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Mutual Gelation of Gelatin and Water-in-Oil Microemulsions

Christian Quellet and Hans-Friedrich Eicke*

A thorough understanding of the gelation process is a challenge to colloid and polymer scientists. Thus an experimental study is presented in which the polymer-colloid interactions nicely elucidate some of the subtle details of the gelation phenomenon. – Varying amounts of gelatin were solubilized in thermodynamically stable water-in-oil microemulsions consisting of aqueous «nanodroplets», i.e. nanometer-sized droplets, covered by a monomolecular layer of Aerosol-OT (sodium bis(2-ethylhexyl) sulfosuccinate) and dispersed in isooctane (2,2,4-trimethylpentane). The mutual gelation process of gelatin and such a microemulsion can be resolved into three well distinguishable steps, i.e. (i) the formation of «nanogels», which means gel formation inside the water pools of the nanodroplets, (ii) coagulation of the nanodroplets into an infinite cluster (nucleation step of gelation, formation of a «gel macromolecule»), and (iii) the bulk gelation. The latter is brought about by cross-linking the nanodroplets via gelatin helix-helix interactions. – The resulting weak solids offer many potential applications, e.g. they may be considered as model systems for medical, agro- and food-chemical or pharmaceutical technologies. The transparency of these four-component systems may also stimulate photochemical investigations.

1. Introduction

In the last decades efforts have repeatedly been made to fully comprehend the problem of polymer gelation. Particular emphasis was laid on the elucidation of the nucleation step of gel-formation. The latter was frequently linked up with inhomogeneities of the system from which nucleation is expected to start. It is difficult, apparently, in polymer-solvent systems to determine such heterogeneities uniquely[1–3]. Quite recently the necessity was pointed out again[4], to prepare and study polymer networks with well-defined heterogeneities.

In this account we present a particularly interesting type of heterogeneity which is not only well-defined but also conforms surprisingly well to a hypothetical model put forward by *Burchard* et al.[4]. Such heterogeneities are represented by spherical aqueous *nano*meter-sized *droplets* (nanodroplets) dispersed in a continuous oil phase and which are spontaneously and reversibly formed by a mixture of water, Aerosol-OT, and isooctane, a so-called water-in-oil microemulsion. To these microemulsions we add increasing amounts of gelatin which will initially be confined to the aqueous nanophases (due to the insolubility of gelatin in apolar media). Hence we begin our experiments with perfectly defined nanoheterogeneities from which the gelation process has to start. By varying the gelatin concentration we expect to obtain a detailed picture of the gelation of the polymer. The progress of gelation can be followed via the formation of helices, i.e. by optical rotation and circular dichroism.

As soon as the nucleation step of gelation, which occurs in the nanophases, is terminated the polymer will start to extend into the continuous dispersion medium. A gelation throughout the bulk of the system requires the formation of polymer links between the nanodroplets of the microemulsion. Such a process leads necessarily to a gelation of the nanodroplets, i.e. of the microemulsion. The whole system has to be called a lyogel in which gelatin and nanodroplets form the network, i.e. three-dimensionally cross-linked colloidal «necklaces» which are dispersed in the continuous oil phase. In this sense one can describe the overall process as a simultaneous gelation of the polymer and the microemulsion.

It is easily to foresee that such systems imply several scientific and technological applications.

Hans-Friedrich Eicke is Professor of Physical Chemistry at the Universität Basel. His research interests center on micelles, microemulsions, supermolecular fluids, gels, and ultraweak solids. He has published more than 100 research papers and contributions to scientific series and books. He recently edited «Modern Trends of Colloid Science in Chemistry and Biology» (Birkhäuser, Basel) and he is co-editing with the late Prof. G. Parfitt «Interfacial Phenomena in Apolar Media» (Dekker, New York). H.-F. Eicke received his diploma in Physics from the Universität Göttingen, FRG, in 1958 and his Ph. D. from the Max-Planck-Institut of Biophysical Chemistry in Göttingen (1961) which was followed by a postdoctorate with the late Prof. Peter J. W. Debye at Cornell Universtity, USA.

