Colloid Polym Sci 273:1101–1137 (1995)

LEADING CONTRIBUTION

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Analytical ultracentrifugation of gels

Received: 1 June 1995
Accepted: 25 August 1995

Dedicated to Prof. Dr. W. Borchard on occasion of his 60th birthday

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Abstract Analytical ultracentrifugation is a powerful tool for the determination of thermodynamic, elastic and molecular parameters and structural properties of gels. Although gelling systems are an important class of mixtures, only a few researchers have studied their properties by means of analytical ultracentrifugation. This might be due to the extreme experimental difficulties concerning the detection of the polymer concentration in turbid gels, adhesion problems, etc. Nevertheless, the potential benefit of such experiments has led to several investigations in this field during recent years. These resulted in the introduction of a theoretical treatment of the sedimentation of even multicomponent gels and an improved experimental set-up which allows the characterization of a gel/solvent system in a limited concentration range in a single sedimentation equilibrium experiment. For microgels prepared and crosslinked in emulsions, an interesting rapid sedimentation velocity technique is available for their characterization. This review article describes the capabilities of the experimental method and what has been achieved with it in the past. Furthermore, it gives an outlook on applications which may be possible in the future.

Key words Analytical ultracentrifugation – gels – microgels – sedimentation velocity – sedimentation equilibrium

Introduction

Throughout recent decades, analytical ultracentrifugation proved to be one of the best methods for the physical characterization of gels as they can be studied in a continuous equilibrium (which can be varied by for example the rotational speed) or by means of rapid sedimentation velocity experiments which furthermore provide information about uncrosslinked molecules. Since the introduction of a new generation of analytical ultracentrifuges, specifically, the Optima XL-A from Beckman, a renaissance of analytical ultracentrifugation can be observed especially in the fields of biophysics/biochemistry. Simultaneously, an increasing interest in colloidal systems can be observed. As the analytical ultracentrifuge has been one of the classical devices for colloid characterization since its early days, it is expected that analytical ultracentrifugation will again play an important role in the characterization of colloidal systems. One important class of colloids are gels, either physically or chemically crosslinked. This review will describe the capabilities of the analytical ultracentrifuge for the characterization of gels.

The first experiments with gels in an ultracentrifugal field were reported already in the early years of the technique by Mc Bain and Stuewer [3] and the pioneer Svedberg [4]. Afterwards, in more than 20 years only two studies were published using the ultracentrifuge as a tool

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for the quantitative detection of microgels. In the 1960s and 70s, Johnson and coworkers carried out basic investigations on the behavior of gels in the centrifugal field [10, 13, 17, 18, 20–22]. After a further 10 years of relative stagnation, researchers became interested once more in using the analytical ultracentrifuge as a tool for the characterization of gel properties. Over the years the characterization of gels in the analytical ultracentrifuge has been improved from both the theoretical and the experimental side. Today, the ultracentrifuge can be more effective than any other method known for the characterization of gels. Up to 70 samples can be characterized simultaneously in terms of thermodynamic, elastic, molecular and structural parameters. Another big advantage of the ultracentrifugal investigation of gels is the continuous equilibrium which can be determined by the selection of the rotational speed. Therefore the analytical ultracentrifuge should be applied much more for gel characterization than has been the case up to now. This review will cover what has been achieved in the past and what can be achieved in the future. The article is divided into (i) a general part, (ii) the description of what has been done over several decades of ultracentrifugation, and (iii) an outlook to the future. The literature is reviewed in historical order and covers all subjects related to the analytical ultracentrifugation of gels (theory, instrumentation, experimental approaches, results, etc.). As some of the reviewed publications contain not just studies about the analytical ultracentrifugation of gels, only the appropriate parts of these studies have been reviewed here. It was further tried to keep the numbers of cross-references not dealing with the analytical ultracentrifugation of gels at a minimum.

General part

General behavior of a gel in an ultracentrifugal field

If a gel is placed in an ultracentrifugal field in the sector shaped ultracentrifuge cell, two cases can be distinguished which are schematically presented in Fig. 1. The first case a) is the beginning of the experiment or an experiment at low rotational speed where no sedimentation of the macroscopic gel phase occurs (as sedimentation of the gel phase, the sedimentation of the gel meniscus is understood). Nevertheless, a concentration gradient of the polymer in the gel phase will occur at these lower speeds due to the sedimentation of the crosslinked polymer. The gradient indicates the locally dependent deswelling of the gel which is caused by the swelling pressure generated by the centrifugal field. The concentration gradient changes until a final equilibrium gradient is established.

