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Poly(*N*-isopropylacrylamide) coated gold nanoparticles as colourimetric temperature and salt sensors

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Thermoresponsive poly(*N*-isopropylacrylamide) (PNIPAM) coated gold nanoparticles (AuNPs) are reported having residual citrate groups on the surface resulting from their synthesis via the Turkevich method. These PNIPAM coated AuNPs have dual stabilisation by the polymer chains and the charges, which we exploit for the development of temperature and salt sensors of which the sensing regime can be tuned by variation of salt concentration and temperature, respectively.

Nanoparticles often have distinct physicochemical properties that can serve as basis for developing sensing tools.[1,2] Gold nanoparticles (AuNPs) are frequently utilized due to their unique optoelectronic properties and their straightforward synthesis.[1] Well dispersed AuNP solutions exhibit a strong localized surface plasmon resonance (SPR) due to the collective oscillation of the conduction electrons across the nanoparticle and display intense size-dependent colour in the visible spectrum.[1,3] Aggregation of small red-coloured AuNPs induces electronic coupling of the SPR leading to a visible colour change from red to purple or blue.[4] AuNP based colourimetric sensors are abundant, whereby the colour changes are utilized as a quantification tool. Such AuNPs have been used for the design of a wide range of sensors that can detect target analytes, such as DNA, proteins, small organic molecules, and inorganic ions.[5] Driven by the need for monitoring and measuring a wide range of physical parameters and analyte concentrations, the most popular and versatile sensor systems that have been developed include fluorescence-based sensors,[6] electrochemical sensors[7] and colourimetric sensors.[1,8] The major advantage of colourimetric sensors is the very simple and inexpensive analytical tool (UV-vis) that is needed for the detection of changes in the AuNP SPR band upon binding to the target or upon variation of the environmental conditions. Furthermore, these colour changes can easily be observed by the naked eye.[9] The clear change in colour due to the association and redispersion of AuNPs offers an absolute basis for the design of absorption-based colourimetric sensor for all kinds of target analytes, such as metal ions, anions[10] small organic molecules as well as biomolecules like proteins and nucleic acids,[11] that directly or indirectly trigger the AuNP aggregation or redispersion

behaviour. In aqueous medium AuNPs coated with thioglucose groups have, for example, shown high selectivity for fluoride anions against other anions such as Cl^-, Br^-, I^-, AcO^-, and NO_3^- over a relatively narrow concentration range (20−40 mM).[10b] An AuNP-embedded plasticized poly(vinyl chloride) (PVC) membrane has been demonstrated to be highly selectivity for sensing of I^- anions in the presence of other anions such as F^-, Cl^-, Br^-, N_3^-, NO_2^-, NO_3^- and AcO^-.[10c]

Recently, thermoresponsive polymer coated AuNPs were also considered as promising materials for the design of colourimetric sensors as they exhibit strong response to external stimuli, such as temperature,[12a,13] pH[8a,12b,c] and salts,[8a,12d-g] which are all three important parameters for monitoring of biological systems.

Recently, it has been reported that PNIPAM coated AuNPs – prepared via citrate reduction of $HAuCl_4$ – exhibit a reversible colour change from red to blue purple when heated from 25 °C to 40 °C, only in presence of NaCl.[12d, 12e] Morishima reported that temperature-induced PNIPAM-AuNP aggregation with 50 mM NaCl while we reported such thermoresponsive behaviour with 100 mM NaCl, ascribed to the necessity of charge screening to account for the negative charges resulting from residual citrate groups on the PNIPAM-AuNP surface due to incomplete exchange.

Inspired by this requirement of salt for the temperature-induced AuNP aggregation and colour change (Scheme 1), we focused our attention to the development of tunable colourimetric temperature and salt-sensors in the current work. Temperature sensing is envisioned by the temperature-induced colour change, whereby the transition temperature may be tuned by variation of the salt concentration in the solution. Similarly, under isothermal conditions, increasing the amount of salt can potentially induce the AuNP aggregation and associated colour change, being the basis for salt sensing. The latter has been studied with two salts from the Hofmeister series, where one shows 'salting out' effect (NaCl) and another 'salting in' effect (NaSCN) to evaluate whether the system purely responds as ionic strength sensor based on salt screening or that the salt-induced PNIPAM (de)hydration also plays a role.

