coma, it may be only a small part, with the remainder having too low an optical depth to be detectable in these data. ☐

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Graft copolymers that exhibit temperature-induced phase transitions over a wide range of pH

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THERE are many potential applications of 'intelligent' aqueous polymer systems[1–8] in medicine, biotechnology, industry and in environmental problems[9–13]. Many of these polymer systems undergo reversible phase transitions—for example, abrupt changes in volume—in response to external stimuli such as temperature, pH or the nature of the solvent. Most of the polymers studied previously are responsive to only one kind of stimulus. But for some applications, independent responsiveness to several factors, such as temperature and pH, may be required. Here we describe a polymer that undergoes marked solubility changes in water in response to temperature and/or pH changes. The polymer is prepared by grafting temperature-sensitive side chains onto a pH-sensitive backbone. We also find that block copolymers, in which the temperature- and pH-sensitive units alternate along the chain, show similar behaviour.

When the temperature of a polymer solution is raised above the lower critical solution temperature (LCST) or cloud point (CP), the polymer will phase-separate[14]: hydrophobic groups in the polymer form insoluble aggregates, turning the solution cloudy. We have used this principle previously[15] to prepare a copolymer hydrogel which exhibits temperature-sensitive swelling–deswelling changes over a limited pH range. This gel is composed of a random copolymer of monomers that are pH-sensitive (acrylic acid, AAc) and temperature-sensitive (*N*-isopropyl acrylamide, NIPAAm). For a composition of less than 10 mol% AAc, the gel exhibits a CP at pH 7.4. For higher AAc content the CP disappears, because then the AAc components (which are ionized at pH 7.4) convey sufficient solubility to offset the aggregation of the hydrophobic temperature-sensitive components. High molecular weight PAAc is a well-known bioadhesive polymer, which 'sticks' to the hydrated mucosal cells coating the eye, nose, mouth, lungs, gastrointestinal tract, vagina and anus. In order to prolong the residence time of a drug delivery vehicle in contact with such mucosal surfaces, PAAc is often incorporated into a delivery formulation. Further, if it is also desirable to slow the rate of drug release from the bioadhesive formulation, a more hydrophobic component, such as the temperature-sensitive polymer, may be added. Random copolymers of AAc and NIPAAm will not work, because of the loss of the temperature sensitivity when the content (that is, molecular weight) of the AAc component is increased to the point where bioadhesive properties are obtained. Physical mixtures of these two types of polymers are also possible, but they tend to separate physically and release drug too rapidly. Thus, the graft copolymer structure, which combines both behaviours in a single molecule and does not permit physical separation, is most effective. This is the rationale for the work described here.

Copolymers[16] and interpenetrating networks[17] of two polymers that form hydrogen-bonded complexes involving carboxylic acid groups may also exhibit temperature sensitivity, but this usually disappears when the pH is raised above the pK of the acid groups, ionizing them to carboxylate. For example, Klier *et al.*[16] prepared hydrogels of graft copolymers of polyethyleneglycol (PEG) on a poly(methacrylic acid) (PMAAc) backbone, which form hydrogen-bonded complexes between –O– and –COOH groups at low pH. Although these gels gradually shrank as temperature was raised at pH 4, this temperature-sensitivity would not occur at pH 7.4 where the gels were highly swollen owing to ionization of the carboxyl groups and the consequent disruption of the complex[16].

To obtain polymers that retain their temperature-induced transition over broad and useful compositional and pH ranges, especially at physiological conditions of pH 7.4 in a saline solution, we have synthesized graft copolymers composed of side-chains of a temperature-sensitive polymer (PNIPAAm) grafted onto a pH-sensitive backbone polymer (PAAc). We have used two methods for synthesizing the NIPAAm–g–AAc graft copolymers (Fig. 1*a–d*). In Fig. 1*e* we illustrate possible hydrogen-bonding interactions between the grafted NIPAAm and backbone AAc groups; these hydrogen-bonded structures are similar to those proposed to exist between carboxyl and amide groups in interpenetrating networks of PAAc and an acrylamide copolymer[17].

