Faraday Discuss., 1996, **101**, 207–223

GENERAL DISCUSSION

Prof. Tanaka opened the discussion of Prof. Khohklov's paper: Microphase segregation occurs in homopolymer gels. The essential difference between the phenomenon in homopolymer and heteropolymer gels is that in the latter the special arrangement of microphase-separated domains may be memorized as a thermodynamically stable state. Do you think this is correct?

Prof. Khokhlov responded: For heteropolymers there should be an additional effect connected with the microsegregated structures formed owing to the different 'primary structures' (*e.g.* different composition of the copolymer) in different parts of the gel. This kind of microstructure will reappear after transition to a highly swollen state and subsequent shrinking. From this viewpoint it is possible to speak about the 'memorizing' of the microstructure in the collapsed gel.

Prof. Tanaka said: You have shown the result of a polyelectrolyte gel–surfactant complex. There may be many possible combinations. For example, if you have a hydrophilic gel plus neutral surfactant in water you may have a similar situation to your system. Have you observed this?

Prof. Khokhlov answered: We have studied the interactions of the gels of poly(methylacrylic acid) (PMAA) with a poly(ethylene glycol) (PEG)-based non-ionic surfactant, Triton X-100. When the surfactant is added to the external solution the gel first collapses owing to the formation of the hydrophobic H complexes between PMAA and PEG chains and aggregation of hydrophobic 'heads' of Triton. When all the hydrogen bonds in the system are saturated, further addition of the surfactant results in a swelling of the gel, possibly owing to the increase in the amount of hydrophobic surfactant 'tails' in the gel that are not incorporated in the formation of hydrophobic H complexes with PMAA carboxyl groups. The physical effects for gel–neutral surfactant complexes are controlled mainly by the hydrophobic–hydrophilic balance, while the factor connected with the translational entropy of counter ions is absent.

Dr. Beelen said: In your paper you mentioned the remarkable effect that the mesh size of your gel is much smaller than the structural ordered surfactant aggregates, characterized by the sharp bands in the SAX spectra. How big are your structural ordered aggregates and is there an upper limit? How is the size of the aggregates controlled by the 'templating' gel? For example, is there a steering effect of the ionic groups in the gel present? Is it possible, by very slow addition of surfactant, to minimize the number of 'nuclei' in the gel and therefore to grow very big aggregates?

Prof. Khokhlov replied: I estimate that the regions with practically perfectly ordered aggregates have a size of 40 nm. The change in the cross-link density in the range of 0.2% to 2% mole fraction essentially does not affect the X-ray scattering pattern. The general trend is that the microstructure of the surfactant aggregates become more perfect with the increase of the charge content of the gel. We are now trying to test the idea that the degree of order in the gel–surfactant complex depends on the rate of the addition of surfactant to the external solution.

Dr. Schosseler said: In your paper, the influence of cross-links is not discussed, although it could modify the ability of the chains in the gels to rearrange. Heterogeneities of cross-linking might also play a special role. Do you have any ideas about these effects?

Concerning the case of PDADMAB gels, what is the cross-linking agent? Are the charges located randomly, close to cross-links or whatever?

You spoke about a mesh size much smaller than the characteristic distance for the ordering of micelles. How do you estimate this mesh size? If the answer is by stoichiometry, then do you think that, upon swelling, large 'voids' could be created in the gel by disinterpenetration of cross-linking heterogeneities? This could explain why the gel does not disturb the micellar order, *i.e.* micelles essentially occupy the regions with small polymer concentrations.

Prof. Khokhlov responded: The cross-linking agent was *N*,*N*′-methylenebisacrylamide. Our SAXS experiments were performed for the conditions when all monomer links were charged. As I have already answered, the cross-link density was raised in the range from 0.2 to 2 mol%, and this had no effect on the emerging microstructure.

I do not know much about the heterogeneities of cross-linking, but it is surely strongly heterogeneous. In particular, I agree with you that the existence of large 'voids' in the gel might possibly explain the sharpness of the X-ray scattering peaks.

Prof. Keller said: In my lecture, I referred to experiments on polystyrene sulfonate (PSS) where we measured the extensibilities of the chain, as influenced by multivalent cations, by our elongational methodology.[1,2]

Here we observed molecular contraction (as reflected by reduced extensibility) competing with molecular associations (as reflected in increased extensibility), both effects being most pronounced with Al^{3+} cations. We associated both effects with multifunctional ionic bridging which can be either intramolecular or intermolecular. The former can lead to precipitation (due to hydrophobic interactions) the latter to gelation. The former is realised in dilute polymer concentrations at sufficiently high cationic concentrations, which is closely stoichiometric, and the latter for sufficiently high polymer concentrations. Once a transparent gel is formed at high polymer and low cation concentration, then on addition of further Al^{3+} cations the gel becomes turbid indicating precipitation within the gel.

How does the effect we observed fit into your classification of collapse structures within polyelectrolyte gels, or alternatively, is it something quite different, or even new, falling outside it?

1 K. A. Narh and A. Keller, *J. Polym. Sci. Phys. Ed.*, 1994, **32**, 1697.
2 K. A. Narh and A. Keller, *J. Polym. Sci. Phys. Ed.*, 1993, **30**, 335.

Prof. Khokhlov responded: In the presence of multivalent counterions there are several reasons for the appearance of inhomogeneities. First, multivalent counterions frequently form ions pairs with the co-ions, and these ion pairs in turn form multiplets. So in this case, we have the ion-pair character of microheterogeneities. Secondly, multivalent ions lead to a bridging effect, *i.e.* to the effective decrease of the quality of solvent for the gel. It is possible to imagine the situation of heterogeneous distribution of bridges in the gel leading to alternating collapsed and swollen regions. However, these kinds of microstructure have not yet been analysed theoretically.

Prof. Hoffmann communicated: I would like to draw attention to a phenomenon that can occur in the systems of Prof. Khokhlov and that were not mentioned in the lecture.

Polyelectrolyte–bivalent metal ion systems can form gels with a well defined supramolecular architecture if the gels are formed by interdiffusion of the reactants. A well defined pore structure develops in the gel if Cu^{2+} ions diffuse into an alginate solution

in the sol state. The diameters of the capillaries formed depend on the concentrations of the reactants and are in the range 8–300 μm. A theoretical model which can describe the structure formation was recently proposed by Kohler;[1] general information may be found in ref. 2.

1 H. H. Kohler, *Chem. Phys.*, submitted.
2 T. Heinze, D. Klemm, F. Loth and B. Philipp, *Polymerica*, 1990, **41**, 259.