Christian Quellet: Born 1958 in Neuchâtel. Dipl.-Chem. Ing. 1984 at the Université de Neuchâtel. Post-diplom research with Prof. Raphaël Tabacchi and at Philip Morris Laboratories in the field of the analysis of flavours and fragrances by gas-chromatography/mass spectrometry. Since 1984, Ph. D. Student at the Institute of Physical Chemistry in Basel. Present main interests in the physical chemistry of organized systems and their applications.

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* *Correspondence:* Prof. Dr. H.-F. Eicke
Institut für Physikalische Chemie
Universität Basel
Klingelbergstrasse 80, CH-4056 Basel

2. Experimental Remarks

2.1. Materials

Samples of various gelatin qualities have been tested and the most homogeneous and reproducible gels have been obtained with a material (a pig skin gelatin), purchased from Sigma Company, which was selected in view of a high gel strength in water (300 Bloom).

Light scattering measurements on aqueous solutions using the Zimm-plot[5] and the Guinier method[6] yielded a molecular weight of 90000 ± 10000 g/mol and an average radius of gyration $\langle R_g \rangle$ of 19 ± 2 nm for the single strands (α-gelatin). Both values are similar to those determined by *Boedtker* and *Doty*[7] and *Eagland* et al.[8]. All gelatin samples, however, showed a pronounced polydispersity which is usually observed with gelatin solutions.

Comparison with results of the scaling theory for random coils[9] confirmed that, even at 303 K and in solutions containing a hydrogen-bond breaker such as KSCN, gelatin single molecules do not behave like free randomly coiled species but display rigid intracatenar structures. The isoionic pH, determined at the maximum scattering intensity and a scattering angle of 90°[7], was shown to be 9.5 ± 0.1, a value typical of pig skin gelatin[10].

Aerosol-OT (= AOT, i.e. sodium bis(2-ethylhexyl) sulfosuccinate, from Fluka AG) was purified in an active carbon slurry under stirring for about 24 hours. The sample was filtrated, evaporated to dryness, and the residual water was removed under reduced pressure ($3 \cdot 10^{-2}$ Torr). Because AOT is hygroscopic, the purified surfactant was stored in a desiccator.

2.2. Preparation of the Samples

Weighed-in amounts of gelatin were allowed to swell in a calibrated quantity of water for 2 h at room temperature. Then the system was warmed up to 333 K and kept at this temperature for 10 min, in order to obtain a clear solution. Finally this solution was dispersed in a given volume of 0.1 M AOT/isooctane solution, at the above temperature, with the help of a vibromixer. The parameters of this system are: W_0 (mol/mol) = $[H_2O]/[AOT]$, G (%) = gelatin weight per total volume, G_w (%) = gelatin weight per water volume, c_{AOT} = AOT concentration (mol/L).

The samples were quenched at 290 K and allowed to set one week prior to any measurement.

Repeated heating, redispersion, and setting cycles did not show any remarkable variation with respect to the physical properties of the systems within the time scale of the experiments. However, as became apparent from aging of some of the samples, gels obtained by this procedure were metastable and developed slowly a more densely packed structure, releasing small amounts of solvent. Several thermal cycles involving slow heating of the samples up to 305 K and subsequent cooling at the same rate (0.01 K/min) appeared to accelerate the aging process but did not yield reproducible states. Nevertheless, these measurements allowed us to determine the equilibrium concentration threshold of gelatin (gel point) for gelation of the total sample volume, which was 3.1 ± 0.2% gelatin; this value is slightly higher than that necessary for gelation under metastable conditions (G = 2.7 ± 0.2%) and considerably higher than the gel point in pure water (G_w = 0.9%).

2.3. Measurements

Electrical Conductivity

A specially designed conductance cell, consisting of two 30 × 5 mm Pt-electrodes 5 mm apart, was used for the measurements of the specific conductivity σ at various temperatures and gelatin concentrations. Immersion of the electrodes into the system before or after the gelation had occurred did not change the final conductivity values. Gels were sufficiently weak not to be permanently damaged by accommodating the electrodes. Conductivity was measured after an equilibration time of 0.5 to 10 h, depending on the viscosity of the sol or on the gel strength. Measurements were made with a Wayne-Kerr universal bridge model B221 for $\sigma < 10\ \Omega^{-1}\ m^{-1}$ and a Methrom conductometer model E382 for larger σ-values.

