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Dual-Sensing Copolymers

Soluble Polymeric Dual Sensor for Temperature and pH Value**

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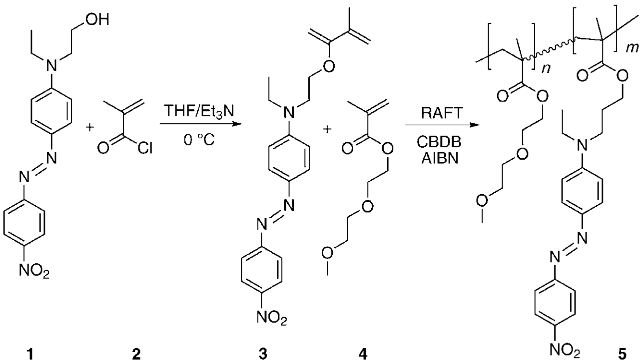
The interest in "smart" functional materials that respond to changes in the environment strongly increased in the last years owing to the desire to control complexity and to create systems that adapt or respond to the environment. Moreover, such "smart" materials are used to design and develop new responsive materials for a wide range of applications in various fields, such as biotechnology,[1–3] drug delivery,[4–6] particle transport,[7] and optical sensing.[8–11] Recently, a thermoresponsive fluorescent nanogel was applied as an intracellular thermometer, that is, to monitor the temperature in living cells.[12]

A current trend in the field of optical sensing is the development of dual sensors that respond simultaneously and independently to different stimuli.[13] In recent years, dual optical sensors have been reported for pressure and temperature,[14] oxygen and temperature,[15–18] oxygen and carbon dioxide,[19–21] as well as oxygen and pH value.[22–24] Surprisingly, no dual sensor has been reported for temperature and pH value, which would be beneficial, for example to monitor chemical reactions and for biological diagnostics.

We have aimed to develop a soluble dual sensor that responds to both temperature and pH value. The solubility of the sensor material allows monitoring in situ while at the same time providing information about homogeneity and local conditions. In contrast to reported dual sensors, which are generally based on two different sensing chromophores, we have combined a pH-responsive solvatochromic dye with a thermoresponsive polymer. Solvatochromic dyes change color in response to changes in solvent polarity.[25–27] Recently, it was reported that combining a solvatochromic dye with a temperature-responsive polymer leads to a color change upon changing the temperature, as in the dissolved state the dye is in contact with water while in the collapsed state the dye is dissolved in the less polar precipitated polymer.[8–10,12]

Herein, we report our efforts to develop a dual sensor that senses temperature by the solubility transition of a thermoresponsive polymer and senses the pH value by a pH-responsive solvatochromic dye, namely disperse red 1 (DR1, **1**; Scheme 1).[28,29] Poly(oligoethyleneglycol methacrylate) (POEGMA) was chosen as temperature sensing polymer on the basis of its biocompatibility and the possibility of tuning the lower critical solution temperature (LCST) by copolymerizing different OEGMA monomers.[30–32]



Scheme 1. The synthesis of DR1-functionalized monomer and the reversible addition fragmentation chain transfer (RAFT) copolymerization with OEGMA (AIBN = azoisobutyronitrile, which is used as initiator; CBDB = 2-cyano-2-butyldithiobenzoate, which is used as chain-transfer agent).

Since the polymer solubility transition can depend on the molar mass distribution of the copolymer, a well-defined polymer is required to ensure homogeneous solubility of the sensing polymer. Therefore, a controlled radical polymerization process, namely reversible addition fragmentation chain transfer (RAFT),[33–35] was applied to prepare well-defined copolymers of OEGMA and a methacrylate monomer functionalized with disperse red 1 (DR1-MA, **3**; Scheme 1).

For this purpose monomer **3** was prepared by esterification of the hydroxy group of **1** with methacryloyl chloride (**2**, Scheme 1).[36,37] In the next step, this dye-functionalized monomer **3** was statistically copolymerized with diethylene glycol methacrylate (DEGMA, Scheme 1) or a mixture of DEGMA and OEGMA with 22–23 ethylene glycol units by RAFT. In both cases the monomer-to-RAFT-agent ratio was 100 using 5% dye-functionalized monomer, aiming for a theoretical degree of polymerization of 100. Different ratios of DEGMA and OEGMA were chosen to investigate whether dual sensors can be prepared that cover very

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Supporting information for this article, including experimental preparations and analysis for **3** and for the copolymers **5** and **6** as well as pictures of DR1 and copolymer **5** in different solvents, is available on the WWW under http://dx.doi.org/10.1002/anie.200901071.

different temperature regimes. Size-exclusion chromatography (SEC) revealed that well-defined polymers were obtained by RAFT as indicated by polydispersity indices (PDIs) close to 1.2 (Table 1). Furthermore, disperse red 1 was successfully incorporated into the copolymers in approximately 5% content as determined by ^{1}H NMR and UV/Vis spectroscopy (Table 2).