Dr. Geissler opened the discussion of Dr. Schosseler's paper: This is a technical question relating to the boundary conditions in the elastic modulus measurements. Do the gels retain their exact shape as they swell by factors of 100 or so?

Dr. Schosseler replied: Yes, they do. Usually we give the gels a finite amount of solvent to take in and wait until all the solvent has disappeared and the gel has reached equilibrium shape. The swelling ratio is obtained through weighing to take into account possible evaporation which is generally small. In this way we have no trouble with the shape of the gels.

Prof. Tanaka said: You have shown in a very systematic manner how the polymerization condition determines the viscoelastic and dynamic properties of gels. You were able to make extremely transparent and homogeneous gels by adding an ionizable group to them. We went in the opposite direction to make very inhomogeneous gels by making gels closer to the spinodal line of the final gel. I thus believe that a general picture can be drawn for the origin of structural inhomogeneities in a gel, namely, that they depend on the relative distance between the gelation condition and the spinodal line. Do you agree with that view?

Because of our interest in the memory effects of polymers, we have made gels at zero osmotic pressure so that when the polymerized gel is placed in water it does not swell or shrink. This may be a very interesting condition to study.

Dr. Schosseler responded: I agree partly with your view. The proximity of the gelation condition to the spinodal line certainly plays a role in building heterogeneities in the gels. This was already stated by de Gennes in his book[1] and probably also conjectured by other people earlier. On the other hand, Bastide and co-workers[2] have shown at length how structural inhomogeneities are inherent to the random nature of the cross-linking reaction even when the quality of the solvent does not play a role. However, these inhomogeneities appear only upon swelling of the gels as an extra-scattering intensity from the gels compared with the solutions in the same conditions. Therefore we could think of distinguishing the two types of heterogeneities according to the origin, *i.e.* random cross-linking or proximity to the spinodal line. This is perhaps pertinent because there seems to be a difference in the shape and size distribution of the heterogeneities present in neutral gels, like those studied by Bastide and co-workers, and in our poly(acrylic acid) gels. It would be interesting to compare with your gels or those studied by Geissler and co-workers. There is, however, a general rule of thumb that up to now seems to summarize all the results: the closer to swelling equilibrium, the larger the effects of heterogeneities. This rule includes gels prepared close to the spinodal line (high cross-linking ratio, poor solvent conditions, low ionization degree, high salt concentration, *etc.*; these gels are already close to their swelling equilibrium in the reaction bath) as well as gels prepared in good solvents and then swollen at equilibrium.

Following the preceding arguments, I would conjecture that there are very large memory effects. However, I would be very curious about the results if these gels were then placed in conditions where large swelling compared with the reaction bath can be achieved, for example by changing the solvent, the ionization degree, the temperature

etc., and then studied at concentrations between the concentration in the reaction bath and the swelling equilibrium.

1 P. G. de Gennes, *Scaling concepts in Polymer Physics*, Cornell University Press, Ithaca, New York, 1979.
2 J. Bastide and L. Leibler, *Macromolecules*, 1988, **21**, 2647; E. Mendes, P. Lindner, M. Buzier, F. Boué and J. Bastide, *Phys. Rev. Lett.*, 1991, **66**, 1595.

Prof. Dušek added: I agree that the highest degree of inhomogeneity is found in gels prepared under conditions close to the limit of thermodynamic instability given by dilution and concentration of the cross-links. In addition to the relative hydrophobicity of the cross-links one should consider the formation of inhomogeneities due to cyclization which is promoted by dilution and increasing concentration of the cross-linker. The cyclized regions shrink. Above the dilution limit only a solution of microgel-like particles is formed.

Dr. Boué commented: You showed that the modulus of your system is decreasing when you remove progressively the added salt. I would have been tempted to imagine the opposite: when you add salt, the electrostatic screening allow large scale polymer concentration fluctuations to appear. Then one could expect such fluctuations to allow some rearrangements leading to a decrease of the modulus. It appears that it is the other way around.

Prof. Khokhlov opened the discussion of Prof. Dušek's paper: Is it possible to calculate, using the theory of branching processes, the amount of possible topological types of clusters, which can be encountered with a probability higher than some small threshold probability? Were such characteristics measured in computer experiments?

Prof. Dušek responded: Within the theory of branching processes (generation from units), it is possible to obtain by cascade substitution in an implicit form the whole degree-of-polymerization or molecular-weight distribution. In the simplest case, the fraction of x-mer can be obtained analytically, but can always be obtained explicitly by successive differentiation of the respective generating function; this is, however, impractical for higher degrees of polymerizations. Normally, topological isomers of an x-mer are not distinguished. However, the cascade substitution makes it possible to distinguish between x-mer compositions in terms of units with 1, 2, 3, ... reacted functional groups and thus to characterize the asymmetry distribution within the x-mer fraction. For example, an x-mer isomer can be linear composed of $x - 2$ units with two reacted functional groups and two end groups with one reacted group, as one extreme, or spherical composed of $1 + \sum_{i=0}^{k} f(f-1)^i$ units reacted with all f functional groups and $f(f-1)^k$ end groups with one reacted functional group, where $x = 1 + \sum_{i=0}^{k} f(f-1)^i + f(f-1)^k$, as the other extreme.

The Monte-Carlo simulation of a general kinetic (coagulation) process makes it possible to retain more detailed information on the x-mer topology, in principle up to a full description by the adjacency matrix. Distinguishing between possible topological isomers when x increases is limited by computer time and memory.

Prof. Ross-Murphy said: The kinetic approach described in your paper is very interesting. In particular I was intrigued to see that non-classical critical exponents could be obtained, by modifying the precise 'coefficients' in your rate equations. Is it possible that such an adapted model could predict and explain the very pronounced concentration dependence of the gel time described in the paper of Clark?

Prof. Dušek replied: All theoretical approaches in which the ability of forming a bond between active groups of two species (molecules, particles) depends not only on the

number and reactivity of groups but also on the size (and structure) of the molecules, offer non-classical critical exponents. This is because the size distribution of molecules is modified. This modification determines the steepness of approaching the divergence limit (gel point). For example, the fraction of groups inside the molecules sterically excluded from the reaction increases with the size and compactness of the molecules. This gives rise to narrowing of the degree-of-polymerization distribution and non-classical critical exponents. Such an effect can be modelled, for example, by the product kernel with exponents lower than unit. Control of structure growth by translational diffusion can be another reason for narrowing the distribution. A correct model of structure evolution should include all important chemical and physical interactions. Some of them become less important when approaching the critical point, so that the critical behaviour is more universal. Large differences in diffusivities of molecules near the gel point and the fact that the largest molecules are interconnected into an infinite structure by bridging with smaller molecules seems to be the dominating feature. We are not entirely free to extend the dominating subcritical bond formation mechanisms to the critical region.

