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Near-infrared light-triggered irreversible aggregation of poly(oligo(ethylene glycol) methacrylate)-stabilised polypyrrole nanoparticles under biologically relevant conditions†

Kin Man Au,‡[a] Mei Chen,‡[b] Steven P. Armes*[a] and Nanfeng Zheng*[b]

We report the use of near-infrared (NIR) radiation to trigger the *irreversible* flocculation of poly(oligo(ethylene glycol) methacrylate)-stabilised polypyrrole nanoparticles in physiological buffer.

Thermo-responsive polymers are a well-studied class of stimuli-responsive materials, with potential applications ranging from drug delivery to tissue engineering.[1] In principle, thermo-responsive polymers can exhibit two types of transitions: an upper critical solution temperature (UCST) or a lower critical solution temperature (LCST). Classical thermo-responsive polymers that exhibit LCST-type phase transitions in aqueous solution such as poly(*N*-isopropylacrylamide) and poly(2-(dimethylamino)ethyl methacrylate) are of particular interest.[2a–c] Such polymers are water-soluble below their LCST because of extensive hydrogen bonding with water molecules. Upon heating to above the LCST, the hydrogen bonds are disrupted and the polymer chains become hydrophobic, leading to phase separation.[1] Poly(oligo(ethylene glycol monomethacrylate)) (POEGMA) is a non-ionic biocompatible polymer.[1] Although POEGMA-based nanomaterials have been investigated for various biomedical applications for more than a decade, the thermo-responsive behaviour of POEGMA was not widely recognised until 2006.[1,3] Thermo-responsive polymers that irreversibly aggregate upon heating above their LCST may offer advantages in new biomedical applications, such as blocking capillaries near tumours to prevent further tumour growth[4a] or repairing ruptured epithelial tissues (*e.g.* intestine).[4b] To prevent unwanted precipitation during systemic delivery, this LCST phase transition must be well above physiological temperature (~37 °C). Although isolated cells are rather sensitive to thermal shock, most healthy tissues can withstand *brief* incubation at moderate hyperthermia temperatures (65–75 °C).[5]

Light irradiation is a widely used method to trigger phase transitions in stimuli-responsive biomaterials.[6] Unlike ultraviolet or visible light, NIR light penetrates deeply into healthy tissue and leads to less photothermal damage.[7] Thus NIR irradiation is an ideal non-invasive and site-specific method for triggering an LCST-type phase transition in a thermo-responsive polymer-coated NIR photothermal transducer. Although such transducers (*e.g.* gold nanorods) have been investigated for various biomedical applications,[8] the use of NIR light to trigger a phase transition under biologically relevant conditions has not yet been achieved because of poor energy conversion efficiencies (≤22%).[9] Polypyrrole (PPy) is a widely investigated organic conducting polymer.[10] PPy nanoparticles (NPs) have been evaluated for various new biomedical applications such as photothermal therapy agent,[11a,b] optical contrast agents for photoacoustic imaging[11c] and photothermal optical coherence tomography.[11d] Thus, thermoresponsive polymer-functionalised PPy NPs that irreversibly aggregate upon NIR irradiation could be used for photothermal cancer therapy; the heat generated from the PPy particle core can in principle ablate cancer cells[11a,b,e] while also triggering irreversible aggregation of NPs to block nearby blood vessels, thereby preventing further tumor growth.[4a] Herein, we demonstrate that thermo-responsive POEGMA-stabilised PPy NPs can act as a biocompatible, high-performance photothermal transducer that efficiently converts absorbed NIR light into heat. More specifically, a NIR laser is utilised to trigger an *irreversible* LCST-type phase transition so as to cause permanent colloidal aggregation of the PPy NPs.