The synthesis of the PNIPAM was accomplished by RAFT polymerization using methyl 2-(((butylthio)carbonothioyl)thio) propanoate (MBTTC) as chain transfer agent (CTA) as reported
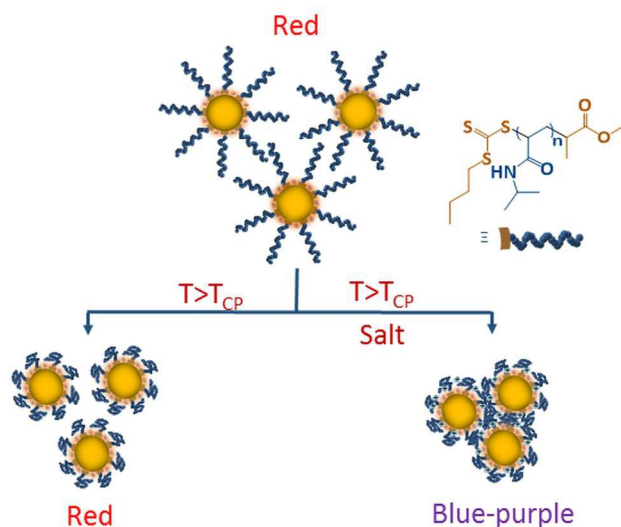
Scheme 1 Schematic representation of the effect of salt and temperature on the PNIPAM@AuNP aggregation and colour change.

earlier.[12e] The detailed procedure for the synthesis of PNIPAM is described in the ESI†. SEC analysis data demonstrated that a well-defined polymer was obtained with M_n =10.6 kg/mol and Đ = 1.1. ^{1}H NMR spectroscopy revealed that the degree of polymerization (DP) of the polymer is 43. Turbidity measurements of the synthesized PNIPAM (5 mg/mL) revealed a T_{CP} of 27 °C, which is slightly lower than the typical value of 32 °C found in literature. This can be ascribed to the presence of hydrophobic end functionality in the relatively low molecular weight polymer.[12e]

A red coloured solution of citrate stabilized gold nanoparticles (AuNPs) was obtained via the Turkevich citrate reduction method where $HAuCl_4$ salt was reduced by sodium citrate in aqueous medium under reflux condition (Scheme 2).[12e-g, 14] UV-vis spectroscopy demonstrated an absorption maximum of the SPR band at 522 nm for the citrate stabilized AuNPs (citrate@AuNPs) measured at 20 °C. The synthesized citrate@AuNPs do not show any significant change of the surface plasmon resonance (SPR) band upon heating from 20 °C to 45 °C (Fig. S1 (left), ESI†). Transmission electron microscopy (TEM) data revealed a mean diameter of ca 15±2 (Fig. 1A) whereas dynamic light scattering (DLS) data revealed a hydrodynamic diameter of 30 nm at 20 °C (Fig. S2, ESI†). Size based on the DLS measurements always shows a higher diameter due to the measurements of hydrodynamic diameter in aqueous medium while TEM was measured in dry state. The citrate@AuNPs had a zeta potential of ca. -36 mV, indicating the effective stabilization via repulsive electrostatic forces.

In order to investigate the effect of the thermoresponsive PNIPAM on the AuNPs, trithiocarbonate functionalized PNIPAM coated AuNPs (PNIPAM@AuNPs) were prepared by exchange of the comparatively weakly bound citrate on the AuNPs via a 'grafting to' approach. It has been reported that the RAFT end group of polymers showed strong affinity towards AuNPs, which can efficiently be used to attach them to the gold surface without the need for prior reduction of the RAFT end groups into thiols.[15] Excess non-grafted polymers were removed from the PNIPAM@AuNPs by three centrifugation-redispersion cycles using MilliQ water. The successful modification of the AuNPs was confirmed by UV-vis spectroscopy, TEM and DLS. UV-vis spectroscopy showed a slight shift of absorption maximum of the SPR band from 522 nm to 526 nm clearly indicating the successful modification of the AuNPs with PNIPAM leading to a slightly less polar microenvironment that