I am not so familiar with the physical gelation of biopolymer solutions as described by Clark, but I would expect a change of cluster size distribution with decreasing polymer concentration. For example, for non-specific gelation, the number of intramolecular bonds is expected to increase with increasing dilution and to be stronger with larger molecules. Also the intermolecular excluded volume may disfavour association between large clusters. These effects are expected to make the concentration power-law dependence steeper.

Prof. Winter said: Our experimental observations on materials at the gel point (critical gels) have been criticized for not showing universal exponent values. Basically, the theoretical explanation for this non-universability seems to be missing. Your studies suggest great effects of (1) the final size of the cross-linking point and (2) the stiffness of the cross-links. It seems that these are not just secondary effects but major contributions. When included in the theory, could they explain the large variations of the relaxation exponent of the relaxation modulus [$G(t) = St^{-n}$].

Prof. Dušek responded: I have already partly discussed this problem in my answer to Ross-Murphy's remark. Various models of kinetically controlled processes of cluster evolution leading to the formation of an infinite cluster offer different critical exponents (*e.g.* ballistic aggregation, diffusion-limited aggregation, Flory–Stockmayer or classical kinetic model, percolation). The generalized Smoluchowski process described by sets of differential equations for time evolution of all distinguishable clusters is one of the ways of modelling the effects of steric obstruction, cluster size dependent cyclization, or cluster diffusion. The critical exponents move away from the classical ones with an increasing degree of non-randomness and the width of the critical region also changes.

There is, however, no proof that the process that dominates the structure evolution in the off-critical region also controls the sol–gel transition at the critical point. For example, experimental data on some systems are well described by the simple statistical model (FS) in the off-critical region, but experimental critical exponents are closer to the percolation values. On the other hand, percolation models describe the off-critical region very poorly. The adherence of experimental data on a conventional covalently gelling system may be caused by immobility of the largest clusters (which are responsible for gelation) close to the critical point.

There may be two reasons for experimentally observed non-universal (*i.e.* non-percolation) critical exponents: (a) the process itself may be of non-percolation type even at the critical point; (b) the near-to-critical region, from where critical exponents are calculated, may be affected by strong deviations from conventional branching processes.

Dr. Lal opened the discussion of Dr. Geissler's paper: Since you have done the experiment on just one molecular weight polymer chain trapped inside the gel, are you completely justified in saying that the size of the chain is smaller owing to a random field of static obstacles (this is true in the limit of large molecular weight chains and dilute, completely random obstacles)? It could also be that the size is smaller because the chains are localized in less cross-linked regions of the gel.

Furthermore, are you justified in using Debye fits on collapsed chains as the measured scattering may not be completely or purely intrachain.

Dr. Geissler replied: There is probably no major difference between the two situations in which the cross-links are separate or form clusters. In both cases the cross-links and their immediate environment act as a repulsive potential. The only information available on the size of the clusters here comes from the SANS spectra of these gels (Fig. 3 of our paper): the typical size of the static structure related to the cross-links is between 80 and 100 Å.

Our expectation is that at the θ temperature the labelled chains are perturbed by neither the solvent nor the uncross-linked polymer, but only by the cross-links. Since our first report (ref. 17 of our paper), other measurements have been published in which, for a given molecular weight of free polymer, the radius of gyration R_g varies with the cross-link density of the gel.[1]

Although the Debye formula is not designed for this situation, it probably remains an acceptable approximation here. The essential result is that the radius of gyration shrinks appreciably. Since the labelled chains inside the gel are highly diluted (*ca.* 0.2% volume fraction), interchain scattering is weak.

1 R. M. Briber, X. Liu and B. J. Bauer, *Science*, 1995, **268**, 395.

Dr. Schosseler said: I am sceptical about using a dilute solution of chains as a reference system to compare with the chains in the gel. Even for experiments performed in Θ conditions, the third virial term is likely to have a different magnitude in both systems owing to the different concentrations.

Also, the use of a different composition for deuteriated/hydrogenated solvent and the fact that you do not know the concentration of chains inside the gel make it very difficult to check the consistency of the $I(0)$ value in the gel with the concentration and molecular weight of the chains. How do you know that you are measuring isolated chain parameters in the gel?

You report no change of the measured radius of gyration for the chains in the gel upon an increase of temperature from 52 to 70 °C. What change would you expect in that quantity for the chains in dilute solution upon such a variation? Have you measured it?

Dr. Geissler responded: The underlying assumption in our experiment is that labelled chains adopt the same unperturbed configuration in a melt as in a low molecular weight solvent in the θ temperature. In this configuration, three-body interactions play a negligible role in the free energy of the chain. In the present intermediate case, were it not for the presence of cross-links, the third virial term should be little different, and therefore the configuration would not change.

The concentration of chains inside the gel, found by weighing the sample, is indeed known (see my previous reply).

The change in radius of gyration for the solution between 52 and 70 °C was not measured in this experiment. However, for the neat gels swollen in a deuteriated solvent, the SANS spectra can be decomposed into a solid-like and a liquid-like part[1] (Fig. 3 of our paper): the resulting correlation length of the thermally excited fluctuations (blob

size) changes from 58 to 24 Å in this temperature range, while the longitudinal osmotic modulus increases by a factor of nearly five. (Use of an alternative decomposition procedure involving a Debye–Beuche expression gives similar answers, namely 53 Å and 26 Å, respectively.) This behaviour and the evidence from the swelling pressure of these gels[2] are both consistent with the fact that excluded volume conditions prevail at 70 °C.

1 F. Horkay, A. M. Hecht, S. Mallam, E. Geissler and A. R. Rennie, *Macromolecules*, 1991, **24**, 2896.
2 F. Horkay, W. Burchard, A. M. Hecht and E. Geissler, *Macromolecules*, 1993, **26**, 4203.

Prof. Dušek said: I wonder whether the small size of the linear molecules within the network phase cannot be explained by a poor 'thermodynamic quality' of the medium (*i.e.* a swollen network). It would then correspond to a dilute polymer solution in a poor solvent. The swollen network disfavours the linear polymer, and in case of a polydisperse polymer, fractionation also occurs so that in the swollen phase only polymers of the lowest molecular weights are present.

Dr. Geissler said: Certainly. As mentioned above, the effect appears to be related not to the interaction with the network chains, but to the cross-links.