In Fig. 2 we plot the effect of AAc content on CP for random and graft copolymers of NIPAAm and AAc at pH 7.4 and 4.0; that is, above and below the pK of AAc, respectively. It can be seen that the CP of the random copolymer is always higher than that of the PNIPAAm homopolymer and rapidly rises as the AAc content increases, especially at pH 7.4. In contrast, the graft copolymers show a constant CP at either pH, independent of AAc content over a wide range of compositions. Their LCST may rise only when the composition of the graft approaches 100% AAc (assuming that the few PNIPAAm graft chains present will still phase-separate individually, but without any visible evidence of turbidity in the solution). This constancy of CP over a broad composition range (especially at pH 7.4) is a clear demonstration of the significant differences in behaviour between the random and graft copolymers studied here, as well as between our graft copolymers and those of Klier *et al.*[16] or the interpenetrating networks of Katono *et al.*[17].

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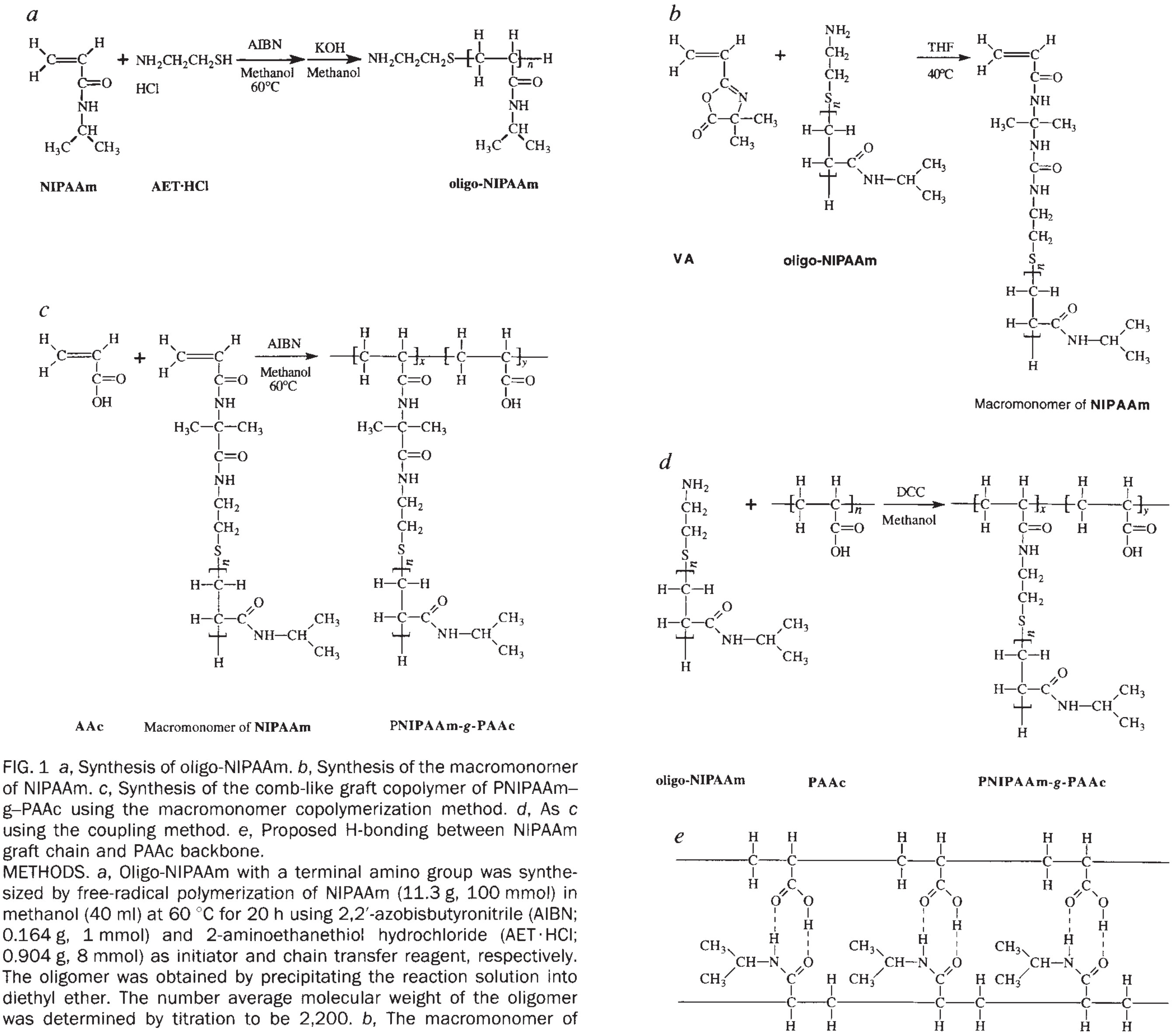
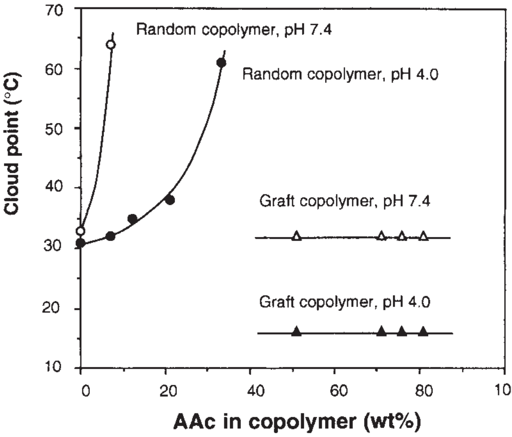