Light Scattering

Light-scattering experiments were carried out using a light-scattering photometer Sofica with thermostated scattering cells. The wavelength was 546 nm and the intensity of the scattered light was measured as function of the scattering angle.

Optical Rotation

Optical rotation of sols and gels was measured with a Zeiss polarimeter, equipped with a mercury lamp, using thermostated 1 and 5 cm thick cells. All measurements were carried out at a wavelength of 546 nm. Gels were warmed up to 305 K before transferring them into the cell. Time-dependent measurements at different temperatures have shown that the equilibrium specific rotation ($[\alpha]_\lambda$-value) was attained after 3 days for all samples.

Circular Dichroism

Because of the high gelatin concentration and the nanometer-sized heterogeneities of the matrix, which induce strong absorption and depolarisation of the CD beam, thin Hellma cells (0.001 cm) had to be used. The apparatus was a Jobin & Yvon Autodichrograph Mark IV equipped with a cell carrier block, which could be thermostated.

3. Results and Discussion: Formation of a «Superpolymer»

A typical phase diagram of our system is shown in Fig. 1. Both, sol-gel transition (curve a) and demixing line of the microemulsion (curve b) are depicted for constant water/surfactant molar ratio, W_0 = 60, and constant total volume.

The curves divide the phase diagram into four main regions labeled I to IV in Fig. 1. Regions I and II correspond to a clear sol and a clear gel separated by a narrow transition domain where gelatineous macroscopic aggregates coexist with the solvent. While the area of this domain decreases with increasing temperature, the position of the (clear)sol–(clear)gel transition is temperature-independent. Region III is heterogeneous

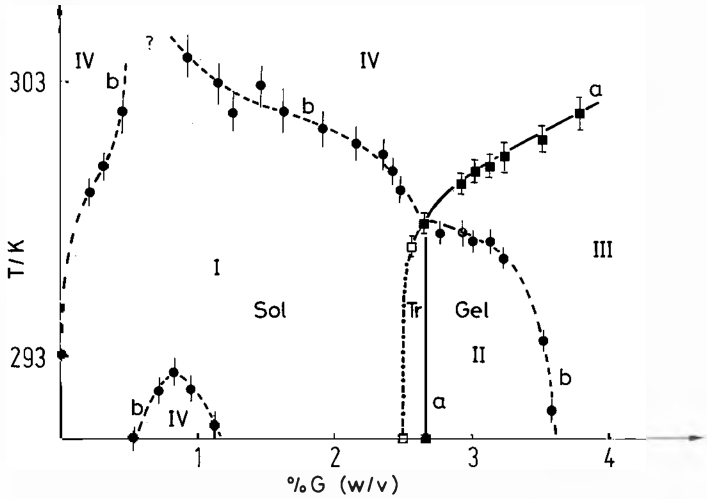

Fig. 1. Phase diagram of the gelatin-water-AOT-isooctane system at constant $W_0 = [H_2O]/[AOT] = 60$, $T = 293$ K, and $c_{AOT} = 0.1$ M. ------: binodale; ——: melting curve; Tr: transition region.

because gelation is accompanied by a liquid-liquid phase separation and preferential aggregation of the polymer in the water-rich phase; in other words, there exists a temperature-dependent gelatin concentration above which the microemulsion is destroyed. Phase separation is also encountered in region IV which corresponds to a two-phase sol. It is difficult to determine the cloud point in this region, probably due to a matching of the refractive indices of the two coexisting phases in region IV.

The homogeneous gel as well as the clear sol regions cover a relatively small range of temperatures and concentrations; it reveals the dependence of the system on various parameters such as polymer-polymer interactions (tendency for coagulation), polymer-surfactant interactions (complex formation), and polymer-solvent interactions leading to excluded volume effects and preferential adsorption. All these interactions control the stability of the microemulsion and the structure of the gel.

All results referred to in this study are obtained exclusively from an investigation of the homogeneous domains of the phase diagram, i.e. sufficiently far from phase boundaries.