induces a red shift in the SPR band as shown in Fig. S3 (ESI†).[16] After the PNIPAM modification, the colour of the PNIPAM@AuNPs solution remained red as the SPR shift was not high enough to change the colour. Moreover, UV-vis absorption spectroscopy revealed that upon heating from 20 °C to 45 °C, (which is above the T_{CP} of the PNIPAM) these PNIPAM@AuNPs do not show any significant change of the SPR band (Fig. S1 (right), ESI†). However, TEM clearly revealed the presence of a polymer corona surrounding the AuNPs (Fig. 1B). DLS also confirmed that the polymer modification was successful as the hydrodynamic diameter increased from 30 nm to 47 nm (Fig. S2, ESI†). No significant change in the hydrodynamic size was observed upon heating from 20 to 45 °C demonstrating the unresponsive behaviour of the PNIPAM@AuNPs. The DLS size distributions of both citrate@AuNPs and PNIPAM@AuNPs showed a second smaller size distribution below 10 nm measured by DLS. It has been reported that colloidal gold can reveal such a second smaller size distribution if the AuNPs have an anisotropic, non-spherical shape, which can be attributed to the rotational diffusion of the AuNPs.[12f, 17] As translational diffusion is angular dependent and rotational diffusion is not, DLS measurements were performed at two different scattering angles (90 deg. and 173 deg.) (Fig. 1C).
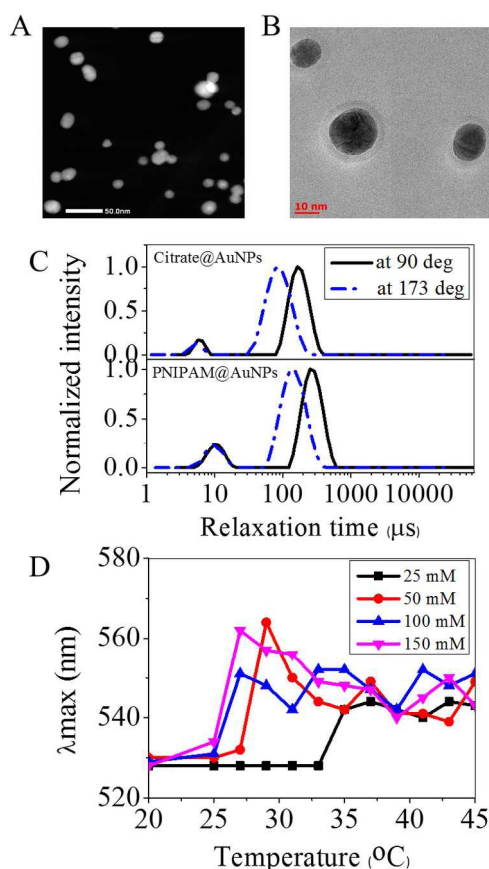
Fig. 1 (A) Bright field scanning transmission electron microscopy (STEM) picture of the citrate@AuNPs (scale bar = 50 nm). (B) Transmission electron microscopy (TEM) pictures of PNIPAM coated AuNPs (scale bar = 10 nm). (C) Normalized intensity vs. relaxation time plot for citrate and the PNIPAM@AuNPs, measured at two different scattering angles (90 and 173 deg) at 25 °C. (D) The λ_{max} peak variation of the PNIPAM@AuNPs as a function of temperature at four different NaCl concentration 25, 50, 100 and 150 mM.
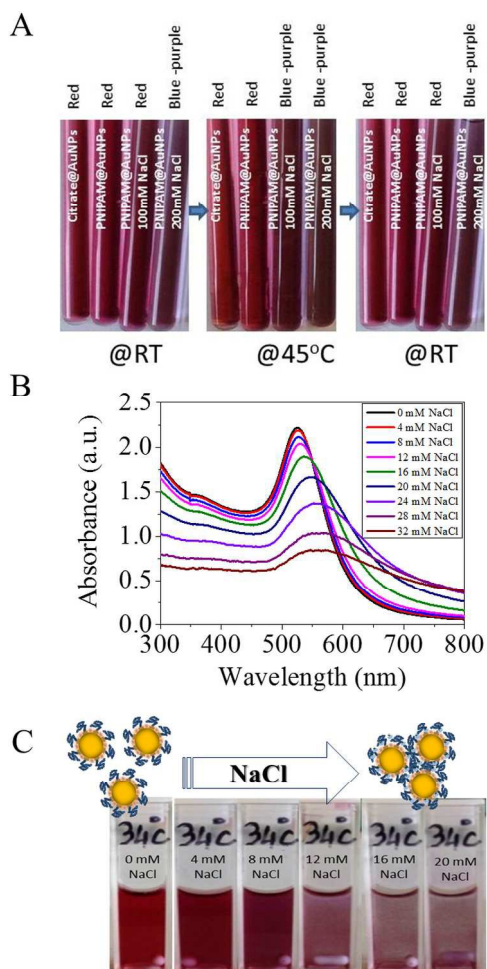
Fig. 2 (A) Colour visualization upon heating (45 °C) and cooling (RT) of citrate@AuNPs and PNIPAM@AuNPs (no salt, 100 mM and 200 mM NaCl). (B) UV−vis spectra of PNIPAM@AuNPs at 28 °C with varying NaCl concentration and (C) Colour visualization of the PNIPAM@AuNPs at 34 °C with varying NaCl concentration.