FIG. 1 *a*, Synthesis of oligo-NIPAAm. *b*, Synthesis of the macromonomer of NIPAAm. *c*, Synthesis of the comb-like graft copolymer of PNIPAAm–g–PAAc using the macromonomer copolymerization method. *d*, As *c* using the coupling method. *e*, Proposed H-bonding between NIPAAm graft chain and PAAc backbone.

METHODS. *a*, Oligo-NIPAAm with a terminal amino group was synthesized by free-radical polymerization of NIPAAm (11.3 g, 100 mmol) in methanol (40 ml) at 60 °C for 20 h using 2,2′-azobisbutyronitrile (AIBN; 0.164 g, 1 mmol) and 2-aminoethanethiol hydrochloride (AET·HCl; 0.904 g, 8 mmol) as initiator and chain transfer reagent, respectively. The oligomer was obtained by precipitating the reaction solution into diethyl ether. The number average molecular weight of the oligomer was determined by titration to be 2,200. *b*, The macromonomer of NIPAAm was prepared by reacting 5.0 g (2.27 mmol) of amino-terminated oligo-NIPAAm with vinyl azlactone (VA; 0.944 g, 6.79 mmol) in 120 ml of dry tetrahydrofuran (THF) at 40 °C for 16 h. To synthesize the comb-like graft copolymers, two methods were used. *c*, The macromonomer of NIPAAm was copolymerized with AAc in methanol (10% w/v) using AIBN as initiator, at 60 °C for 1.5 h, with weight ratios of macromonomer/AAc of 20/80 and 40/60; *d*, The amino terminal group of the oligoNIPAAm was reacted with the carboxyl groups of PAAc in methanol (2% w/v) using dicyclohexyl carbodiimide (DCC) as the activation reagent at room temperature for 24 h, using weight ratios of oligo-NIPAAm/PAAc of 20/80, 25/75, 30/70 and 50/50.

FIG. 2 The phase-separation temperatures, or cloud points (CPs), of random copolymers of NIPAAm and AAc, and of comb-like graft copolymers of PNIPAAm–g–PAAc plotted against the copolymer AAc content at pH 4.0 and 7.4.

METHODS. The CPs of the copolymers were measured by spectrophotometric determination of the turbidity (absorbance at 500 nm) of the copolymer solution in buffer (2.0 mg ml^{-1} in citric-phosphate buffer). The CP was determined at 10% absorbance. The random copolymers of NIPAAm and AAc were prepared by radical copolymerization of NIPAAm and AAc in methanol at 60 °C using AIBN as initiator.