The rotatory dispersion of gelatin and collagen can approximately be described by a single term of the Drude equation[11]

$$[\alpha]_\lambda = A/(\lambda^2 - \lambda_c^2) \tag{1}$$

where A is a constant, λ is the selected wavelength, and λ_c is the wavelength at maximum absorption of the optically active electronic transition. The helix fraction f_h is directly related to the optical activity, measured at one wavelength; it is defined by

$$f_h = \frac{[\alpha]_\lambda^{\mathrm{obs}} - [\alpha]_\lambda^{\mathrm{random\ coil}}}{[\alpha]_\lambda^{100\%\ \mathrm{helix}} - [\alpha]_\lambda^{\mathrm{random\ coil}}} \tag{2}$$

where $[\alpha]_\lambda^{\mathrm{random\ coil}}$ and $[\alpha]_\lambda^{100\%\ \mathrm{helix}}$ describe the specific rotations of completely denatured gelatin and native collagen, respectively. Values of $[\alpha]_\lambda^{100\%\ \mathrm{helix}}$ at 589 and 436 nm are $-400 \pm 20°$/dm and $-800 \pm 10°$/dm[12].

Taking $\lambda_c = 223$ nm for the optically active electronic transition corresponding to the folding of gelatin, $[\alpha]_{546}^{100\%\ \mathrm{helix}}$ is found to be $-465 \pm 20°$/dm.

On the other hand, measurements made with aqueous solutions of gelatin in the presence of KSCN yielded $[\alpha]_{546}^{\mathrm{random\ cell}} = -130 \pm 10°$/dm, a value in agreement with literature[12].

Fig. 2 shows the change of helix fraction f_h of the sample as function of gelatin concentration at 293 K. The left-hand side of this diagram displays a monotonic increase of the specific optical rotation with rising gelatin concentration.

The gelatin concentration corresponding to zero helix fraction has to be obtained by extrapolation. It is lower with respect to the water content of the nanophases G_w(%) than the gel point in pure water. In other words, this is the gel point in the aqueous nanodroplets, which corresponds to the onset of the «nano-gel» formation.

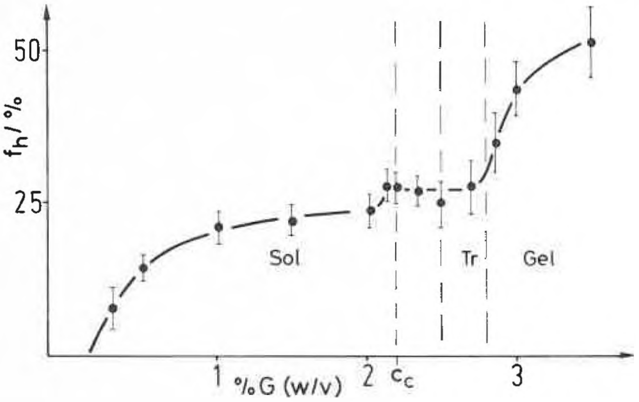

Fig. 2. Variation of the helix fraction (f_h) as function of gelatin concentration. The percolation concentration (c_c) and the phase bondaries are indicated (dashed lines); Tr: transition region.

Conductivity measurements carried out in this concentration range do not show any significant conductivity change during the nanogel formation. This is in accordance with our view that the nanodroplets are the mobile charge carriers moving in the fluid dispersion medium at least at low temperature and gelatin concentrations. The nanogel formation should have no effect on this process.

Increasing amounts of gelatin, however, show a steep rise of the conductivity, up to four orders of magnitude. The gelatin concentration extrapolated from the steep slope of the σ-plot is still lower than that required for (bulk) gelation (Fig. 3).

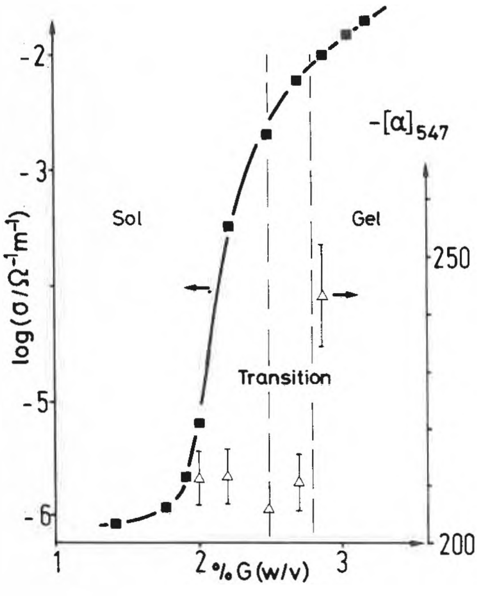

Fig. 3. Logarithm of specific electrical conductivity (σ) and specific rotatory power ($[\alpha]$) as functions of gelatin concentration. $W_0 = 60$, $T = 295$ K, and $c_{AOT} = 0.1$ M.

