Structure–LCST relationships for end-functionalized water-soluble polymers: an "accelerated" approach to phase behaviour studies†

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A novel "high throughput" technique for LCST measurement was developed which is able to identify the effect of subtle changes in end group composition on the aqueous phase behaviour of water-soluble poly(2-(dimethylamino)ethyl methacrylate).

Thermoresponsive polymers are characterized by changes in solubility behavior with temperature as a result of coil-to-globule transitions.[1a,b] The lower critical solution temperature (LCST) for such polymers is defined as the temperature at which the polymer phase-separates from solution (often water) due to the breakdown in hydrogen bonding interactions. Water-soluble polymers such as poly(*N*-isopropylacrylamide) (pNIPAM), poly(ethylene oxide), poly(2-(dimethylamino)ethyl methacrylate) (pDMAEMA) and many proteins exhibit LCST phase behavior.[2] This "intelligent" phenomenon has been exploited in supported catalysts,[3a] drug delivery,[3b] biomaterials,[3c] nanomaterials,[3d] microfluidics[3e] and other applications.[3f,g]

LCST transitions are affected by a number of variables such as the polymer composition, structure and molecular weight, concentration in solution, electrolyte levels, solvent quality and (particularly for pDMAEMA) pH. Typical determination methods, such as cloud point measurements, can be time-consuming and involve slow temperature ramps of around 1–2 °C min^{-1}. Using these manual approaches, detailed multivariate studies can be complex and laborious, particularly in a multidimensional study to investigate the *interactions* between variables. The development of accelerated approaches[4a–d] therefore offers benefits for the rapid discovery of new functional thermoresponsive materials.

We present here a novel "high throughput" (HT) temperature gradient approach for the determination of LCST. Temperature gradients have been exploited previously by Amis and coworkers for combinatorial polymer thin film phase behavior screening,[4d,5] and by Mao *et al*, using a capillary-based gradient device for aqueous solution state LCST measurement.[6a,b] Our approach, however, allows the HT analysis of larger numbers of samples and enables complex multivariate, multidimensional studies through the utilisation of a clear polystyrene 384-well microplate format placed upon a recessed copper base. The base is simultaneously heated and cooled by passing silicone fluid at controlled temperature through each end. This differential heating produces a uniform, linear temperature gradient throughout the well-plate, controlling the temperature of liquids placed in each of the wells.

Fig. 1a shows the consistency of temperatures achieved within the columns and the temperature gradients that have been produced within the rows of the well-plate when one end of the plate is heated to 90 °C and the other is cooled to 30 °C. Fig. 1b demonstrates the flexibility of the approach: many different linear temperature gradients may be achieved by simply adjusting the temperature of each end of the plate.

To demonstrate the applicability of this novel HT measurement, the technique has been validated through the determination of the