The behaviours of the random and graft copolymers at the two pHs studied are also dramatically different. The higher CPs of the random copolymers at higher AAc contents at pH 4.0 are simply due to the hydrophilic character of the AAc monomer units along the backbone. This effect is known to raise the CP of temperature-sensitive random copolymers[18,19]. At this same low pH, the graft copolymers show much lower CPs than homo-PNIPAAm. This is probably due to the efficient formation of hydrogen bonds between relatively long sequences of PNIPAAm and PAAc—as suggested in Fig. 1*e* and as shown for acrylamide and PAAc interpenetrating networks[17]—which could not form in the random copolymer. The hydrogen bonding interferes with the access of water molecules to the NIPAAm amide groups, thus rendering the graft chains of PNIPAAm more hydrophobic, lowering their phase-separation temperature. Based on this mechanism, the CP should be insensitive to the AAc content of the graft copolymer, as observed (Fig. 2). At pH 7.4, the -COOH groups are ionized and the hydrogen bonds are disrupted. At this pH, the pendant PNIPAAm graft chains have the same CP as homo-PNIPAAm.

Figure 3 shows light absorbance versus temperature for 0.2% solutions of the 50% PNIPAAm-g-PAAc graft copolymer at pHs between those shown in Fig. 2. Distinct CPs are observed at all pHs studied, and the transitions tend to be sharper at the lower pHs. These CPs are shown as a function of pH in Fig. 4*a*: four different regions (A–D) are identified. It can be seen that the phase-separation temperature drops sharply as the pH is decreased below the pK of the backbone PAAc. The existence of a CP maximum is not significant. The slight decrease in CP at higher pH is probably due to the slight increase in ionic strength of the solution at the higher pHs[20].

Hypothetical conformations of the graft copolymers in the regions A–D are suggested in Fig. 4*b*. In region A, both the PAAc and PNIPAAm components are at their most soluble (high pH and low temperature) and the sketch of the expanded conformation of the graft copolymer illustrates this. As temperature is raised through the CP at constant pH (to region B), the PNIPAAm chains independently phase-separate, presumably mostly owing to the release of bound water as is the case for homo-PNIPAAm chains in solution[14]. The hydrophobic character of the precipitating chains may cause them to bond together, leading to the clouding of the solution, despite the highly hydrophilic and ionized backbone PAAc. The actual physical state of the 'precipitate' is not well-defined; the sketch of the conformation found in region B (Fig. 4*b*) is only schematic. A random copolymer of the same composition remains soluble over the entire temperature range, as can be deduced from Fig. 2.

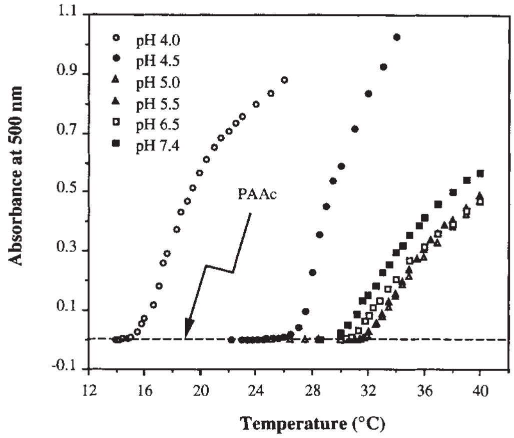


FIG. 3 The absorbance of the 50 wt% NIPAAm graft copolymer in 0.2 wt% solutions (in citric-phosphate buffer) versus temperature at various pH values.