The conductivity increase is also observed with rising temperature (Fig. 4). The onset of this σ-change occurs, in the latter case, well below the demixing temperature of the system.

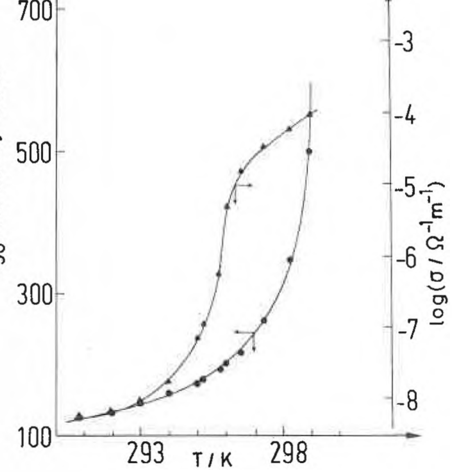

Fig. 4. Intensity of 90° light-scattering ($I_{90°}$) and logarithm of specific electrical conductivity (σ) as functions of temperature. $W_0 = 50$, $c_{AOT} = 0.1$ M, and $G(\%) = 2.51$ (w/v).

As shown by *Eicke* et al.[13], this (quasi) critical behaviour may be described in terms of percolation theory and occurs also in gelatin-free microemulsions with AOT as surfactant. According to this theory, (fractal) cluster formation of aqueous nanodroplets in these microemulsions gives rise, among other things, to a percolation of electric conductivity indicating that an infinite (fractal) cluster has been formed. The existence of such a transition in gelatin containing water-in-oil microemulsions confirms their well-defined nanophase structure, at least in the sol region.

As is apparent from Fig. 2, the percolation threshold coincides approximately with a small conformational change evidenced by a slight increase of the optical activity. The dichroic absorption (measured at 223 nm, see Fig. 5 below) shows that this change is very steep and is due to the formation of additional helical segments. The coincidence of this conformational change and the percolation is partic-