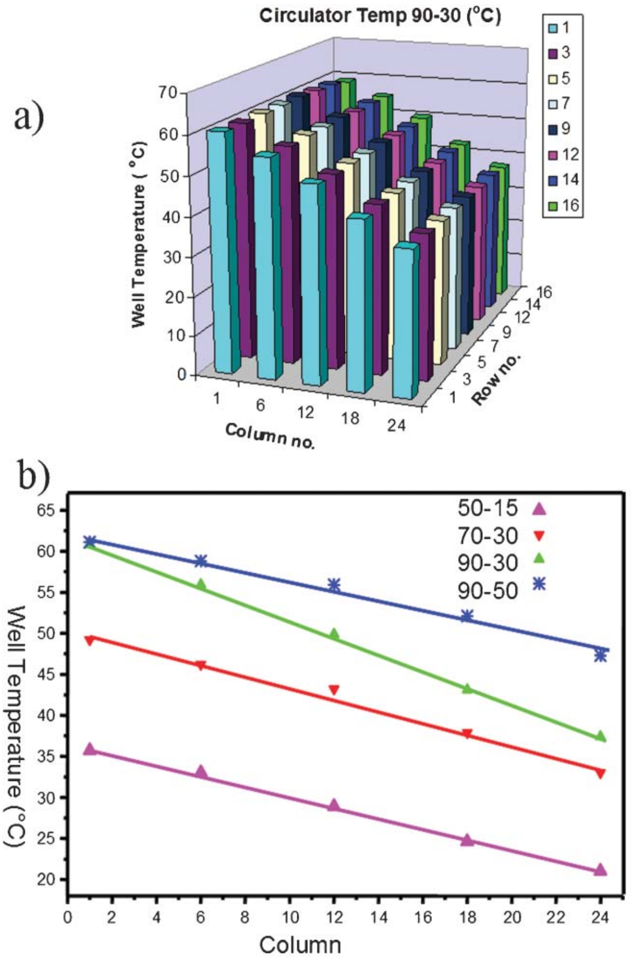

Fig. 1 Generation of static temperature gradients in 384 well-plates (a) Reproducibility of temperature and gradient across rows and through columns, (b) Linear temperature gradients controlled by base-plate temperature (inset shows hot and cold circulator temperatures, °C).

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often reported LCST for pNIPAM (1 wt%, $M_w = 300 \times 10^3$ g mol^{-1}) in neutral distilled water (pH = 9.0–9.5). Importantly, we have taken 16 *static* repeat measurements (Fig. 2a), allowing for a more accurate determination than conventional techniques. The LCST was found to be 30.6 ± 0.4 °C from the data presented in Fig. 2a which is in accordance with the literature.[6a,7] The phase transition can also be analyzed in more detail by "dynamically scaling" the temperature gradient to expand the LCST region (Fig. S4†); a more precise LCST value of 30.7 ± 0.1 °C was obtained in this case. Image analysis (Fig. 2b) allowed the observation of the LCST transition even within a *single well* (Fig. 2c), demonstrating that the working temperature resolution is in fact better than the nominal *R*/24 °C for a given temperature range (where *R* = the overall temperature range and 24 is the number of wells per row).

Processed images (Fig. S4b†) show a distinct similarity with a more standard plot of transmittance *versus* temperature from traditional devices such as UV-visible spectrophotometers,[1b] despite the fact that the data was acquired in all 16 channels (*i.e.*, rows) in "one shot". "Two-dimensional" measurements investigating the effect of KCl (16 concentrations) on the LCST transition were also accomplished (Fig. 2d). There was excellent agreement with the previously reported linear relationship found using conventional cloud point measurements[7] (see data superimposed in Fig. 2d).

The method was developed primarily to allow the investigation of subtle and complex structure-phase behavior relationships for various polymer structures and topologies. The controlled synthesis of a library of 49 pDMAEMA materials with seven systematically varying end groups and seven selected degrees of polymerization (DP), has been accomplished using aqueous atom transfer radical polymerization techniques,[8] (Scheme 1). End group variation was accomplished by using a small library of seven tertiary bromide ATRP initiators, **1**. Since the number average molecular weight (M_n) varies linearly over the timescale of the ATRP polymerization of DMAEMA, **2**, under our conditions, samples taken at specific times have predictable degrees of polymerization (DP) and narrow molecular weight distributions (PDI). It was thus possible to generate well-defined libraries with a range of molecular weights by "temporal sequential sampling ATRP" (see Supplementary Information†). For each end group, narrow polydispersity polymers with DPs in the ranges a = 7–9, b = 12–13, c = 15–16, d = 17–18, e = 19–20, f = 21–22 and g = 22–23 monomer units were produced and fully characterized by both ^{1}H nuclear magnetic resonance spectroscopy (NMR) and gel permeation chromatography (GPC), Fig. 3. Thus, a library of pDMAEMA polymers, **3**, varying systematically in molecular weight and end group was generated.