It is clear that the relaxation time of the smaller size distribution does not show angular dependence confirming that it is a measuring artefact resulting from rotational diffusion of the AuNPs.[12f] The zeta potential of the PNIPAM@AuNPs was determined to be -26 mV, indicating the presence of remaining citrate groups on the PNIPAM@AuNPs surface that may effectively stabilize the AuNPs via repulsive electrostatic interactions in addition to the steric stabilization by the grafted polymer chains.

To get a first insight in the potential sensing behaviour of the PNIPAM@AuNPs,

their thermoresponsive aggregation behaviour was studied in presence of 25, 50, 100, 150 mM NaCl, respectively. UV-vis spectroscopy data (Fig. 1D) reveals that in presence of 25 mM NaCl, the PNIPAM@AuNPs showed a clear red shift of λ_{max} around 33 °C, while with 150 mM NaCl a red shift of λ_{max} was already observed at 25 °C. These UV-vis absorbance results clearly demonstrate that the inter-particle association can be controlled by changing the aqueous NaCl concentration and the temperature, providing a sound basis for colourimetric sensors as the aggregation is associated with a colour change from red to purple. The observed dual responsive

behaviour of the PNIPAM@AuNPs is attributed to the required screening of the electrostatic colloidal stabilisation resulting from the presence of remaining citrate moieties on the surface of the AuNPs. DLS measurements of the PNIPAM@AuNPs in presence of different NaCl concentrations confirmed the formation of large aggregates at the temperatures where an increase in SPR band was found (Fig. S4, ESI†). DLS results confirmed the UV-vis data.

The colourimetric sensing ability of PNIPAM@AuNPs is visualized in Fig. 2A that compares the colour of the citrate stabilized AuNPs and PNIPAM coated AuNPs (without salt, 100 mM salt and 200 mM NaCl) at room temperature (RT) and at 45 °C. The red colour of the citrate stabilized AuNPs and the PNIPAM coated AuNPs (without salt) remains unchanged upon heating to 45 °C and cooling to RT revealing that both PNIPAM and salt are required. At 100 mM NaCl the red colour of the PNIPAM@AuNPs solution switched to purple-blue when heated to 45 °C and back to red when cooled back to RT, demonstrating that interparticle association and dissociation is reversible (Scheme 2). However, PNIPAM@AuNPs in presence of 200 mM NaCl already showed a blue-purple colour at RT suggesting that this high NaCl concentration induces collapse of the PNIPAM chains (salting out) leading to aggregation at RT.