POEGMA-stabilised PPy NPs of about 50 nm diameter were synthesised *via* aqueous dispersion polymerisation of pyrrole in the presence of a POEGMA-based statistical copolymer stabiliser containing pendent thiophene groups, hereafter denoted as poly(2TMOI-OEGMA), as previously reported (Fig. S1, ESI†).[12] The number-average and intensity-average diameters (D_h) of the POEGMA-stabilised PPy NPs were found to be 47 nm (Fig. 1a and Fig. S2a, ESI†) and 109 nm (polydispersity index, PDI = 0.184; Fig. S2, ESI†), as determined by transmission electron microscopy (TEM) and dynamic light scattering (DLS), respectively. By comparing the microanalytical nitrogen contents of the poly(2TMOI-OEGMA), PPy bulk powder and lyophilised POEGMA-stabilized PPy NPs it was

[a] *Department of Chemistry, The University of Sheffield, Brook Hill, Sheffield, Yorkshire, S3 7HF, UK. E-mail: s.p.armes@shef.ac.uk*

[b] *State Key Laboratory for Physical Chemistry of Solid Surfaces and Department of Chemistry, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China. E-mail: nfzheng@xmu.edu.cn*

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‡ K.M.A. and M.C. contributed equally to this work.

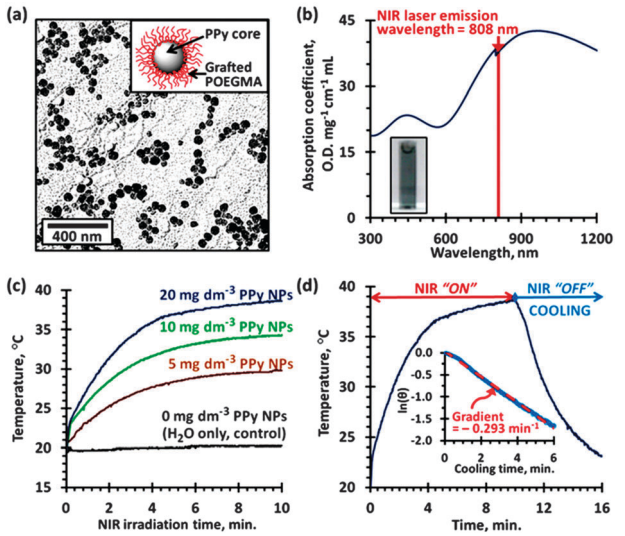

Fig. 1 (a) TEM image obtained for the POEGMA-stabilised PPy NPs. The inset cartoon schematically illustrates their structure. (b) UV-visible-NIR absorption spectrum recorded for the POEGMA-stabilised PPy nanoparticles. The inset digital photograph shows a 20 mg dm^{-3} aqueous dispersion of PPy NPs. (c) Concentration-dependent photothermal responses of 0–20 mg dm^{-3} of dilute aqueous dispersions of POEGMA-stabilised PPy NPs recorded during NIR irradiation (808 nm, 1.0 W cm^{-2}). (d) Steady-state data recorded both during and after NIR irradiation for a 20 mg dm^{-3} of PPy NP aqueous dispersion. The inset shows the semi-log plot of temperature, θ, (extracted from the cooling curve) as a function of cooling time. The gradient of this plot was used to calculate the thermal time constant for cooling (τ_c) and hence the photothermal transduction efficiency (Suppl. cal. 2, ESI†).[14] The thermal time constant (τ_c) for the cooling curve was calculated to be 205 s (since $\tau_c = -1/\text{gradient}$).