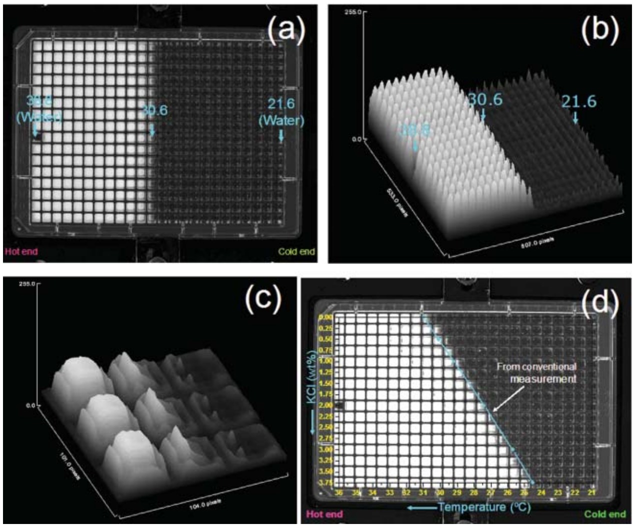
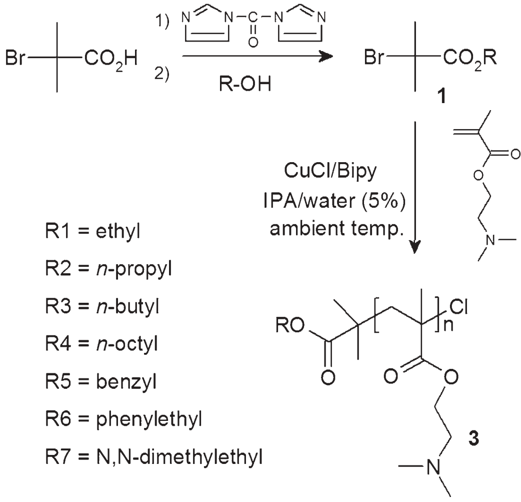


Fig. 2 High throughput determination of poly(*N*-isopropylacrylamide) phase behaviour (a) LCST in distilled water, 16 repeats; (b) image analysis of 384 well plate (temperatures referred to are in °C); (c) phase transitions observed within single wells; (d) static "two dimensional" phase transition measurement (temperature *versus* KCl concentration).[7]



Scheme 1 Synthesis of end-functionalized pDMAEMA using aqueous ATRP.

Fig. 4a shows the variation in LCST with changing end group for two sub-libraries; the lowest DP samples (R1a–7a, DP = 7–9) and those with the highest DP (R1g–7g, DP = 22–23). Fig. 4b illustrates the image processing used to extract the LCST values. The higher DP polymers (R1g–7g) generally have higher LCST values for a given end group. This is probably due to the effect of the hydrophobic end group becoming less pronounced with increased polymer chain length. This was supported by a more detailed study of the effect of DP on LCST for the ethyl- and propyl- end-functionalized series of samples (Supplementary Information, Fig. S9†). Likewise, it was shown previously that a

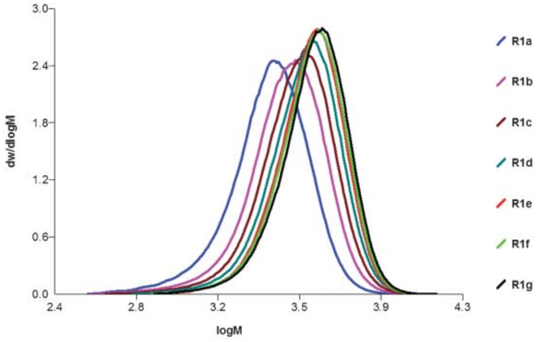


Fig. 3 Overlaid GPC chromatograms of the ethyl end-group pDMAEMA sub-library (polymers R1a–g).