Table 1: Overview of SEC analysis data of the synthesized copolymers.

Polymer	Monomers	[DEGMA]/[DR1]/[OEGMA]	M_n [Da][a]	PDI[a]
5	DEGMA/DR1	95/5	23100	1.18
6	DEGMA/DR1/OEGMA	45/5/50	35100	1.22

[a] Number-averaged molecular weight calculated from SEC with polystyrene standards.

Table 2: Compositions and cloud points of the synthesized copolymers.

Polymer	[M]/[DR1][a]	Composition NMR [%][b]	Composition UV/Vis [%][c]	Cloud point [°C][d]
5	95/5	95.5/4.5	95.5/4.5	17.3
6	(45+50)/5	97.0/3.0	97.0/3.0	92.1

[a] M = unfunctionalized monomer (DEGMA or DEGMA and OEGMA). [b] Calculated from the integrals of the aromatic DR1 signals and the backbone signals in the ^{1}H NMR spectra. [c] Obtained from the extinction coefficient (ε) of the UV/Vis spectrum using the Beer–Lambert Law. [d] Determined at 50% transmittance in the second heating run at 1 mg mL^{-1} in water.

A first test of the sensing ability of copolymer **5** was performed by heating aqueous solutions at different pH values above the cloud point of 17.3 °C (Figure 1). At pH 7, no clear color change was observed upon heating, but the intensity of the red color increased in the precipitated state. In contrast, a characteristic bathochromic shift from 491 to 532 nm occurs at pH 1 in concert with the solubility transition of the polymer, thus indicating a polarity change around the dye upon copolymer precipitation. It is important to note that neither the color intensity nor the color of an aqueous solution of **1** at pH 1 changes significantly with temperature. This finding clearly demonstrates that the temperature-induced color change of copolymer **5** is induced by the polymer solubility transition. Furthermore, **1** and **3** are not soluble in water at pH 7.

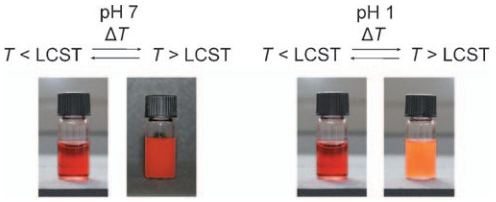
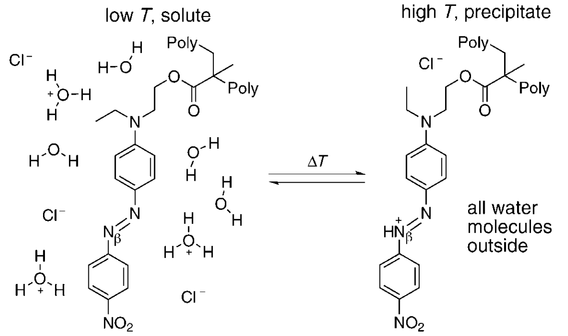


Figure 1. Observed color shift of aqueous solutions of copolymer **5** at pH 7 (left) and pH 1 (right).

The color of **1** can be influenced by the solvent, which is known as solvatochromism and can be defined as the influence of the medium on the electronic absorption spectra of molecules. The solvatochromic shift of **1** results from the interactions between the solute and the solvent causing a shift in the tautomeric equilibrium accompanied by a change in the energy difference between the ground and excited states.[29] The observed solvatochromic shifts of **1** and **5** revealed that λ_{max} increases with solvent polarity (Supporting Information, Figures S1 and S2 and Table S1).