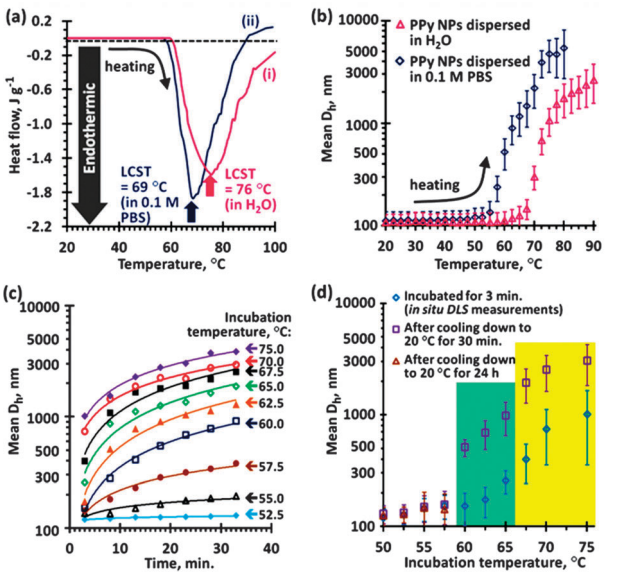

Fig. 2 (a) DSC curves obtained for the POEGMA-stabilised PPy NPs dispersed in (i) water and (ii) 0.10 M PBS. (b) *In situ* variable temperature DLS measurements of the PPy NPs dispersed in water and 0.10 M PBS. (c) *Time-dependent* DLS measurements of the POEGMA-stabilised PPy NPs (dispersed in 0.10 M PBS) recorded at 53–75 °C. (d) The plot of mean D_h for POEGMA-stabilised PPy NPs (dispersed in 0.10 M PBS) recorded after incubation at 50–75 °C for 3 min, and after cooling to 20 °C for 30 min and 24 h. The green and yellow highlighted areas represent partial and complete sedimentation of the aggregated PPy NPs after cooling to 20 °C for 24 h, respectively.

calculated that the PPy NPs contained approximately 21 wt% steric stabiliser (stabiliser adsorbed amount, Γ = 3.0 mg m^{-2}; see Fig. S2 and Supp. cal. 1, ESI†). The POEGMA-stabilized PPy NPs exhibited good colloidal stability in 0.10 M phosphate buffered saline (PBS) and excellent anti-biofouling character under physiological conditions (Fig. S3 and S4, ESI†). *In vitro* toxicity studies performed on HeLa cells indicated that relatively high concentrations (up to 500 mg dm^{-3}) of POEGMA-stabilised PPy NPs dispersed in 0.1 M PBS are non-cytotoxic (cell viability $\geq$95%; Fig. S5, ESI†).

It is well known that PPy is a strong NIR absorber.[13] Fig. 1b and Fig. S6 (ESI†) show the UV-visible-NIR absorption spectra recorded for various concentrations of POEGMA-stabilised PPy NPs. The strong NIR absorption band, which overlaps with the NIR laser emission wavelength at 808 nm, corresponds to the bipolaronic electron transfer in doped PPy.[13] *In vitro* photothermal heating studies indicated that the temperature of aqueous dispersions of PPy NPs increased with both NP concentration and the NIR irradiation period, as expected (Fig. 1c and Fig. S7, ESI†). After achieving a thermal equilibrium of 39 °C on NIR irradiation, the photothermal transduction efficiency of a 20 mg dm^{-3} aqueous dispersion of PPy NPs was calculated from the rate of released heat to be 47% (see Supp. cal. 2, ESI† and Fig. 1d).[14] This is more than twice as efficient as other NIR photothermal transducers ($\leq$22%) irradiated at similar wavelengths reported in the literature.[9]

POEGMA-stabilised NPs typically exhibit classical LCST-type phase behaviour.[1,3] We investigated the thermo-responsive behaviour of the POEGMA-stabilised PPy NPs using differential scanning calorimetry (DSC). At a concentration of 50 mg dm^{-3}, POEGMA-stabilised PPy NPs dispersed in either water or 0.10 M PBS undergo first-order endothermic phase transitions[15] with LCSTs of 76 °C and 69 °C, respectively (Fig. 2a). It is well known that the presence of salt (*e.g.* PBS) usually lowers the LCST of non-ionic water-soluble polymers relative to that observed in pure water due to the kosmotropic effect.[3,16a,b] It is also noteworthy that these values are somewhat lower than those of the poly(2TMOI-OEGMA) stabiliser alone (83 °C in water and 75 °C in 0.10 M PBS; see Fig. S8, ESI†). Variable temperature DLS studies were performed to investigate the thermo-responsive behaviour of the POEGMA-stabilised PPy NPs. The PPy NPs undergo a LCST-type phase transition (Fig. 2b). The mean D_h (and polydispersity) of the PPy NPs dispersed in water remained at $\sim$110 nm on heating up to 65 °C. Above about 68 °C, the mean D_h of the NPs increased monotonically (Fig. 2b) indicating NP aggregation triggered by gradual dehydration of the POEGMA stabiliser chains, as illustrated in Fig. S9 (ESI†). The PPy NPs dispersed in 0.10 M PBS underwent a similar LCST-type phase transition (Fig. 2b), on heating above 58 °C.[3,16a,b] *Time-dependent* DLS studies confirm that the rate of PPy NP aggregation in 0.10 M PBS increased for longer incubation times above the critical aggregation temperature (Fig. 2c and Fig. S10, ESI†). Further DLS studies confirm that this LCST-type phase transition for the POEGMA-stabilised PPy NPs is irreversible, even if the NPs are incubated for just 3 min above the critical aggregation