Prof. Tanaka commented: I would like to go back to Prof Dušek's comments. It will be very interesting to change the chemical structure of the host network in which you place polymers to measure the radius of gyration. Highly concentrated polymer solutions are used to partition molecules in gels for controlled release. The chemical structure, as well as the degree of cross-linking, will have a dramatic effect on this partitioning, and thus maybe on the radius of gyration of the guest polymers.

Prof. Dušek responded: I just want to add to your remark that polymer solutions containing polymers of $M > 10^4$ were used for de-swelling studies because their concentration in the swollen phase was negligible. If linear polymers are added during the network formation they are very effective in phase separation, if it occurs before the gel point. Beyond the gel point the system becomes thermodynamically unstable but cannot usually phase separate owing to steric and diffusivity limitations. The systems can also become thermodynamically unstable if it is in equilibrium with the outer solution at one temperature and the temperature is changed.

Dr. Ettelaie addressed Dr. Geissler: You indicated that in your experiments the radius of gyration of free polymer chains was larger or of the same order of magnitude as the mesh (blob) size of the network. When the size of free polymers is much smaller one might expect that the free chains will not be as strongly perturbed as when in the network. As a result the degree of collapse for small chains might not be as large. Can you comment on the relation between the degree of collapse of the chains in the network and their size when in the solution.

Dr. Geissler said in reply: It is probably too early to give a definitive answer to this question. According to theory (ref. 22–26 of our paper), the degree of collapse involves a product of the density of obstacles and their repulsive potential. The repulsive potential in the present system is unknown.

The geometrical considerations are that in a theta solvent the blob size is roughly equal to the distance between cross-links, while in good solvent conditions the former is of course smaller (see above). Another important length scale is the distance between cross-links, R_x. This can be estimated from the elastic modulus $G = CkT\varphi^{1/3}/R_x^3$, where C is a constant of the order of unity, and φ the polymer volume fraction. The measured value of G for our sample was 8.8 kPa. Taking C equal to unity yields $R_x \approx 65$ Å, *i.e.* roughly the measured size of the free chain in the network.

Prof. Vincent added: An alternative explanation of the lower R_g values you report in Table 1 for the PVAc polymer chains in the gel network, compared with free solution, is that there is preferential absorption of a lower molecular weight fraction into the gel. In this regard it would be helpful to know the polydispersity of your sample of PVAc.

Dr. Geissler said: The polydispersity of the linear PVAc was found from GPC to be $M_w/M_n = 1.5$. The neutron scattering measurements gave $M_w = 1.8 \times 10^5$ in the free solution and $(1.4 \pm 0.2) \times 10^5$ for the free chains in the gel. This preferential absorption effect is too small to explain the observed difference in radius of gyration.

Prof. Khokhlov said to Dr. Geissler: Personally, I think that your explanation of the collapse of free chains in the gel is very appealing. If this is indeed the case, it becomes possible to probe the inhomogeneities of the structure of the gel by incorporating free chains in the gels. The trapping of the free molecules by the gel inhomogeneities would also result in changes of the diffusion properties of such molecules and in the change of the intramolecular structure factor. It is worthwhile checking whether these effects indeed take place, in order to confirm your explanation.

Dr. Boué commented: You have shown a fit of the signal from labelled free chains diluted in a polymer gel contrast-matched with the solvent, and assumed that this signal was close to the form factor, *i.e.* from the intrachain scattering only. This must correspond to a very weak concentration of free chains in the system network plus solvent. Though this may not be the case for your measurements, one can actually keep in mind that the interchain effect may be important: the localization of short chains in a gel can reflect the heterogeneity of the network even at a low fraction of labelled chains. This was observed, for example, in our work quoted as ref. 5 in your paper, where chains of d-polystyrene (d-PS) are trapped in h-PMMA gels. For each molecular weight M, the fraction ϕ was chosen such that $\phi M \approx 400$. For large M, this leads to values 10 times smaller than the overlap concentration ϕ^*, yet a very strong interchain effect is visible. No demixing is observed but a very strong scattering with an exponent $[S(q) \approx 1/q^{2.5}]$ independent of the molecular weight. The mobile chains seem to localise inside some weakly cross-linked regions of the gel. However, when replacing the d-PS chains by D-PMMA, which should be more compatible, these effects were reduced at least for short chains [a few 10 000 (unpublished observations)].

We also observed deuteriated free chains localized inside a network in the bulk. There no solvent is present. Free chains were trapped inside a non-deuteriated network prepared by aminomethylation (this method is described in our paper). The total concentration of free chains was kept fixed (10%); the fraction ϕ_D being deuteriated was varied between 100% and zero. Even if the chains are not diluted, extrapolation to zero ϕ_D of the results yields exactly the form factor $S_1(q)$. For chains of molecular weight 32 000, trapped in an aminomethylated network of maximum swelling around 12 (*i.e.* of medium average cross-linking density, typically 100 monomers between two cross-links), we observed a form factor quite close to that of an isotropic Gaussian chain, with a value of the radius of gyration (54 Å) close to that found [49.4 Å $= 0.275\ (32\,000)^{1/2}$] for the same molecular weight in a melt. That is to say, no effect was found in this case on the shape of the individual chain; the only effect was on the interchain scattering. In summary, effects on the individual shape should probably strongly depend on the average cross-linking density and on the structure of the fluctuations.

Prof. Keller opened the discussion of Dr. Boué's paper: First I wish to ascertain whether I understood (or remembered) your transparency showing hard silica spheres embedded in the soft rubber silica. Do they really show a stratification on deformation?

If so, is this the consequence of the particle being hard compared with the matrix? If this is so, it could have a significant consequence in the stretching of polymers, *e.g.* in fibre drawing with particulate fillers which are frequently added, *e.g.* TiO_2 particles as delustrant. In the stretched fibre, these particles would then have some stratified, clustered, distribution which could be of consequence in itself. In addition and significantly, such particles often act as centres for crystal nucleation. The clustering of such particles into layers would then induce oriented crystal (spherulite) growth which would impart a macroscopic orientation to the sample as a whole purely by directionally selective primary crystal growth, *i.e.* without any deformation of the crystals themselves, in turn affecting physical properties.

Dr. Boué replied: I actually showed some results (not shown in the paper) for hard particles in a soft matrix, as a case close to the one of hard regions in polymer network or glassy polymer rich regions in IPNs. The butterfly patterns can then be more directly attributed to the fact that hard regions are not deformable at all. The stratification is initiated by affine deformation of the centres of mass, which become closer and closer together in the transverse direction. They come into contact and may interpenetrate in the case of 'softer' harder regions or aggregates. The second step is not yet clear: whether they stick together along the transverse direction and form a stronger stratification, or start to avoid each other for larger deformations is not completely solved.

