213-217.
19. Do, J. S.; Chen, M. L., *Journal of Applied Electrochemistry* **1994,** *24* (8), 785-790.
20. Canizares, P.; Martinez, F.; Jimenez, C.; Lobato, J.; Rodrigo, M. A., *Environmental Science & Technology* **2006,** *40* (20), 6418-6424.
21. Stackelberg, P. E.; Gibs, J.; Furlong, E. T.; Meyer, M. T.; Zaugg, S. D.; Lippincott, R. L., *Science of the Total Environment* **2007,** *377* (2-3), 255-272.
22. Sandeman, S. R.; Gun'ko, V. M.; Bakalinska, O. M.; Howell, C. A.; Zheng, Y. S.; Kartel, M. T.; Phillips, G. J.; Mikhalovsky, S. V., *Journal of Colloid and Interface Science* **2011,** *358* (2), 582-592.
23. Oliveira, L. C. A.; Rios, R.; Fabris, J. D.; Garg, V.; Sapag, K.; Lago, R. M., *Carbon* **2002,** *40* (12), 2177-2183.
24. Sanghi, R.; Bhattacharya, B., *Coloration Technology* **2002,** *118* (5), 256-269.
25. Jiraratananon, R.; Sungpet, A.; Luangsowan, P., *Desalination* **2000,** *130* (2), 177-183.
26. Liu, H. L.; Chiou, Y. R., *Chemical Engineering Journal* **2005,** *112* (1-3), 173-179.
27. Wu, J. N.; Eiteman, M. A.; Law, S. E., *Journal of Environmental Engineering-Asce* **1998,** *124* (3), 272-277.
28. van der Zee, F. P.; Villaverde, S., *Water Research* **2005,** *39* (8), 1425-1440.
29. Panswad, T.; Luangdilok, W., *Water Research* **2000,** *34* (17), 4177-4184.
30. Sponza, D. T.; Isik, M., *Enzyme and Microbial Technology* **2002,** *31* (1-2), 102-110.
31. El-Desoky, H. S.; Ghoneim, M. M.; Zidan, N. M., *Desalination* **2010,** *264* (1-2), 143-150.
32. Bayram, E.; Ayranci, E., *Environmental Science & Technology* **2010,** *44* (16), 6331-6336.
33. Nayak, S.; Debord, S. B.; Lyon, L. A., *Langmuir* **2003,** *19* (18), 7374-7379.
34. Lutz, J. F.; Weichenhan, K.; Akdemir, O.; Hoth, A., *Macromolecules* **2007,** *40* (7), 2503-2508.
35. Dupin, D.; Fujii, S.; Armes, S. P.; Reeve, P.; Baxter, S. M., *Langmuir* **2006,** *22* (7), 3381-3387.
36. Amalvy, J. I.; Wanless, E. J.; Li, Y.; Michailidou, V.; Armes, S. P.; Duccini, Y., *Langmuir* **2004,** *20* (21), 8992-8999.
37. Plunkett, K. N.; Kraft, M. L.; Yu, Q.; Moore, J. S., *Macromolecules* **2003,** *36* (11), 3960-3966.
38. Serpe, M. J.; Rivera, M.; Kersey, F. R.; Clark, R. L.; Craig, S. L., *Langmuir* **2008,** *24* (9), 4738-4742.
39. Nayak, S.; Lyon, L. A., *Chemistry of Materials* **2004,** *16* (13), 2623-2627.
40. Miyata, T.; Asami, N.; Uragami, T., *Nature* **1999,** *399* (6738), 766-769.
41. Schild, H. G., *Progress in Polymer Science* **1992,** *17* (2), 163-249.
42. Wu, C., *Polymer* **1998,** *39* (19), 4609-4619.
43. Wu, C.; Zhou, S. Q., *Macromolecules* **1996,** *29* (5), 1574-1578.
44. Matsuo, E. S.; Tanaka, T., *Journal of Chemical Physics* **1988,** *89* (3), 1695-1703.
45. Serpe, M. J.; Jones, C. D.; Lyon, L. A., *Langmuir* **2003,** *19* (21), 8759-8764.
46. Gong, X. J.; Wu, C.; Ngai, T., *Colloid and Polymer Science* **2010,** *288* (10-11), 1167-1172.
47. Hu, T. J.; You, Y. Z.; Pan, C. Y.; Wu, C., *Journal of Physical Chemistry B* **2002,** *106* (26), 6659-6662.
48. Jones, C. D.; Lyon, L. A., *Macromolecules* **2000,** *33* (22), 8301-8306.