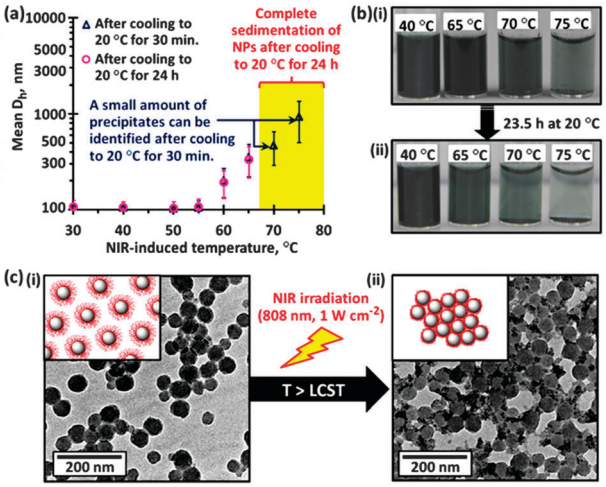

Fig. 3 (a) Mean D_h and (b) corresponding digital photographs obtained after NIR irradiation of POEGMA-stabilised PPy NPs dispersed in 0.10 M PBS to attain various temperatures followed by cooling to 20 °C for either 30 min or 24 h. (c) TEM images recorded for POEGMA-stabilised PPy NPs after NIR irradiation to attain temperatures of (i) 40 °C (still colloidally stable) and (ii) 70 °C (formation of NP aggregates).

temperature (Fig. 2d). Sedimentation of aggregated PPy nanoparticles was observed 24 h after cooling to 20 °C (Fig. 2d and Fig. S11, ESI†). Usually, LCST thermal transitions are fully reversible. The irreversible aggregation of the PPy NPs is most likely because of the relatively high Hamaker constant of the PPy cores,[17] which leads to strong van de Waals forces between neighbouring NPs once the collapsed, dehydrated POEGMA chains can no longer provide effective steric stabilisation.

Finally, a relatively high concentration of PPy NPs (500 mg dm^{-3}) dispersed in 0.10 M PBS was subjected to NIR irradiation in order to mimic the likely higher concentration of PPy NPs within the target tissue after systemic delivery or local administration. In these experiments, PPy NPs were irradiated to attain desired temperatures in the 30–75 °C range before being cooled to 20 °C for 30 min for *ex situ* DLS measurements (Fig. 3a). As for the NP aggregation induced by bulk heating (Fig. 2b and c), the mean D_h (and polydispersity) of the NIR-irradiated PPy NPs gradually increased on heating above the critical aggregation temperature of 55 °C (Fig. 3a and Fig. S12, ESI†). The mean D_h of the NIR-irradiated PPy NPs heated up to 55 °C retained reasonable colloidal stability on cooling to 20 °C. In contrast, the PPy NPs heated above their LCST *via* NIR irradiation slowly sedimented after cooling to 20 °C (Fig. 3b and Fig. S13, ESI†). The irreversible nature of this NIR-triggered LCST-type phase transition was further confirmed by TEM studies, since relatively large NP aggregates are observed after NIR irradiation to above 70 °C (Fig. 3c). This indicates that NIR irradiation is as efficient as bulk heating for triggering an LCST-type phase transition in an aqueous dispersion of POEGMA-stabilised PPy NPs.

In conclusion, POEGMA-stabilised PPy NPs can act as a biocompatible, high-performance photothermal transducer. NIR irradiation is strongly absorbed by the PPy particle cores, which causes efficient localised heating. This leads to irreversible particle aggregation due to an LCST-type phase transition. This is observed at approximately 20 °C above physiological temperature, which is sufficiently high to prevent aggregation being accidentally triggered during systemic delivery. This work is expected to lead to the development of a range of POEGMA-functionalised photothermal transducers for biomedical applications.

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