A scenario like your 'transverse crystal nucleation' seems to me (although I am not an expert in semicrystalline polymers) to be possible and interesting. It is also possible that the crystalline parts (and maybe stress-induced crystallites) present in the genuine semicrystalline sample, before the beginning of the deformation, could play the role of hard particles.

Prof. Khokhlov asked: Could you comment more on the physical reasons for the segregation of hard particles in the soft matrix under stretching?

Dr. Boué replied: When the sample is elongated along the stretching direction, the lateral dimensions are diminished along the transverse direction. If the hard regions, or hard particles, cannot be deformed, let us assume that their centres of mass are affinely displaced. Then they will soon be brought very close together along the transverse direction, becoming more and more separated along the stretching direction. This will lead to apparent bands. If we assume that the hard regions stick together when coming into contact, and behave later on as a single aggregate, affine displacement will apply to the centre of the aggregate only, and this stratification will be enhanced. However, the formation of very long bands (along the transverse direction) should, in turn, lead to rearrangement (see ref. 2 and 3 of our paper).

Dr. Bot communicated: I would be interested to know whether you also performed some large deformation rheological studies. Does this give any additional information regarding your neutron scattering experiments?

Dr. Boué communicated in response: The polymer networks samples were elongated up to maximum breaking for all of them. The stress σ_{eq} was measured for each deformation, *i.e.* for each elongation ratio, after typically 10 min of relaxation. Except for a few cases (mostly binodal networks not reported here), the apparent rubber elastic modulus $G = \sigma_{eq}/(\lambda^2 - 1/\lambda)$ is always close to a linear function of $1/\lambda$, $G = 2C_1 + 2C_2/\lambda$. This is the so-called Mooney–Rivlin non-linear behaviour (the linear behaviour would correspond to $C_2 = 0$). The majority behaviour is thus just that this linear increase of G with $1/\lambda$ goes on with the same slope until the sample breaks. The variation of the scattering

is also a progressive increase without change when approaching breaking (see ref. 9 of our paper). While the values $(2C_1 + 2C_2)$ and $2C_1$ can be reasonably related to the cross-linking ratio, the values of $2C_2$ are not (see our paper, and ref. 9 of it). One could naively expect that a non-linear coefficient C_2 could be related directly to the butterfly effect of non-affine rearrangement. As far as we checked, no correlation can be established.

The interpenetrated networks were close to breaking or ill-defined deformation as soon as λ was slightly larger than the one studied in this paper. We can relate their low strain modulus at low strain to their composition and structure. For example, when a glassy polymer is the majority component and is observed to form a random structure, the modulus is very high, and the butterfly patterns are different in shape (more elongated along the stretching axis).

In the other composites such as silica in polymer matrix, reinforcement effects are visible as a function of silica content and structure; the large deformation behaviour will be reported soon elsewhere.

In summary the modulus usually decreases with strain, but we have not been able to relate it to the rearrangement observed by SANS.

Prof. Tanaka opened the discussion of Dr. de Kruif's paper: You have shown the phase diagram that exhibits both the coexistence curve and gelation line. These two lines appear to coincide at the critical point. Did you observe any peculiar critical behaviour at the intercept?

Dr. de Kruif responded: The critical point is determined by the first and second derivative of the osmotic pressure. The percolation line is determined by the pair connectedness function and depends on the range of the potential. Therefore, the actual location of the percolation line depends on the particular system at hand.

For the 'Baxter' adhesive-sphere model the percolation line intersects the binodal near the critical point. We have not observed any peculiar behaviour near this point except that gelation may inhibit the exchange of matter between the two coexisting phases.

Mr. Aymard said (**Dr. Gimel, Dr. Nicolai** and **Dr. Durand** in part communicated): In our study of β-lactoglobulin aggregation at pH 7–pD 7, we have also proposed a two-step process (see ref. 1), but with some differences. We have studied the effect of the interactions on the aggregation process at pH 7–pD 7 on a space scale of *ca.* 1–300 nm. In screened conditions (*i.e.* 0.1 mol dm^{-3} solvent ionic strength), fractal aggregates are formed, with a fractal dimension of 2.05 ± 0.02.[2] SANS experiments show that aggregates formed at different concentrations and temperatures have the same local structure, units of *ca.* 60 monomers with a radius of 10 nm. Moreover, this local structure is not rod-like (Fig. 1).

In conditions of strong interactions (*i.e.* low solvent ionic strength 0.003 mol dm^{-3} and in D_2O), only small particles of *ca.* 60 monomers with a Stokes radius of 11 nm are formed, which we will refer to as 'globules'. The globules aggregate very slowly in a second step. The local structure of large fractal aggregates formed at pD 7, 0.01 mol dm^{-3} is the same as that of the globules formed at 0.003 mol dm^{-3} (Fig. 2). Thus, we showed that the globule is the elementary unit of the fractal aggregates.[3]

We believe that at pH 7–pD 7 the aggregation occurs in two steps (Fig. 3); first small aggregates (globules) are formed which subsequently aggregate to form fractal structures. The globules are the same for all the concentrations and the temperatures tested. In conditions of strong interactions, globules of the same size are still formed but the rate of the second step is strongly reduced. Interchange of disulfide bonds, leading to intermolecular covalent bonding, is probably involved in the formation of the globule,

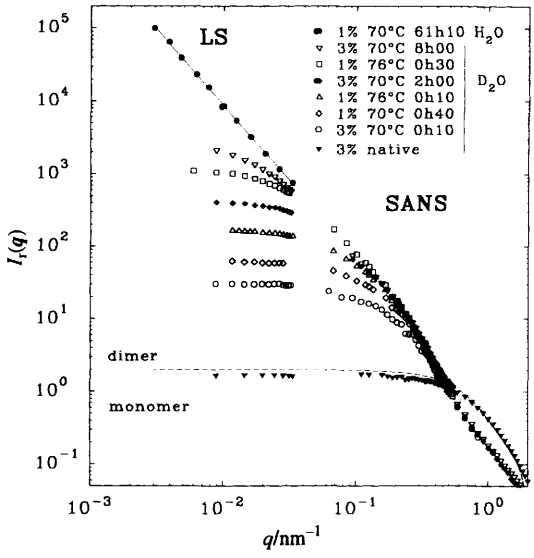

Fig. 1 Static properties of β-lactoglobulin aggregates formed in conditions of screened interactions (pH 7 and pD 7 with 0.1 mol dm^{-3} solvent ionic strength)

whereas a physical aggregation of globules would be involved in the formation of the fractal aggregates.