49. Pelton, R., *Advances in Colloid and Interface Science* **2000,** *85* (1), 1-33.
50. Saunders, B. R.; Vincent, B., *Advances in Colloid and Interface Science* **1999,** *80* (1), 1-25.
51. Lyon, L. A.; Debord, J. D.; Debord, S. B.; Jones, C. D.; McGrath, J. G.; Serpe, M. J., *Journal of Physical Chemistry B* **2004,** *108* (50), 19099-19108.
52. Murray, M. J.; Snowden, M. J., *Advances in Colloid and Interface Science* **1995,** *54*, 73-91.
53. Suzuki, D.; McGrath, J. G.; Kawaguchi, H.; Lyon, L. A., *Journal of Physical Chemistry C* **2007,** *111* (15), 5667-5672.
54. Hoare, T.; Pelton, R., *Langmuir* **2004,** *20* (6), 2123-2133.
55. Brugger, B.; Richtering, W., *Langmuir* **2008,** *24* (15), 7769-7777.
56. Snowden, M. J.; Chowdhry, B. Z.; Vincent, B.; Morris, G. E., *Journal of the Chemical Society-Faraday Transactions* **1996,** *92* (24), 5013-5016.
57. Schmidt, S.; Hellweg, T.; von Klitzing, R., *Langmuir* **2008,** *24* (21), 12595-12602.
58. Kratz, K.; Hellweg, T.; Eimer, W., *Colloids and Surfaces a-Physicochemical and Engineering Aspects* **2000,** *170* (2-3), 137-149.
59. Sorrell, C. D.; Carter, M. C. D.; Serpe, M. J., *Advanced Functional Materials* **2011,** *21* (3), 425-433.
60. Thivaios, I.; Bokias, G., *Journal of Applied Polymer Science* **2010,** *116* (3), 1509-1514.
61. Morris, G. E.; Vincent, B.; Snowden, M. J., *Journal of Colloid and Interface Science* **1997,** *190* (1), 198-205.
62. Wu, Q. L.; Tian, P., *Journal of Applied Polymer Science* **2008,** *109* (6), 3470-3476.
63. Ozkahraman, B.; Acar, I.; Emik, S., *Polymer Bulletin* **2011,** *66* (4), 551-570.
64. Jin, X. Y.; Jiang, M. Q.; Shan, X. Q.; Pei, Z. G.; Chen, Z. L., *Journal of Colloid and Interface Science* **2008,** *328* (2), 243-247.
65. Laszlo, J. A., *Environmental Science & Technology* **1997,** *31* (12), 3647-3653.
66. Anandan, S.; Lee, G. J.; Chen, P. K.; Fan, C.; Wu, J. J., *Industrial & Engineering Chemistry Research* **2010,** *49* (20), 9729-9737.
67. Guilherme, M. R.; da Silva, R.; Rubira, A. F.; Geuskens, G.; Muniz, E. C., *Reactive & Functional Polymers* **2004,** *61* (2), 233-243.
68. Guilherme, M. R.; Silva, R.; Girotto, E. M.; Rubira, A. F.; Muniz, E. C., *Polymer* **2003,** *44* (15), 4213-4219.
69. Parasuraman, D.; Serpe, M. J., *ACS Applied Materials & Interfaces* **2011**, *3*, 2732.
70. Meng, Z. Y.; Smith, M. H.; Lyon, L. A., *Colloid and Polymer Science* **2009,** *287* (3), 277-285.
71. Alvarez-Lorenzo, C.; Concheiro, A., *Journal of Controlled Release* **2002,** *80* (1-3), 247-257.