After establishing the colourimetric sensing potential of the PNIPAM@AuNPs and that both PNIPAM and salt are required, we focused our attention to more in depth evaluation of the sensing capabilities. It is well reported that salts from the Hofmeister series can influence the solubility and the behaviour of the polymer in solution.[18] In the present investigation two different salts were studied, namely NaCl as kosmotrope (salting out) and NaSCN as chaotrope (salting in).

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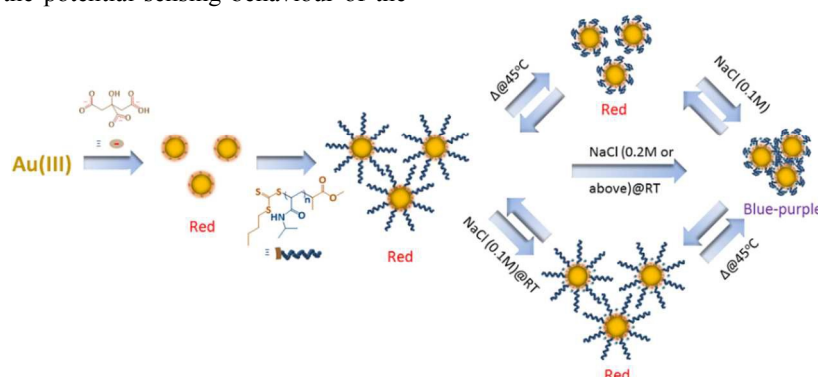
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Scheme 2 Schematic representation of the thermoresponsive behaviour of the PNIPAM coated AuNPs in aqueous solution in presence of NaCl.
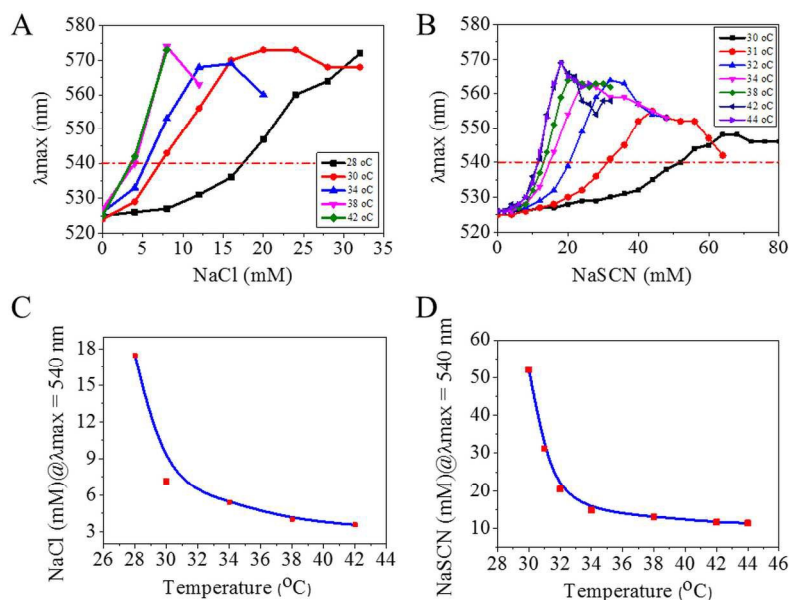
Fig. 3 The λ_{max} peak variation of the PNIPAM@AuNPs at different (A) NaCl and (B) NaSCN concentration with variation of temperature. (C) NaCl and (D) NaSCN concentration at λ_{max} peak = 540 nm vs. temperature plot.