There are clear discrepancies between our model and one presented in your paper and in more detail in a previous paper.[4] We believe that the discrepancy is partly due to a different analysis of the dynamic light scattering data: the use of the inverse Laplace transform instead of the cumulants for the determination of the mean relaxation times seems more appropriate in the case of an aggregation process, as the existence of two

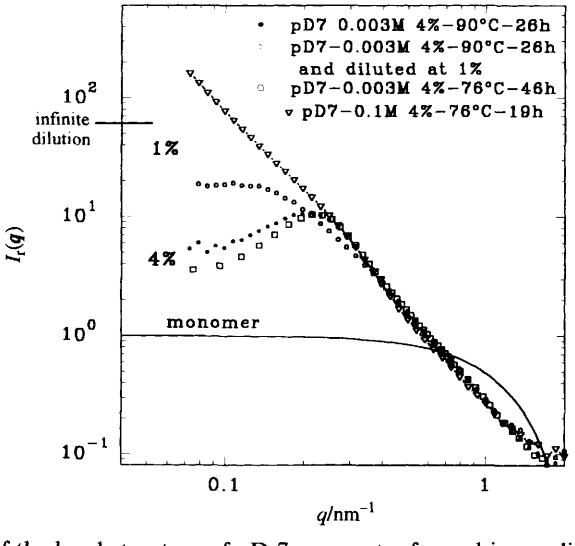

Fig. 2 Comparison of the local structure of pD 7 aggregates formed in conditions of strong interactions (0.003 mol dm^{-3} solvent ionic strength) with one of the aggregates formed in screened conditions (0.1 mol dm^{-3} solvent ionic strength)

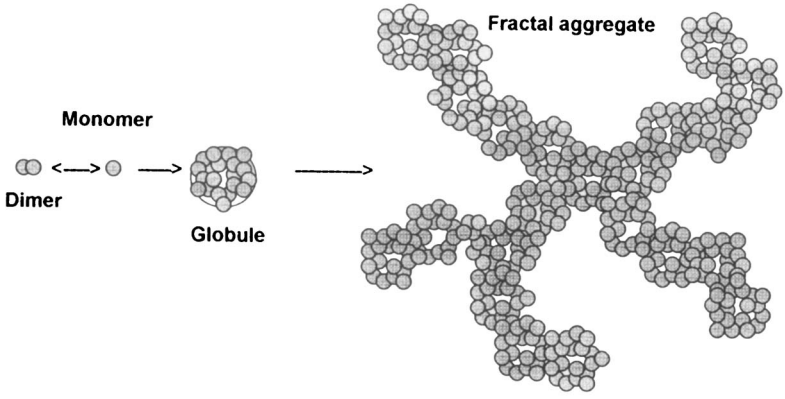

Fig. 3 Scheme of the aggregation process for β-lactoglobulin at pH 7

populations (residual monomers and aggregates) is obvious. Moreover, scattering data should be corrected to take into account the effect of the interactions, which are strong at low ionic strengths.

1. J. C. Gimel, T. Nicolai, D. Durand and P. Aymard, *J. Chim. Phys.*, 1995, in the press.
2 J. C. Gimel, D. Durand and T. Nicolai, *Macromolecules*, 1994, **27**, 583.
3 P. Aymard, T. Nicolai and D. Durand, *J. Int. Polym. Anal. Characterization*, in the press.
4 S. P. F. M. Roefs and C. G. de Kruif, *Eur. J. Biochem.*, 1994, **226**, 883.

Dr. de Kruif responded: Aymard *et al.* have made an extensive series of light-scattering and SANS measurements on β-lactoglobulin (β-lg) aggregates. They propose that the aggregates contain building blocks of what they call 'globules' and that at low salt concentration only the globules are formed. We agree that their data could be interpreted as such.

In our work, we predicted the formation of 'polymeric' particles at low salt concentration. The essence of the theory was that these particles would consist of a chain or necklace or string of β-lg monomers which are connected by intermolecular disulfide bonds. We called this a linear chain as is done in polymer physics, indicating that there are few, if any, side chains. The suggestion that these chains form globular substructures as real polymers do is still consistent with our model. The polymeric particles are predicted to be polydisperse. Initial analyses of aggregates by gel permeation chromatography and polyacrylamide gel electrophoresis confirm the prediction. According to Aymard *et al.* the globules should be quite homodisperse.

Our polymerization model predicts that the size of the polymeric particles is proportional to the square root of the initial concentration of β-lg. This was confirmed by a large body of light-scattering experiments in the concentration range 1.5–10%; the analyses with gel permeation chromatography and polyacrylamide gel electrophoresis also indicated that aggregate size increases with initial concentration. At low salt concentration (comparable to our experiments) Aymard *et al.* present the data for only one concentration, *i.e.* 4%. From these results it would be difficult to establish size differences in the polymeric particles in view of the square root dependence on β-lg and polydispersity effects. When salt concentration is increased to 0.1 mol dm^{-3}, we also observed a two-step aggregation process.[1] First, primary particles are formed and subsequently at a critical concentration of primary particles they aggregate to much larger secondary aggregates.

In conclusion we think that the two sets of results are complementary rather than conflicting.

1 M. Verheul, S. P. F. M. Roefs and C. G. de Kruif, in *Food Macromolecules and Colloids*, ed. E. Dickinson and D. Lorient, Special Publication No. 156, The Royal Society of Chemistry, Cambridge, UK, 1995, p. 437.

Dr. Horne said: There is a considerable body of literature, recently reviewed by Dalgleish,[1] on the modelling of the aggregation reaction of casein micelles induced by the enzymic proteolysis of κ-casein. Much of this theory was originated by the late T. A. J. Payens, formerly of NIZO. In these studies the casein micelles are always attractive and the removal of the steric stabilizing barrier provided by the κ-casein leads to an irreversible aggregation and ultimately gel formation. These theories are therefore attempting to model the kinetics of what is essentially, in these terms, an irreversible process. The adhesive sphere model, which you present in this paper, is, I believe, an equilibrium theory and as such should be reversible. One could imagine that in a more diluted suspension where differences in viscosity from the medium would be less readily measurable, the attraction between micelles, dependent on your well depth and the extent of proteolysis, would lead to transient aggregates detectable by dynamic light scattering or turbidity, say, and that further dilution would produce a decrease in average size as the reversible bonds, once broken, would rely on the micelles meeting once more for re-formation, a now less likely event. Of course, if irreversible aggregates had been formed then there would be no change in aggregate size on dilution. Have you been able to demonstrate that the system is reversible, as expected of equilibrium behaviour? How far up the viscosity growth arm of the viscosity curve does this apply?