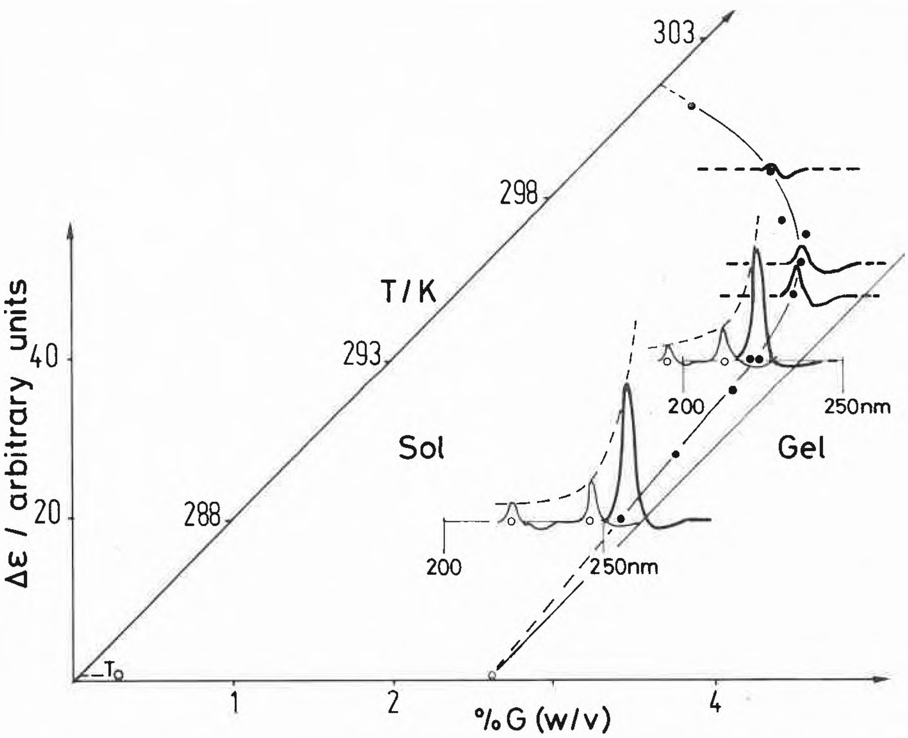

Fig. 5. Plot of percolation thresholds and dichroic absorption (at λ = 223 nm) as functions of gelatin concentration. The shape of the dichroic spectrum between 200 and 250 nm is also indicated. ○: *measurements below the percolation threshold;* ●: *measurements at the percolation threshold; T_0: extrapolated temperature, where percolation and gelation coincide; $W_0 = 60$; $c_{AOT} = 0.1$* M.

ularly interesting; it reflects the fact that both, macroscopic (cluster formation) and microscopic (molecular and nanophase structure) properties change in this concentration region. It is supposed that gelatin itself participates in the cluster formation by modifying the immediate environment of the nanodroplets. The building up of polypeptide segments with helical structure extending into the oil phase may be responsible for this effect which induces a *polymer-controlled percolation*. Such a behaviour is strongly affected by the hydrophobic nature of the helices and by the presence of hydrophobic amino acids distributed along the polypeptidic skeleton.

A plot of percolation thresholds and dichroic absorption data as functions of gelatin concentration is shown in Fig. 5, in which the shapes of the dichroic absorption bands at 223 nm have also been indicated.

The percolation thresholds define the concentrations where infinite clusters appear. From the shape of this curve, which resembles an order-disorder transition, we infer a controlling effect of the helix-coil transition on the percolation process.

Extrapolation of the curve to higher amounts of gelatin yields the temperature (T_0) where percolation threshold and gel point coincide. This temperature is lower than the lower demixing temperature of the system. The situation at this gel point should be physically similar to that encountered during gelation in a normal binary system consisting of, for example, polyfunctional (f > 2) monomers which are allowed to gel in a single solvent by three-dimensional polymerization. The theory of gelation[14] predicts the same behaviour at the critical gel point, independent of the particular nature of the particles which are involved in the gelling process. The theory assumes that this behaviour is directly related to the coagulation of the sol-species into an (infinite) gel-macromolecule, i.e. percolation is identical to gelation.

In our case, this condition is only encountered at T_0. At higher temperature, we find that the gelling process is preceeded by the percolation of the nanophases; the latter has to be distinguished from the proper gelation and may be interpreted as a nucleation step.

Thus, the classical as well as the so-called percolation theory of gelation[15] do not apply to the particular case of gelation in one component macrofluid-like[21] microemulsions in the presence of polymers.

Beyond this transition region (see Fig. 2) the helix fraction of the polymer increases markedly at the gel point and tends to level off with growing concentration. This result suggests that intercatenar helices are responsible for the gel-network formation; this is, actually, observed with all gelling polypeptides during gelation in pure water, although the change of the optical activity is somewhat smoother in the latter case.

The helical structure of the crosslinks is revealed by the CD-spectrum of the gel (Fig. 6), which is qualitatively identical with that of native collagen[16]. A quantitative evaluation of the dichroic absorption in this phase is, however, difficult because of the high gelatin concentration in the sample and depolarization effects due to the nanoheterogeneous matrix.

The change of [α] as function of temperature (Fig. 7) displays a hysteresis, typical of helix-coil transitions. The decrease of [α] coincides with the lowering of the gel strength. However, only a portion of the curve can be shown since the melting point