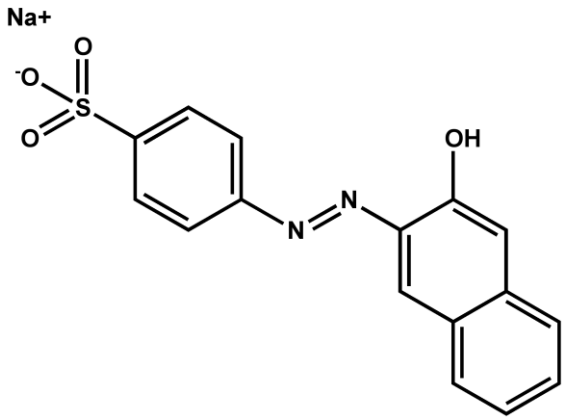

Figure 1. Structure of Orange II

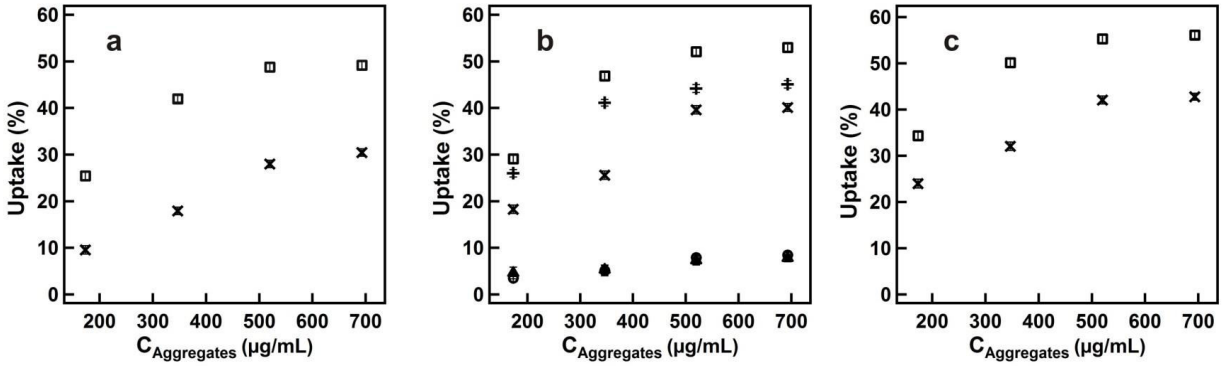

Figure 2. Uptake of Orange II as a function of the concentration of aggregates (a) 100 mg BIS aggregates; (b) 500 mg BIS aggregates and ; (c) 750 mg BIS aggregates using (**x**) pNIPAm-*co*-AAc-1 microgels, and (□) pNIPAm-*co*-AAc-3 microgels. Panel (b), shows results for (**+**) pNIPAm-*co*-AAc-2 microgels.[2] Panel (b) also contains the control experiment results; aggregating BIS, then adding the microgels, from (▲) pNIPAm-*co*-AAc-3 microgels and (■) pNIPAm-*co*-AAc-1 microgels. Each point on the plot represents an average of three replicate experiments of uptake studies and the error bars denote the standard deviation.