As pH of the solution is lowered at constant temperature, the PAAc backbone chain will become less ionized and its expanded molecular coil should begin to shrink, but still remain in solution, as in region D (Fig. 4). In this state, some intramolecular hydrogen bonding between the grafted PNIPAAm chains and non-ionized sequences along the PAAc backbone may occur, as described above and shown in Fig. 1*e*. As pH continues to decrease from region A to D, the formation of hydrogen-bonded sequences will become increasingly probable. Eventually, a critical level of hydrophobicity of the grafted PNIPAAm chains will be reached at which they will precipitate, even at temperatures well below the CP of homo-PNIPAAm.

These graft copolymers represent a new family of 'hybrid intelligent' polymers. Furthermore, they may be fabricated in the form of soluble polymers, gel-coated surfaces or free-standing

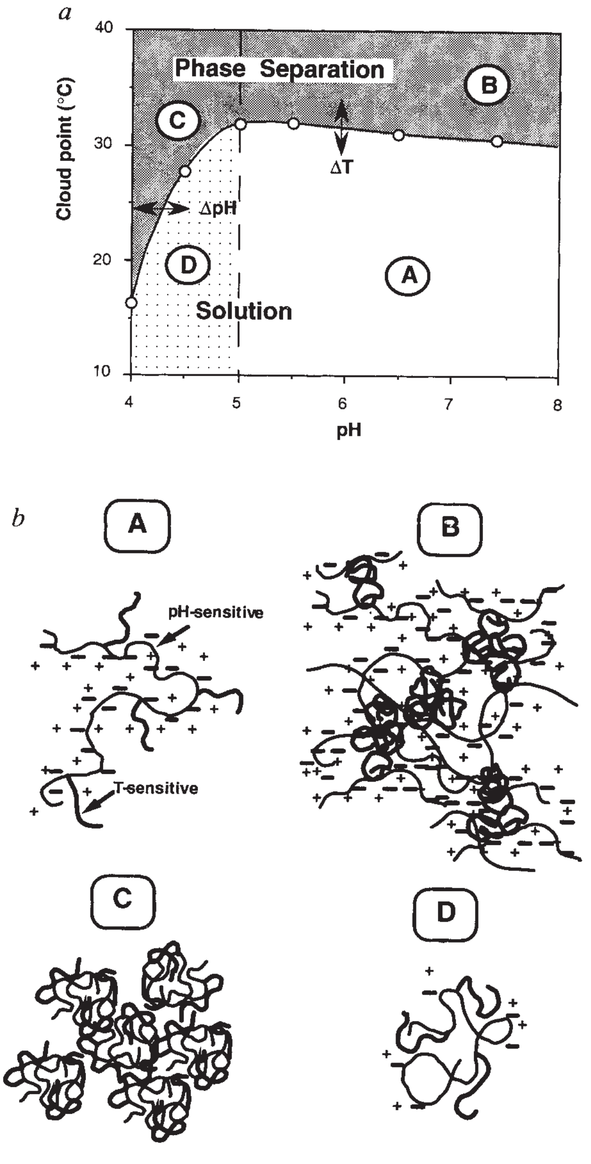


FIG. 4 *a*, The CPs of the graft copolymer (50 wt% NIPAAm) versus pH, showing four regions A–D. Two of the regions (B and C) represent conditions where the graft copolymer has phase-separated, whereas A and D are regions where the copolymer remains in solution. Arrows indicate reversible phase separations induced between regions A and B by a small change in temperature (ΔT) at constant pH, or between regions C and D by a small change in pH (ΔpH) at constant temperature. The dashed vertical line is placed at a pH near the pK of the PAAc, separating regions of relatively high and relatively low extents of ionization. *b*, Sketches of the molecular conformations in the four regions shown in *a*.

hydrogels. Block copolymer structures, including coating and hydrogel forms, are also possible. In addition, graft or block copolymers having components with sensitivities to different stimuli could become even more 'intelligent' by combining them with a variety of biomolecules. Applications of these copolymer-biomolecule systems include drug delivery, diagnostics, separations, cell culture and bioreactions. In addition to physical mixtures, if biomolecules are covalently conjugated onto some of the block or graft polymer chains, a 'hybrid intelligent' copolymer with amplified biological activity would be possible. □

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Dominant influence of atmospheric circulation on snow accumulation in Greenland over the past 18,000 years