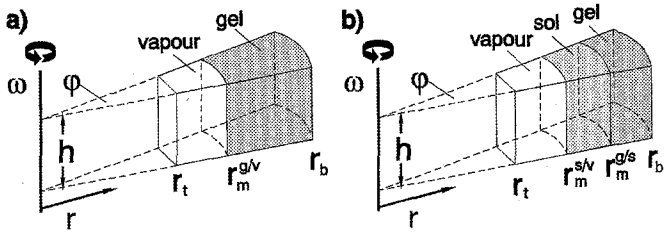

Fig. 1 Gel in an ultracentrifuge cell. a) At the beginning of the experiment or at low speeds where no sedimentation of the gel phase occurs, b) At high speeds where the gel phase has sedimented establishing a sol phase. ω is the angular velocity, h the height of the ultracentrifuge cell, φ the sector angle of the ultracentrifuge cell and r the distance to the axis of rotation with the indices t = top, m = meniscus, b = bottom, g/v = boundary gel/vapor, s/v = boundary solvent/vapor and g/s = gel/solvent. Redrawn from Cölfen H., "Bestimmung der thermodynamischen und elastischen Eigenschaften von Gelen mit Hilfe von Sedimentationsgleichgewichten in einer Analytischen Ultrazentrifuge am Beispiel des Systems Gelatine/Wasser"; Verlag Köster Berlin (1994) with kind permission of Dr. Köster, Berlin, FRG

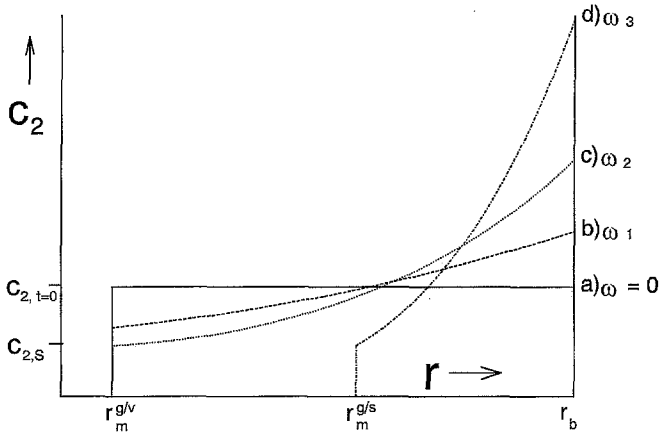

Fig. 2 Radial dependence of the local polymer concentration in the gel c_2 at different angular velocities ω_i. $c_{2,s}$ = concentration of the maximum swollen gel. Redrawn from Cölfen H., "Bestimmung der thermodynamischen und elastischen Eigenschaften von Gelen mit Hilfe von Sedimentationsgleichgewichten in einer Analytischen Ultrazentrifuge am Beispiel des Systems Gelatine/Wasser"; Verlag Köster Berlin (1994) with kind permission of Dr. Köster, Berlin, FRG

The second case b) is observed at higher rotational speeds, i.e., above 10 000 rpm (revolutions per minute) for the system gelatin/water. Again, the polymer concentration is increased at the cell bottom whereas it is decreased at the meniscus gel/vapor. These processes are illustrated in Fig. 2.

At the beginning of the experiment ($\omega = 0$), the polymer concentration in the gel is constant. It is increased at the cell bottom at ω_1 which leads to a decrease of c_2 at the meniscus

gel/vapor. At a critical angular velocity ω_2 the polymer concentration has dropped to the value of the maximum swollen gel $c_{2,s}$. As the polymer concentration in the gel cannot be lower than $c_{2,s}$, a sol phase is introduced as soon as the polymer concentration has reached this lower limit and the meniscus gel/sol begins to sediment (ω_3). This corresponds to case b) in Fig. 1. The sol phase might consist of pure solvent as well as of a solution of ungelling material. The gel phase sediments in certain cases until an equilibrium is reached. From the equilibrium states of both case a) and case b) in Fig. 1, information about thermodynamic, elastic, structural and molecular parameters of the gel can be obtained.