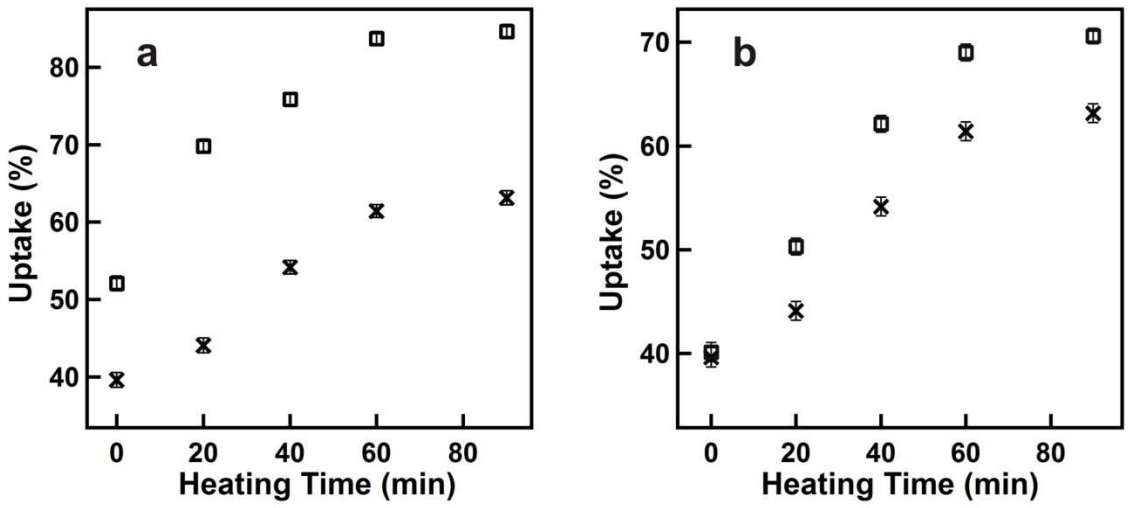

Figure 3. (a) Uptake of Orange II as a function of the time the microgels were held at elevated temperature for a single hot cycle (**x**) pNIPAm-*co*-AAc-1 aggregates and (□) pNIPAm-*co*-AAc-3 aggregates. Panel (b), uptake for pNIPAm-*co*-AAc-1 microgel aggregates after (**x**) a single hot cycle and (□) two hot cycles. Each point on the plot represents an average of three replicate experiments of uptake studies and the error bars denote the standard deviation.

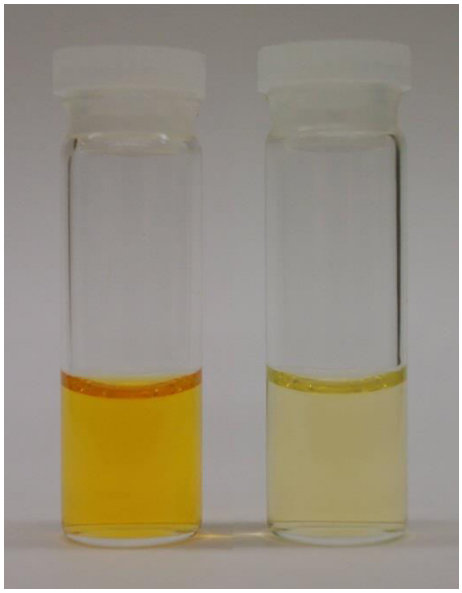

Figure 4. Solutions of 114 μM Orange II (left) before and (right) after the addition 500 mg BIS of (aggregate concentration of 693 μg/mL of reaction solution) pNIPAm-*co*-AAc-3 aggregates. Here, single cycle heating cycle was performed for a period of 90 minutes (hot time).

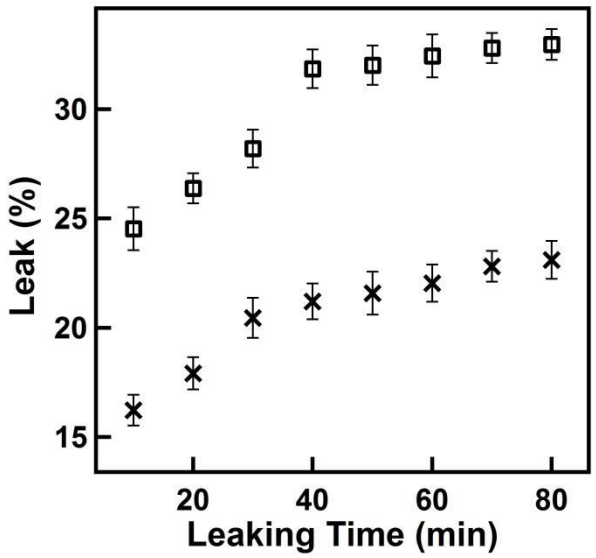

Figure 5. The percent leak of Orange II as a function of leak time for (□) pNIPAm-*co*-AAc-1 aggregates, and (**x**) pNIPAm-*co*-AAc-3 aggregates. Each point on the plot represents an average of three replicate experiments of uptake studies and the error bars denote the standard deviation.