Surprisingly, the λ_{max} of copolymer **5** is not significantly influenced by the pH value of the solution below the LCST transition. Moreover, the red color of the solution of copolymer **5** below the LCST is significantly different from the purple color of a solution of **1** at pH 1 (see the Supporting Information). Therefore, it can be concluded that the azo dye in the copolymer is not well hydrated (i.e. not dissolved) below the cloud point, thus obstructing protonation of the basic amine group. Instead, DR1 might interact with the polar DEGMA units in copolymer **5**, as indicated by the lower λ_{max} of the $\pi^*\leftarrow\pi$ transition compared to **1** in a range of solvents (Supporting Information, Table S1). Upon heating beyond the cloud point of copolymer **5**, the λ_{max} value at pH 1 shifts to 532 nm, thus indicating a high polarity around the dye. This result is in clear contrast with the anticipated lower polarity of the precipitated polymer compared to the aqueous solution, which was also previously reported.[8–10] This discrepancy is proposed to be due to solubilization of DR1 in the precipitated polymer above the cloud point, thus facilitating protonation of DR1 (Scheme 2). This proposed mechanism is supported by the fact that at pH 7, 10, and 13 no shift of λ_{max} is observed for **5** when the system passes the polymer phase transition. At these pH values, the dye cannot be protonated, and, therefore, no shift in λ_{max} is observed. Even though the pK_a of DR1 is not reported in water owing to low solubility in the unprotonated state, the pK_a of related compounds is around 2 in a water/ethanol (50:50 vol %) mixture.[38]



Scheme 2. Schematic representation of the proposed mechanism for the temperature-induced color shift of copolymer **5** at pH 1.

Furthermore, the λ_{max} of copolymer **5** above the cloud point (532 nm) is higher than the λ_{max} of **1** (515 nm) at pH 1. The absence of hydration of the protonated dye in the precipitated polymer apparently shifts the equilibrium towards protonation of the β-nitrogen atom rather than the amino nitrogen atom, thus causing a red shift of the $\pi^*\leftarrow\pi$ transition compared to **1** (Scheme 2).[39]

To further quantify the temperature and pH-responsive properties of the copolymers, UV/Vis absorption spectra were recorded as a function of temperature. A representative example of the resulting absorption spectra is given in Figure 2 for copolymer **5** at pH 7. This 3D representation clearly demonstrates that the intensity of both the absorption maxima at 292 (π*←π(n)) and 487 nm (π*←π) increase when the cloud-point temperature is crossed, which is most likely due to solvation of the DR1 moiety in the precipitated polymer. However, the intensity at 292 nm increases more steeply than the intensity at 487 nm.

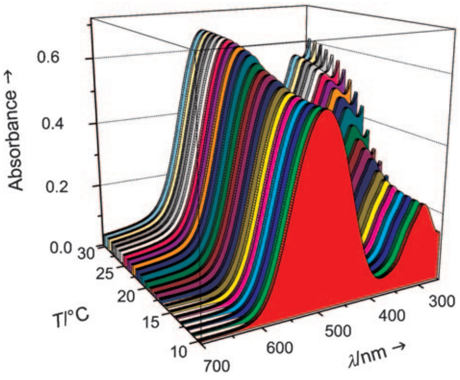


Figure 2. Temperature dependence of the UV/Vis absorption spectrum of an aqueous solution of copolymer **5** (0.1 mg mL^{-1}) at pH 7.

The absorption maximum of the π*←π transition and the intensity ratio of the π*←π(n) and π*←π transitions of **5** are plotted as functions of temperature and pH value in Figure 3. These quantitative plots correspond well to the earlier observation that at pH 7, 10, and 13 only the intensity of the color changes, as indicated by the change in the π*←π(n)/π*←π ratio (Figure 3 b, together with a negligible shift in the absorption maximum (Figure 3 a)). Furthermore, both the ratio and the absorption maximum significantly shift upon heating solutions of **5** at pH 1 and 4, thus indicating both a change in intensity as well as color upon passing the cloud-point temperature. The intensity and color transitions occur in a temperature region from 10 to 20 °C, which can be regarded as the temperature-sensing regime.

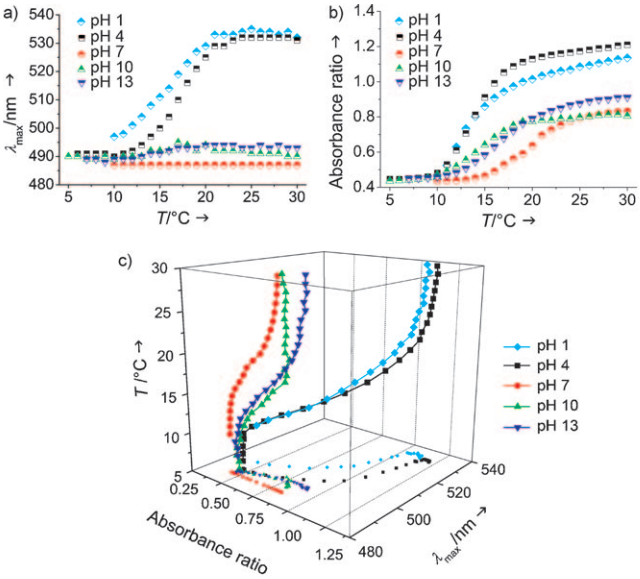


Figure 3. a) Temperature dependence of λ_{max} (π*←π transition of disperse red 1), b) temperature dependence of the absorption ratio (π*←π(n)/π*←π), and c) 3D representation of the responsiveness (λ_{max} vs. absorbance ratio vs. temperature) of poly(DEGMA-*stat*-DR1-MA) copolymer **5** (0.2 mg mL^{-1}) at different pH values.