If the rotational speed is chosen very high, a sedimentation velocity experiment can be performed in analogy to a sedimentation velocity run with a polymer solution. In this case the movement of the boundary gel/sol towards the cell bottom can be measured as a function of time although recently it has been pointed out that the movement of the center of mass has to be considered rather than that of the meniscus gel/sol [1, 2].

Results reported in the literature

The early years up to 1940

The first pioneering investigations of gels in an ultracentrifugal field were reported by Mc Bain and Stuewer [3]. These workers used an air-driven spinner capable of rotating at speeds up to 210 000 rpm to generate centrifugal fields as high as 1 200 000 g. The spinner was filled with agar sol. After gelling, the sedimentation of the gel phase was followed visually with the help of a contact lever, by taking samples during centrifugation or by stopping centrifugation and weighing of the gel phase in the spinner and the sol phase separately. By means of this simple device it could already be derived that low concentration agar gels in the range of 0.31–1.6% by wt. and short maturation times reached the swelling pressure equilibrium (named equilibrium between the sedimentation velocity of the upper gel meniscus ($r_m^{g/s}$) and the speed of swelling at lower centrifugal fields in this study). A simple formula for the calculation of the swelling pressure was given [3] which differs from that derived from thermodynamic considerations. The gel concentration was assumed to be constant at different radial positions. This is not true, as could be shown in many of the later works reviewed in this chapter.

Theoretically, it was stated that the sedimentation velocity of a gel cannot be constant. It was suggested [3] that the sedimentation of a gel is influenced not only by the centrifugal force but by syneresis, swelling due to chemical solvation and orientation of the solvent as well as thermal molecular movements.

When the movement of the meniscus gel/sol ($r_m^{g/s}$) was plotted against the time of sedimentation for different concentrated agar gels, the sedimentation velocity of the gel was found to be constant in the beginning of the experiment, but then, to decrease to zero with time. This state is the swelling pressure equilibrium. From the sedimentation velocities of the gel a molar mass of 1 million was estimated for the gel, a wrong value as it is well known today that a gel consists of a network with an infinite molar mass. But the statement that this molar mass was found larger for lower initial gel concentrations hints at the point that the sedimentation velocity of the gel is related to the gel structure; that is to say, the molar mass of the network chains between two crosslinks in the gel. Nevertheless, these experimental findings have basic character and were studied in more detail by Johnson 30 years later.

Probably due to the poorly defined experimental conditions (temperature constancy, rupture of the gel upon deformation, etc.) and the limited accuracy of detection, the swelling pressure was found to be independent of the initial gel concentration and the prehistory of the gel. The concentration dependence was observed to be linear with very low swelling pressures in the range of only a few millibars. The linear concentration dependence at the low polymer concentrations was seen to be analogous to the osmotic pressure behavior of solutions.

As in many other ultracentrifugal works, Svedberg carried out the pioneering investigations on the behavior of a gel in an ultracentrifugal field [4]. But in comparison to the large amount of information presented in his book, only four pages are devoted to gels. One of his basic findings was that a gel shows behavior in the ultracentrifuge differing from solutions. The latter show a constant sedimentation velocity, independent of the column height whereas this is not the case for gels. This provided the verification of the considerations of Mc Bain and Stuewer [3]. Furthermore, Svedberg had already stated that two cases have to be distinguished carrying out ultracentrifuge experiments with gels: either some measureable changes in terms of the sedimentation of the gel phase occur, or they do not. This is exactly the situation shown in Fig. 1.

Svedberg also derived an equation to represent the so-called "hydrostatic partial pressure" of a gel which is the swelling pressure at equilibrium [4]:

$$\Pi_S = \omega^2 \int_{r_m^{g/s}}^{r} c_2 (1 - \tilde{V}_2 \rho_{01}) r dr, \qquad (1)$$

with Π_S = swelling pressure, r = distance from the axis of rotation with the indices m for meniscus and g/s for the boundary gel/solvent, c_2 = polymer concentration (usu-

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ally expressed as partial density of the polymer in the gel in g/ml), ω = angular velocity, $\bar{V}_2$ = partial specific volume of the polymer and ρ_{01} = density of the pure solvent.