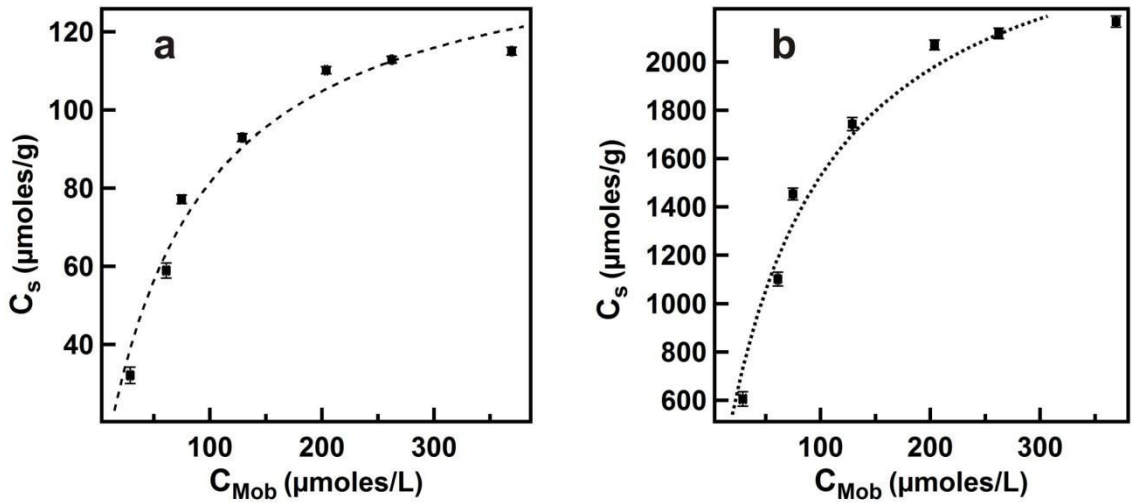

Figure 6. Langmuir sorption isotherms for the removal of Orange II by (a) the aggregates with pNIPAm-*co*-AAc-1 microgels and (b) considering only the mass of microgels in the aggregates after correcting for the mass of BIS in the aggregates. C_{Mob} is the concentration of the dye remaining in the aqueous phase and C_s is the concentration of Orange II sorbed on the aggregates/microgels. Each point on the plots represents an average of three replicate experiments and the error bars denote the standard deviation.

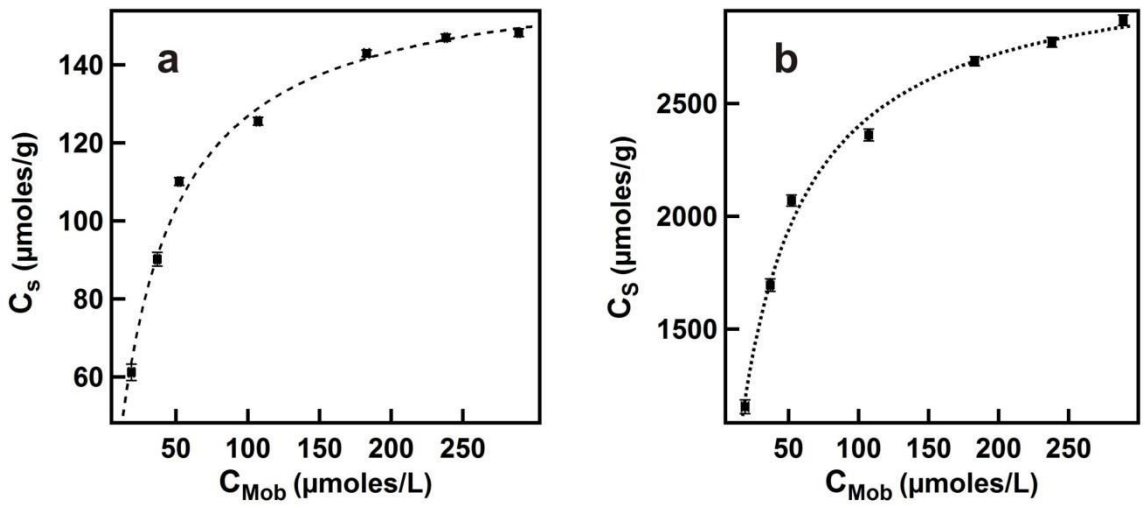

Figure 7. Langmuir sorption isotherms for the removal of Orange II by (a) the aggregates with pNIPAm-*co*-AAc-3 microgels and (b) considering only the mass of microgels in the aggregates after correcting for the mass of BIS in the aggregates. C_{Mob} is the concentration of the dye remaining in the aqueous phase and C_s is the concentration of Orange II sorbed on the aggregates/microgels. Each point on the plots represents an average of three replicate experiments and the error bars denote the standard deviation.