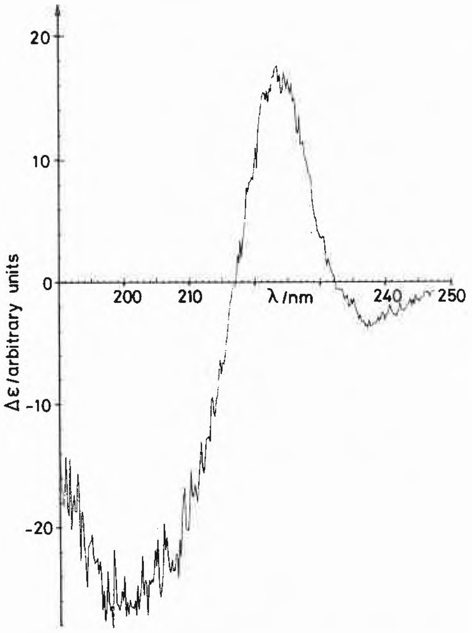

Fig. 6. Circular dichroic absorption spectrum of the gel. $W_0 = 60$, $T = 293$ K, $G(\%) = 3$ (w/v), and cell thickness = 0.01 mm.

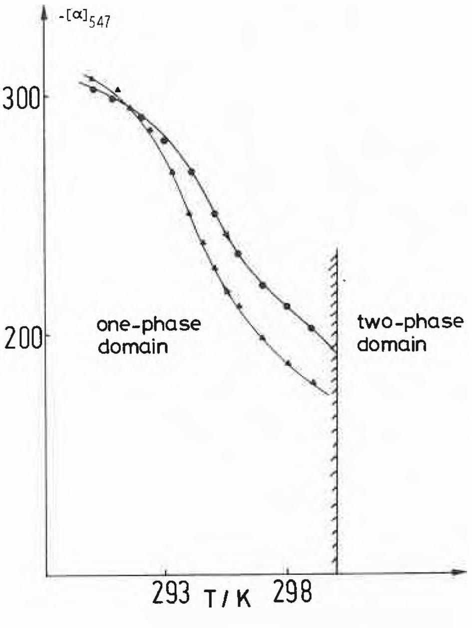

Fig. 7. Temperature dependence of the specific rotatory power of the gel phase as function of the temperature. $W_0 = 60$, $c_{AOT} = 0.1$ M, *and $G(\%) = 3.29$ (w/v).*

of the gel occurs above the binodale and cannot be observed by optical measurements.

Conductivity measurements in the gel phase (right-hand side of Fig. 3) reveal that the conductivity increases exponentially with the gelatin concentration. Such a behaviour differs from that met with aqueous gelatin gels, where the conductivity has been found to change linearly with concentration[17].

The overall gelation process of gelatin in our particular macrofluid can now be resolved into three different steps each associated with a conformational change of the molecule:

– *The first step* is the gelation of the solubilized polypeptide in the water pool of the nanophases, i.e. the formation of nanogels. The mechanism is identical to the gelation in pure water; it involves the formation of intra- and intermolecular crosslinks due to the formation of collagen-like helical segments (cf. Fig. 8). Taking a radius of about 100 Å for the gelatin-free water droplets at $W_0 = 60$ and $T = 303$ K[18] and assuming that the size of the latter remains approximately constant on addition of low amounts of gelatin, the onset of this intramicellar gelation corresponds to a concentration of two gelatin molecules per nanophase. Below this concentration the polymer may thus be considered to behave as a random coil in the water pool of the nanophase.

– *The second step* of the gelling process is the percolation of the nanophases. At this point, the system undergoes a drastic change of its structure and of its connectivity properties, since an infinite fractal cluster of nanophases is formed. This step corresponds, according to our interpretation scheme, to a nucleation step with respect to the gelation.

We claim that the percolation of the nanophases is related to the organization of the gelatin molecules at the water-oil interface with formation of helical segments extending into the oil phase. This assumption is supported by the fact that such segments are considerably more hydrophobic than the unfolded ones, because of the shielding of the charged side-groups[19].

The concomitant decrease of the percolation temperature is due to an increasing contact probability because of increasing attraction between the nanophases. The percolation (at 293 K) occurs at a formal concentration of about 15 gelatin molecules per water droplet. It should be emphasized that this concentration appears to be rather large and, thus, the nanogels become densely packed. The nucleation occurs in a well-defined range of gelatin concentrations. This is a consequence of our particular «solvent», which prevents the polymer from cross-linking in the apolar phase before a sufficient gel concentration is reached. Consequently, a retardation of gelation is observed, unknown in binary gelling systems, where nucleation and gelation can hardly be distinguished. Thus we conclude that it is a typical feature of this macrofluid-like microemulsion.