Equation (1) is a special case of the generalized Svedberg–Pedersen equation for binary, low concentration and highly swollen gels presented later in this review [43]. Svedberg pointed out, that it is very often impossible to determine the polymer concentration gradient in the whole gel phase with the optical detection system of the ultracentrifuge due to the high turbidity of the gel. Therefore, he introduced an approximation method based on a mass balance and the assumption that the concentration in the middle of the gel column is equal to the average concentration of the gel column.

Svedberg found that the formula of Freundlich and Posnjak [5] $\Pi_S = \Pi_0 \cdot c_2^k$ (Π_S = swelling pressure, Π_0 = swelling pressure at unit concentration of the polymer, c_2 = polymer concentration in the gel and k = characteristic constant between 2 and 5) does not describe the dependence of the swelling pressure upon polymer concentration in every case. Instead it was found for agar gels that the swelling pressure of the gel is roughly proportional to the polymer concentration in the gel.

The period 1940–1960

The analytical ultracentrifuge was used for the determination of the amount of microgel formation resulting from emulsion polymerisations by Shaskoua and van Holde [6]. The microgels studied consisted of styrene crosslinked with divinylbenzene, methyl acrylate crosslinked with divinylbenzene and acrylonitrile crosslinked with methylene-bisacrylamide. Afterwards, styrene and acrylonitrile were grafted onto the polymers. The success of the grafting was studied employing the Schlieren optical system of the ultracentrifuge. In incomplete grafting reactions two components were found in the Schlieren patterns. The fast component was the microgel, the slow one the linear polymer. The concentrations of the individual components have been determined by measuring the areas under the Schlieren peaks. Then they have been corrected taking the concentration dependence of the sedimentation rates into account. The additional fact has been taken into account that for two chemical species separated into two components which sediment with different speeds, the faster component contains both species whereas the slower component consists of one single species. A mixture of microgel and 25% of linear polymer was analyzed with this method [6] yielding 26% of linear polymer. This proved that the ultracentrifuge is a quantitative method for analyzing linear polymer/microgel mixtures.

In another study this method has been applied to characterize the crosslinking efficiency of an emulsion polymerization [7]. The components for the polymerization of a microgel described in [6] have been used in different combinations and amounts to find the minimum quantity of crosslinking agent necessary to form the microgel. The ratio of the microgel and the linear polymer has been determined with the ultracentrifuge as described before [6].

The period 1960–1965

The so-called ultracentrifugal field relaxation method was applied by Kegeles et al. to distinguish between systems which gel at the cell bottom and those which do not [8]. As this technique was used only for the detection of a gel at high concentration at the cell bottom rather than for its analysis, the results given are not considered more closely here.

The density gradient technique has been applied to detect a microgel in an acrylonitrile-vinylacetate copolymer using a dimethylformamide-bromoform density gradient in the ultracentrifuge cell [9]. The copolymer solution could be separated into three fractions. One of the fractions was assumed to be the linear copolymer, the second one to be highly branched and the third to be weakly crosslinked. The structural differences between the highly branched and the crosslinked fraction were found to be rather small, causing a difference in the apparent partial specific volumes of only about 0.0005 ml/g which could still be resolved with the density gradient in the analytical ultracentrifuge.

The first basic study to be published on the behavior of a macroscopic gel in the ultracentrifugal field came from P. Johnson in 1964 [10]. He investigated agar and gelatin gels in a phosphate-NaCl-buffer at temperatures between 10 ° and 25 °C using very short maturation times of only 30 min for the agar gels. The concentration of the gels was determined to a low degree of accuracy by compressing the gel at 60000 rpm and determining the mass of the compressed gel phase. After drying to constant weight the concentration of the original gel could be obtained with the knowledge of its mass.