You refer to κ-casein as a polyampholyte brush and suggest that this collapses as the system pH approaches pK_a, causing the loss of the steric stabilization component. Though we have yet to carry out a similar exercise for κ-casein, Leermakers *et al.*[2] have carried out a calculation of the conformation of an adsorbed β-casein 'look-alike' molecule at an interface using the self-consistent-field theory of Scheutjens and Fleer. Among the results of these calculations is the prediction that the extended layer does not collapse as the pH is lowered from 7.0 to 5.5, admittedly still above the isoelectric point of the casein. This prediction is in qualitative agreement with some unpublished results of ours on adsorbed layer thickness of β-casein on polystyrene lattices measured as a function of pH using dynamic light scattering. In the casein micelle, of course, the situation on acidification is further complicated by the dissolution of the micellar calcium phosphate and the possibility of 'freeing-up' intra-micellar bonding prior to isoelectric precipitation effects.

1 D. G. Dalgleish, in *Advanced Dairy Chemistry, 1-Proteins*, ed. P. F. Fox, Elsevier Applied Science, 1992, p. 579.
2 F. A. M. Leermakers, P. W. Atkinson, E. Dickinson and D. S. Horne, *J. Colloid Interface Sci.*, submitted.

Dr. de Kruif responded: The adhesive-sphere model is a statistical mechanics theory.[1] Therefore, we agree with Horne that this theory describes reversible or, better, equilibrium processes. In the literature, as reviewed by Dalgleish, models are based on Smoluchowski-type approaches and are therefore kinetic theories in which the aggregate formation is usually a one-way process. The adhesive-hard-sphere approach only applies if the attractions are not too strong, *i.e.* of the order of a few kT. For larger interaction energies the probability of breaking a bond becomes so small that it is permanent on the timescale of the experiment.

The theory incorporates the concentration dependence of macroscopic variables such as light scattering, turbidity and viscosity.[2,3] We have shown that on dilution the experimentally accessible quantities vary according to theory.

Concerning the collapse of the polyelectrolyte brush, κ-casein is somewhat different indeed from β-casein. Yet we would expect a collapse of β-casein as well on approaching the isoelectric point or better, according to theory, the 'theta conditions' for the brush expressed in a second virial coefficient v_{crit}, of the brush. This v_{crit}, however, depends on charge density, brush density and salt concentration. In order for collapse to occur it is further necessary that the backbone chain is in a poor solvent.

In conclusion, we would indeed expect a collapse of the brush; however, it will be difficult to predict *a priori* the conditions of collapse.

1 D. A. McQuarrie, *Statistical Mechanics*, Harper and Row, New York, 1976.
2 C. G. de Kruif, *J. Colloid Interface Sci.*, 1993, **155**, 38.
3 C. G. de Kruif, *Proceedings of Food Colloids and Polymers*, April 1992, Lunteren, ed. E. Dickinson and P. Walstra, Royal Society of Chemistry, Cambridge, 1993, No. **113**.

Mr. Bos said: At the end of your paper, you measure a decreasing D, and mention that this conflicts with theory. We have, both in simulation and experiment, found decreases in D and have been able to explain this by coarsening of an already formed network. Of course, the development of D is not the key point here, more the development of correlation length and size of the basic building blocks. In a log–log plot, these are to be found in the intercept, whereas D only gives the slope. The theories with which your experiments conflict are wrong, because they only consider scaling, whereas in rheology and permeability you are actually interested in absolute values. I think that these theories can be modified simply, incorporating not just the slope, but also the intercept.

Dr. de Kruif agreed.

Prof. Djabourov asked: You make a very interesting comparison between syneresis and spinodal demixing. What are the experimental facts that can support this analogy?

Dr. de Kruif responded: We have made a comparison between syneresis and spinodal demixing on the basis of two facts. (i) Theory predicts a coarsening (demixing) of the two continuous phases if the attraction is strong enough so that the system is in the two-phase region. (ii) In practice, one observes a coarsening of the structure, which for instance is very manifest in the permeability of the gels.

Dr. Poon said: I would like to comment on what might happen to the percolation line inside the two-phase region (Fig. 6 of your paper). Lattice-gas simulations of Binder and co-workers[1,2] suggest that deep inside a region of two-phase coexistence there should be a cross-over from spinodal decomposition (S) to 'dynamic percolation' (DP). Some time after quenching, the 'second-phase' spans space. The space-spanning structure, a 'transient gel', lasts for a finite time, τ, and then collapses. The gel lifetime, τ, increases with the quench depth.

I would also like to draw attention to the experimental work of the Pavia group[3] which suggests that a homogeneous sample with composition in the two-phase region but to the left of the DP line (filled square in Fig. 4) would 'phase separate' into a dilute phase (1) and a gel (2).

1 S. Hayward, D. Wottermann and K. Binder, *J. Stat. Phys.*, 1987, **49**, 1053.
2 G. Lironis, D. W. Heermann and K. Binder, *J. Phys. A: Math. Chem.*, 1980, **23**, L329.
3 R. Piazza and G. di Pietro, *Europhys. Lett.*, 1994, **28**, 445.

Dr. de Kruif agreed.

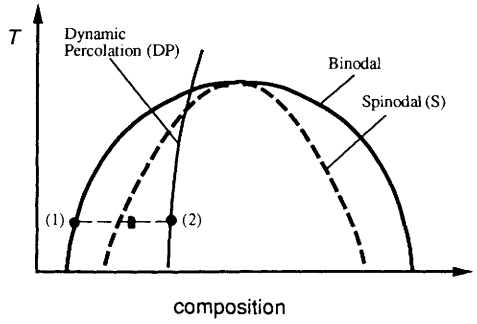

Fig. 4 Dynamic percolation line according to Binder and co-workers indicated schematically in relation to the binodal and spinodal

Prof. Nishinari communicated: We reported that casein micelles with small sizes formed gels faster than those with large sizes.[1] Do you think that the viscosity increase shown in Fig. 2 of your paper begins earlier for casein micelles with small sizes than for those with large sizes?

You have not described the method of viscosity measurement for Fig. 2 of your paper. Does the viscosity measurement destroy the structure being formed?

1 R. Niki, K. Kohyuma, Y. Sano and K. Nishinari, *Polym. Gels Networks*, 1994, **3**, 105.

Dr. de Kruif communicated in reply: Whether the increase in viscosity starts earlier for small particles than for large particles would be an interesting subject, because it will provide insight into the details of the model and into the colloidal stability of the casein micelles. The answer can be predicted with the help of the theory, provided that the following values can be specified: strength of the attraction as a function of size, number of κ-casein molecules per surface area (roughly constant), enzyme activity and volume fraction. Our intuitive guess is that with all variables unchanged except for the micellar size, the small particles would be less attractive because the steric layer has a longer range compared with the particle size.