To evaluate the dual sensing capabilities of copolymer **5**, a 3D plot of absorption maximum, absorption ratio (π*←π(n)/π*←π), and temperature is depicted for different pH values in Figure 3 c. A projection of the absorption maximum and the absorption ratio is displayed in the *xy* layer, demonstrating that each temperature in the transition regime results in a unique projection point at pH 1, 4, and 7. As a result, it can be concluded that **5** represents the first reported (soluble) dual sensor for both temperature and pH value with sensitivity for temperatures from 10 to 20 °C and pH values from 1 to 7. Above pH 7, the projection points overlap and thus the temperature and pH value cannot be distinguished.

The temperature-sensing capability of copolymer **5** is at rather low temperature but should be tunable to higher temperatures by incorporating a more hydrophilic OEGMA monomer. Therefore, copolymer **6** was prepared with DEGMA, OEGMA, and **3**, which has a significantly higher cloud point at 92.1 °C (Table 1). Surprisingly, heating poly(OEGMA-*stat*-DEGMA-*stat*-DR1-MA) **6** above the cloud point did not result in a color shift at pH values ranging from 1 to 13.

The quantitative measurement of the UV absorption at different temperatures and pH values clearly demonstrates that the absorption maximum is unaffected by the temperature at all investigated pH values, while the ratio π*←π(n)/π*←π increases when the cloud point is crossed (Figure 4; see the Supporting Information for 3D plot). The temperature transition regime ranges from 86 to 96 °C; it can thus be concluded that the width of the solubility transition is not affected by the transition temperature, as both copolymers **5** and **6** show a 10 °C transition regime. Figure 4 a and the 3D representation (Supporting Information, Figure S3) clearly demonstrate that the more hydrophilic copolymer **6** cannot be used as a dual sensor, because the λ_{max} values fall onto the same line at different pH values.

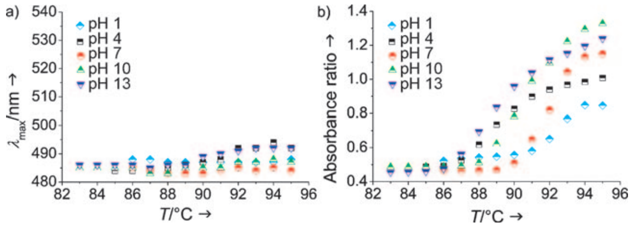


Figure 4. a) Temperature dependence of λ_{max} (π*←π transition of disperse red 1) and b) temperature dependence of the absorption ratio (π*←π(n)/π*←π) of poly(OEGMA-*stat*-DEGMA-*stat*-DR1-MA) **6** (0.3 mg mL^{-1}) at different pH values.

Even though it is not possible to prepare a dual-sensing copolymer with a temperature transition around 90 °C using the concept presented herein, these results indicate a substantial difference in the cloud-point transition phenomena of hydrophilic and hydrophobic (co)polymers. The hydrophobic polymer expels water in the collapsed state, thus resulting in a large difference in polarity and causing a color shift of **1** owing to the formation of a weakly hydrated protonated DR1. In contrast, the hydrophilic polymer apparently remains well-hydrated even in the collapsed state; thus, **3** is not well-dissolved even in the precipitated polymer, and the solvatochromic shift does not occur.

In conclusion, the synthesis and evaluation of a thermoresponsive DEGMA copolymer bearing disperse red 1 as solvatochromic dye revealed that these materials can act as soluble dual sensors for both temperature and pH value. The dual-sensitive polymeric material shows responsiveness in a temperature range from 10 to 20 °C and a range of pH values from 1 to 7. However, when the hydrophilicity of the copolymer is increased by incorporating OEGMA as comonomer, the solvatochromic color change was lost, making the polymer unsuitable as dual sensor. Nonetheless, this latter result clearly shows the potential of including solvatochromic dyes into thermoresponsive polymers to gain a better fundamental understanding of hydration/dehydration phenomena during polymer solubility transitions. As such, the impact of this work will be twofold, namely for the design and development of new sensor materials and as a new research tool to evaluate the hydration of thermoresponsive polymers.

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