Studying both a kosmotrope and a chaotrope will allow to assess whether the salt responsive behaviour of the PNIPAM@AuNPs is purely charge screening (salt independent) or also due to a change in the PNIPAM collapse temperature (salt dependent). At first isothermal UV-vis titrations were performed by stepwise increase of the NaCl concentration at different temperatures, namely 28, 30, 34, 38 and 42 °C. A representative series of UV-vis absorbance spectra of the PNIPAM@AuNPs, titrated at 28 °C with NaCl is shown in Fig. 2B. It is evident that with increasing NaCl concentration the λ_{max} of the SPR red-shifted due to aggregation, accompanied by a decrease in absorption and an increase in scattering. UV-vis absorbance spectra measured at 30, 34, 38 and 42 °C titrated with aqueous NaCl are shown in Fig. S5 (ESI†). The clear visual change in colour during the titration of PNIPAM@AuNPs with aqueous NaCl, measured at 34 °C is shown in Fig. 2C. All the snap shots were taken during the titration demonstrating that with 4 mM NaCl the colour already turned dark red and then it changed to blue–purple at 8 mM NaCl. Further addition of NaCl does not change the colour, but does induces macroscopic precipitation of nanoparticle aggregates. Moreover, UV-vis absorbance spectra were also measured at 30, 31, 32, 34, 38, 42 and 44 °C upon titration of aqueous NaSCN and results are shown in Fig. S6 (ESI†). To investigated the significance of these data, we have measured the λ_{max} peak variation of the temperature responsive PNIPAM coated AuNPs at different salt (NaCl) concentration at 30 °C in triplicate revealing small standard deviations, especially up to a

λ_{max} of 550 nm (Fig. S7, ESI†). Note that the time between measurements is crucial for all the isothermal salt (NaCl and NaSCN) titrations. At every addition of salt, PNIPAM@AuNPs was incubated for 2 min to assure thermal equilibration.

The wavelength of maximum absorption (λ_{max}) of the PNIPAM@AuNPs is plotted as a function of salt (NaCl and NaSCN) concentration at different temperatures in Figs. 3A and 3B, clearly demonstrating that the required salt concentration for the colour change is tuneable by temperature. Furthermore, the temperature sensing regime is shifted to higher temperatures with NaSCN (28 to 42 °C for NaCl and 30 to 44 °C for NaSCN) while higher salt concentrations are required compared to NaCl, indicating that the PNIPAM@AuNPs aggregation is not simply due to charge screening, but also the Hofmeister effect plays an important role. Next, the required salt concentration to obtain a λ_m of 540 nm was taken as the transition between red and purple-blue visual colours of the solution. This allows plotting of salt concentration versus temperature of the colour transitions for both NaCl and NaSCN, being the calibration curves for the sensors (Fig. 3C and 3D). Practically, we propose that determination of unknown NaCl or NaSCN concentrations in solution can be determined by addition of the PNIPAM@AuNPs followed by a temperature sweep to determine the transition temperature of the solution. Within the sensing boundaries, the transition temperature can be directly correlated to the salt concentration using the calibration curves in Fig. 3C and 3D. Unfortunately, due to the influence of the Hofmeister effect on our system, the presence of other additives

or salts will alter the calibration curve. Temperature sensing can, however, be done in a more robust way; in analogy with the Galilean floating thermometers based on density of the solution. Such a thermometer is proposed based on a series of PNIPAM@AuNPs solutions with different salt concentrations that are placed next to each other in a strip. By choosing the right salt concentrations, the transition temperature can increase from left to right so that the colour changing position in the strip is a direct measure for the temperature.

In summary we have successfully demonstrated that modification of the citrate stabilized AuNPs with thermoresponsive PNIPAM have dual stabilisation: one by the polymer chains and another by charges due to the presence of excess citrate and revealed that these PNIPAM coated AuNPs can act as temperature and salt sensors where sensing regime of the can be tuned by variation of salt concentration and temperature, respectively. The temperature sensing regime was found to be shifted to higher temperatures with NaSCN (28 to 42 ^{o}C for NaCl and 30 to 44 ^{o}C for NaSCN) while higher salt concentrations was required compared to NaCl, indicating that the PNIPAM@AuNPs aggregation is not simply due to charge screening, but also the Hofmeister effects play an important role. However, due to the influence of the Hofmeister effect on our system, the presence of other additives or salts will alter the calibration curve. It should also be noted that variations in polymer chain length and grafting density will require new calibration of the sensors (as we experienced during resynthesis of the PNIPAM@AuNPs). Temperature sensing can, however, be done in a robust way, in analogy with the floating thermometers based on density of the solution.

Notes and references

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