The viscosity measurements were made with an Ubbelohde viscometer. It can be shown that at weak interactions and/or low shear the system is not disturbed by the shear field. However, if attraction becomes stronger, the distribution of the particles and thus viscosity will be changed by the shear flow

Prof. Vincent opened the discussion of Prof. Tanaka's paper: I was interested in the observation you report that ions are only absorbed by your poly(NIPAM/acrylic acid) gels when the gels are in the collapsed state. Martin Snowden and I[1] have been studying the ion uptake/release properties from aqueous solution by poly(NIPAM) microgel particles (diameter *ca.* 0.5 μm in the collapsed state). We find, in contrast with your work, that ions such as Pb^{2+} are absorbed at low temperatures when the microgels are in the swollen state and then released (partially) on heating above the transition temperature, *i.e.* in the collapsed state. Since then we have carried out further experiments[2] with copolymers of NIPAM and acrylamidepropanesulfonate (AMPS), and find similar results, with the preferential uptake of ions such as Pb^{2+} or Cd^{2+}, from mixtures with Mg^{2+} or Ca^{2+}, being enhanced when AMPS is present.

1 M. J. Snowden, D. Thomas and B. Vincent, *Analyst*, 1993, **118**, 1367.
2 J. Martin, B.Sc. Thesis, Bristol University, 1995; to be published.

Prof. Tanaka replied: The difference between your results and ours may be due to the difference in the chemical compositions of the gels used in the operations. In the case of poly(NIPAM) gels, the mechanism of absorption may be an incorporation of the metal solution by swelling and squeezing of the solution upon collapsing.

As to the copoly(NIPAM/AMPS) gel I do not have a quick answer to the question of why the gel absorbs the metal ions in a swollen state and releases them in a collapsed state. Again the chemical compositions for AMPS and acrylic acid are different, as are their affinities to metal ions.

Dr. Ettelaie said: The schematic Helmholtz-energy diagram that was shown in your talk indicated the existence of an intermediate state separated by the Helmholtz-energy barriers from the swollen and also the collapsed phases. Is this intermediate state a long living metastable or a true equilibrium thermodynamic state?

Regarding the intermediate state, it was mentioned that it has very few configurations (hence it maintains a memory effect). This implies that the state has a very low entropy, which has to be compensated by low energies, if the state is to be stable at finite temperatures. You also pointed out the existence of replica symmetry breaking, a phenomenon usually associated with disordered systems. Can you explain what are the main features of the interaction in your system that allow the system to display this type of behaviour?

Prof. Tanaka responded: The diagram illustrates the possible existence of a phase different from collapsed or swollen phases known to homopolymer gels. Depending on the external condition these Helmholtz-energy minima become thermodynamically stable phases or metastable phases.

The phase in a frozen state has a near zero entropy. One of the main features of the interaction needed for a freezing phase transition is the extent of heterogeneity of monomer–monomer interactions. The phase transition temperature of freezing into a random conformation is determined by the variance and flexibility of the heteropolymers. The freezing temperatures to the original conformation upon preparation is higher than the random freezing temperature and depends on the variance and the difference between preparation and measurement temperatures

Dr. Bot asked: For practical applications of these interesting materials, fast switching between swollen and deswollen states is desirable. How fast is this transition in the present system, and are there any methods to accelerate it or slow it down?

Prof. Tanaka replied: The kinetics are very important for practical and experimental purposes. The characteristic time for swelling and shrinking of a gel depends on two factors; the size and shape of the gel and the collective diffusion coefficient of the polymer network. Roughly, the time is given by the square of the smallest characteristic length of the gel divided by the collective diffusion coefficient. The collective diffusion coefficient can become zero on the spinodal line including the critical point where the kinetics become infinitely slow. The strategy to make the molecular recovery faster is to move the operation condition away from the spinodal line and to make the gel smaller.

A typical time for a 1 μm gel bead may be 1 ms and for a 1 cm gel it will be a week.

Prof. Khokhlov asked: I understand that there is a selectivity in the absorption of metal ions in the temperature range around 40 °C; Cu^{2+} ions induce collapse, while Ca^{2+} do not. What about at 60 °C when the collapse will be reduced by both sorts of ions. Will the selectivity remain?

May I guess that the selectivity will probably be less pronounced because of the

irreversibility of the structure in the collapsed gel. This would then resolve the contradiction mentioned by Prof. Vincent.

Prof. Tanaka responded: This is a very important suggestion. We have not done such an experiment in a systematic way and wish to do so in the future. I have the same feeling that the selectivity will become less pronounced as you move away from the transition threshold. The selectivity should be optimum between the two transition temperatures of the metals involved.

I am not sure that the reduction of selectivity could explain the discrepancy between Prof. Vincent's observation on poly(NIPAM/AMPS) gels and ours on poly(NIPAM/acrylic acid) gels, since the difference is in the opposite temperature dependence.

Prof. Vincent commented: We have measured[1] the response time of poly(NIPAM) microgel particles, collapsed diameter *ca.* 0.3 μm dispersed in water, to temperature changes in the region of the swollen/collapsed state transition temperature (*ca.* 35 °C). We monitored changes in the particle diameter, using dynamic light scattering, as the temperature was ramped from below the transition temperature to above, and *vice versa.* The particle diameter appears to change 'instantaneously' as the temperature is varied, *i.e.* the rate-determining step is the transfer of thermal energy into and out of the microgel particles. It is difficult to give a precise timescale for this process.

1 B. Saunders, M. J. Snowden and B. Vincent, to be published.

Prof. Tanaka added: I think that your gels have submicron size and should have a characteristic time of the order of 1 ms.

Prof. Aguilera communicated: Do you know of any examples in nature where the mechanism you presented for collapsible gels operates?

Are you familiar with the vegetative/spore 'transition' of some bacteria, whereby they become 'drier' and highly resistant to heat?

Prof. Tanaka communicated in response: Prof. Pedro Verdugo of University of Washington, Seattle discovered that mast cells secrete histamine-containing gel granules and release them upon a swelling phase transition. Histamine is accumulated into the granule in the collapsed phase *via* the phase transition.

He has also been studying mucin gels of slugs. The best ones are, of course, from Seattle. The granules are used to absorb water around them to retain a moist atmosphere. The question is how such highly swellable gels can be stored, and why they do not swell in the slug's body, which is filled with water. He solved the question by demonstrating that the gels undergo a collapsing phase transition in response to calcium ions. His observation is directly related to what we are shown here.