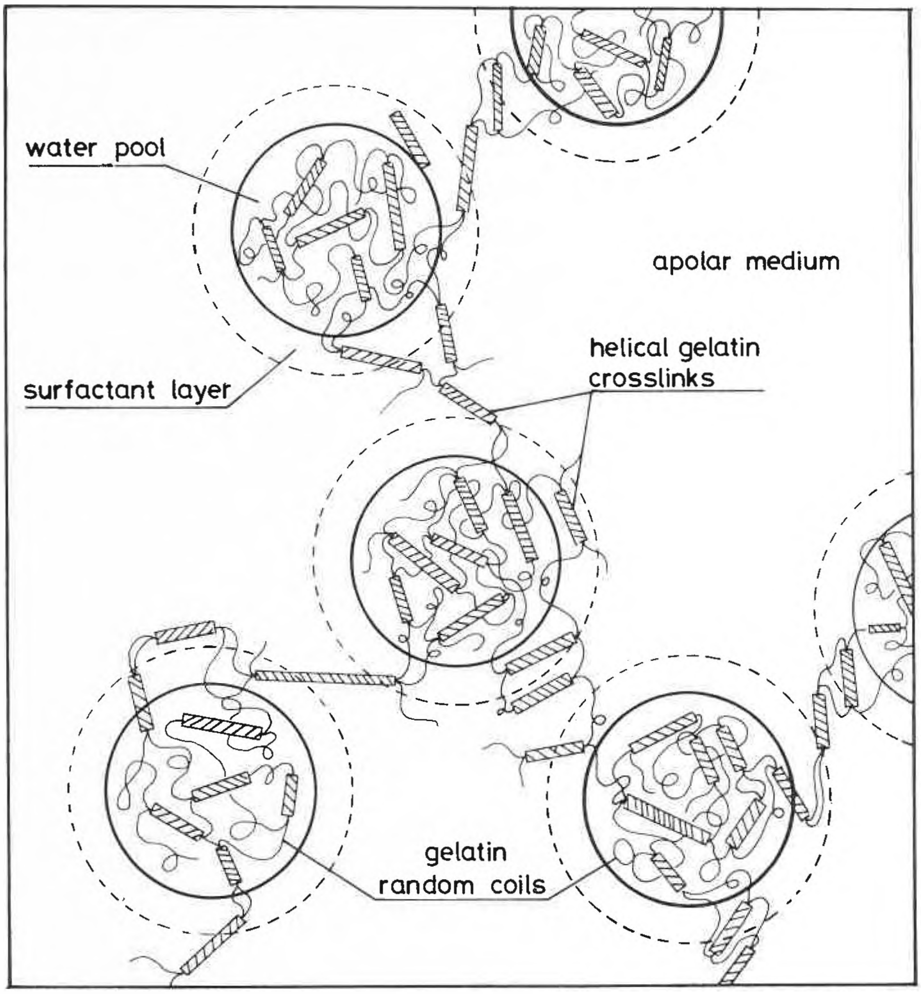

Fig. 8. Proposed model structure of gelatin-W/O microemulsion-gels with AOT as surfactant (W/O = water-in-oil).

– The sol-gel transition is the *third and last step* of the gelling process. This step involves the formation of a three-dimensional infinite network consisting of nanophases interconnected by bridges of collagen-like helices. An interesting aspect is to consider the gel as a «frozen» microemulsion, where a fluid gelatin-rich water phase is dispersed in the apolar medium. The system may be regarded as a gelatineous solid with a particular local order being composed of well-defined aqueous nanophases: The long-range order is brought about by an association of helices in the apolar dispersion medium, which are thought to bridge the gap between neighbouring nanophases. The result is the formation of a «superpolymer»[20]. The just mentioned association of helices which links up the nanodroplets has to be considered as a result of attractive forces between the nanophases. Hence the «superpolymer» might be also described as a stabilized state of coagulated nanodroplets.

In conclusion we hope to have shown that these four-component systems reveal interesting physical-chemical details of the polymer gelation which are otherwise not easily detectable. On the other hand such weak solids offer many potential applications, e.g. they may be considered as model systems for medical, agro- and food-chemical, or pharmaceutical technologies. The transparency of these systems may also stimulate photochemical investigations.

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