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PROJECTIONS of sea-level rise due to greenhouse warming often involve the assumption that increased water vapour pressure will enhance snow accumulation in cold regions of ice sheets, partially offsetting the increased melting of low-latitude and low-altitude ice[1–3]. To test whether this has been true in the past, we compare accumulation rates[4] and temperatures derived from the oxygen isotope composition[5] of ice in the deep core obtained by the Greenland Ice Sheet Project II (GISP2). We find that atmospheric circulation, not temperature, seems to have been the primary control on snow accumulation in central Greenland over the past 18,000 years. During both warm (Holocene) and cold (Younger Dryas, Last Glacial Maximum) climate regimes, the sensitivity of accumulation to temperature changes is less than expected if accumulation is controlled thermodynamically by the ability of warmer air to deliver more moisture. During transitions between warm and cold climate states, in contrast, accumulation varies more than can be explained in purely thermodynamic terms, probably because of changes in storm tracks. Thus, in a world warmed by the greenhouse effect, circulation changes may be more important than direct temperature effects in determining snow accumulation in Greenland and its contribution to sea-level change.

The GISP2 core was dated by counting annual layers[4,6,7,35], with an estimated accuracy of 1–2% over century-length timescales in the Holocene period, and probably better than 5% accuracy in the late glacial[4]. After making ice-flow corrections[8], we can determine the snow accumulation rates[4]. We restrict our analysis to the upper 60% of the ice sheet where ice flow is simple[9] and ice-flow corrections are unlikely to add significant error to estimates of accumulation-rate changes over decades or centuries.

Temperatures were estimated from the oxygen isotope composition of ice[5] $\delta^{18}O$ (‰), using a calibration derived from comparison to a borehole temperature (T) profile: $T(K) = [(\delta^{18}O + 18.2)/0.53] + 273$ (refs 10, 11), which is not significantly different from the spatial-gradient calibration for central Greenland[12]. Broad agreement between isotopic and borehole palaeothermometers over the last glacial cycle[13], and close agreement over the most recent millennium[10,11], show that this is a useful palaeothermometer. We show data from the latter part of the Holocene and from the deglacial transition. Isotope data from certain portions of the Holocene have a small offset due to fractionation during storage before analysis; although this offset can be corrected for, inclusion of the corrected data does not affect our main conclusions and we therefore do not include these data.

Three lines of evidence suggest that the slope of this calibration (0.53‰ per °C) might be significantly larger only at the major climate transitions. First, changes in deuterium excess across the Younger Dryas termination indicate the temperature change of the vapour source area[14,15]. Second, general circulation model simulations of the isotopic composition of precipitation suggest that circulation changes affect the relative importance of different moisture sources and thus the isotopic composition of Greenland snow[16]. Last, simulations of the Younger Dryas cooling using general circulation models yield a smaller temperature change in central Greenland[17] than that obtained from the isotopic shift and the usual slope of the calibration[12]. In addition, any possible effect of ice-age dustiness on isotopic composition through changes in cloud supercooling would also increase the slope of the calibration at the major climate transitions.

The accumulation and temperature (°C) records in Fig. 1 show strong covariance for major changes between climate states, but less agreement for smaller changes within climate states. If accumulation (b) is controlled thermodynamically by the ability of warmer air to deliver more moisture, we would expect approximately an Arrhenius dependence on absolute temperature: $b \propto \exp[-Q/(RT)]$, where R is the gas constant and Q is the activation energy[19,20]. Atmospheric models for modern Greenland for the column-averaged moisture content in the

TABLE 1 Sensitivity of snow accumulation rate to temperature change

Time period	Sensitivity* (% per degree K)
Holocene	~0.9 ± 0.64
Preboreal	~6.8 ± 0.58
Younger Dryas	~1 ± 0.86
Bølling/Allerød	~7.5 ± 0.52
Last Glacial Maximum	~0.2 ± 0.69
Entire data set	~9.5 ± 0.28

* 95% confidence limits given.