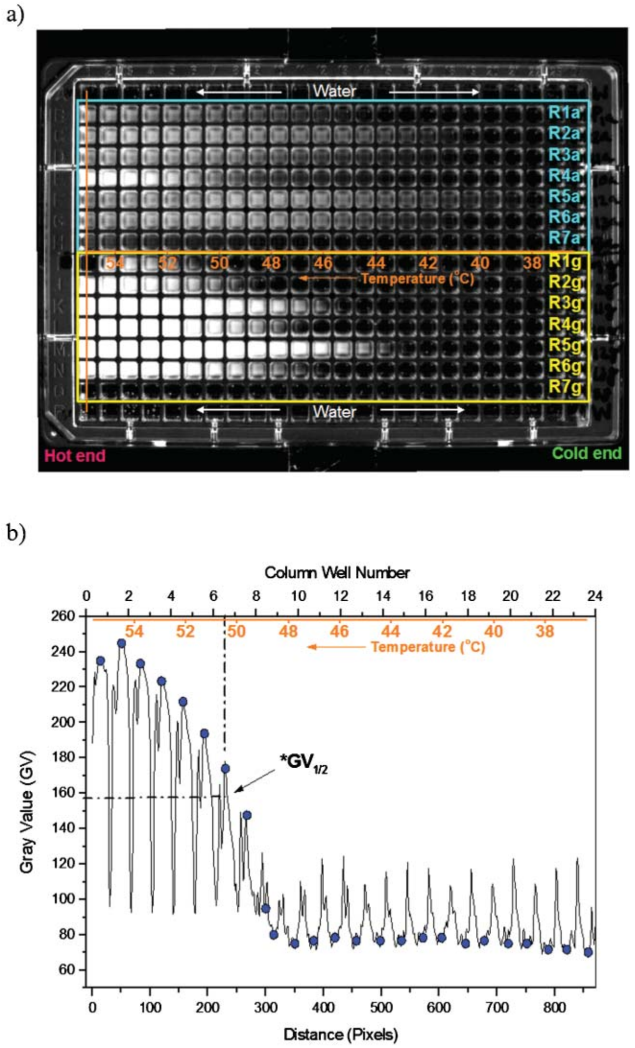


Fig. 4 Determination of the Lower Critical Solution Temperature for poly (2-(dimethylamino)ethyl methacrylate) (1 wt% of polymer and at pH 9.0–9.5) (a) Static observation of the phase transitions polymer libraries. End group variation: R1 = ethyl; R2 = *n*-propyl; R3 = *n*-butyl; R4 = *n*-octyl; R5 = benzyl; R6 = phenethyl; R7 = 2-(dimethylamino)ethyl. Degree of polymerization variation: top DP = 7–9; bottom DP = 22–23 monomer units. Temperature range = 36.4–55.1 °C. (b) Image analysis and half-height gray scale determination of transition temperature for polymer R2g (propyl end group, DP = 22–23 monomer units).

decrease in LCST occurs with increasing molar mass for pDMAEMA with a benzomethoxy end group.[9]

Surprisingly, we have been able to discern marked variations in LCST resulting from very small structural differences in these polymers. For example, three distinct LCST values for the ethyl, propyl-, and butyl- end-functionalized pDMAEMA materials were observed for both the low DP series (R1a–R3a; 47.8, 43.7, 47 °C, respectively) and the high DP series (R1–R3g; 51, 50.2, 47 °C), respectively, despite differences of only a single methylene unit (Table S2).

The least hydrophobic end group, 2-(dimethylamino)ethyl, is an analog of the DMAEMA monomer repeat unit. Polymers initiated from this group (R7a,g) showed only a faint LCST at 53.5 °C for the lowest DP sample (studied from 36.4–55.1 °C). LCST transitions were easily determined for the more hydrophobic end groups at increased DP (Fig. 4a). In general, more hydrophobic end groups led to lower LCST values. The ordering of the butyl- and octyl-end-functionalized polymers (R3g and R4g) is anomalous in this respect and cannot currently be explained, although analysis of the characterization data (NMR, GPC) and validation of these results by conventional LCST measurements suggests that this ordering is correct. In general, the trends are less regular for the low molar mass samples (R1a–R7a) although the lowest LCST values are observed for the most hydrophobic end-groups (benzyl and phenethyl). This can probably be explained by the polymerization process: while the control over molecular weight is very good, even variations of just one DMAEMA repeat unit might be expected to compete with single methylene changes in the end group structure of oligomers with an average DP of just 7–9. With these caveats, the data presented in Fig. 4 and Table S2 represent the basis of a rational "hydrophobicity scale" for structure-induced changes in phase behavior for such polymers.

In conclusion, we have demonstrated a novel HT approach capable of investigating subtle solution structure-phase behavior relationships which may greatly aid in the design of functional thermoresponsive materials. We have used this methodology to show convincingly that the LCST phase behavior for low DP pDMAEMA is affected by very subtle (single methylene group) changes in the structure of the initiating end group.

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