Nevertheless, Johnson [10] could already derive basic findings. By means of sedimentation velocity experiments he was able to show that a gel also shows typical velocity characteristics. He found a sharp gel-solution interface which was sedimenting in the direction of the applied field. But the sedimentation behavior was found to be essentially different from that of a solution (see also [4]). In a sedimenting solution boundary the concentration is continuously decreased due to the radial dilution caused by

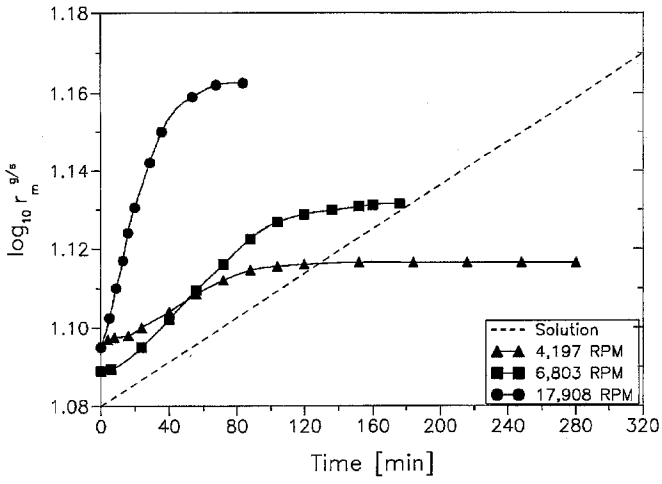

Fig. 3 Plots of $\log_{10} r_m^{g/s}$ against time from gel sedimentation diagrams for 0.5% difco-agar gel at various speeds. The dashed line represents a schematic diagram for a solution. Modified figure from Johnson P., "A sedimentation study on gel systems"; Proc Royal Soc A278 (1964) 527–542 printed with kind permission of the author and the Royal society, London, U.K.

the sector shape of the cell, whereas in a sedimenting gel the mean gel concentration increases as the gel volume is decreased with constant polymer mass. Furthermore, in contrast to a solution, the plot of $\log_{10} r_m^{g/s}$ against time was not linear for a gel but showed a decrease of the slope already after a few minutes. This slope was found to come to zero, defining an equilibrium degree of swelling (see Fig. 3). This equilibrium value was dependent on the rotational speed and the initial gel concentration.

In addition, an "induction" period at the beginning of an experiment was described where no sedimentation of the gel phase occurred if the rotational speed was selected too low. This induction period was found to increase with gel concentration and lower speed. A maximum sedimentation rate could be defined in the $\log_{10} r_m^{g/s}$ vs. time plots (linear portion between rise and decrease of the slope, see Fig. 3) which was approximately proportional to the applied field at several concentrations and inversely proportional to the initial gel concentration between 0.5 and 2% by wt. at the same initial column length.

Johnson [10] observed a slower sedimenting soluble fraction of considerably less than 10% for the agar but about 30% for the gelatin gel. These so-called soluble parts were found to be quite polydisperse in case of the gelatin as deduced from the extensive spreading of the Schlieren peak. In contrast to the gel, the $\log_{10} r_m^{g/s}$ vs. time plot was found to be linear as expected for a solution. The soluble gelatin was detected over a range of experimental conditions. Its sedimentation coefficient was similar to that of the molten gel which gives strong evidence that these soluble parts consist only of gelatin molecules which could not be incorporated into the network due to the very short maturation times of the gels or chemically damaged gelatin. Johnson himself stated that the proportion of soluble parts decreases somehow with allowing a gel to mature, but still very significant soluble portions remained after long times.

Comparing the sedimentation of solutions and gels, he found that in contrast to the plateau region of constant polymer concentration between the sedimenting boundary of a solution and the cell bottom, all of the gelled material remains within the gel. Thus the gel phase must become more and more concentrated during sedimentation. From that point of view, he presented a formula to calculate the mean concentration of the gel phase at a defined time t applying a volume balance. This formula could give at least a rough estimation of the concentrations in the gels during centrifugation. From the average gel concentration at a speed of 59 780 rpm he calculated the mean pore size for a 1% agar gel to be 70–100 Å on the basis of a lattice model, in agreement with previous estimates. Some reflections on the diffusion of small molecules through gels were made.

Johnson then tried to relate the initial slope of the $\log_{10} r_m^{g/s}$ plot to the square of the rotational speed [10] but found only a small range where this could be accomplished. Still, he used this plot to define the conditions under which the gel interface behaves like a solution boundary. For those cases he calculated a so-called effective sedimentation coefficient s considering the movement of the boundary gel/solution towards the cell bottom in analogy to the movement of the boundary in case of a solution by means of the relation:

$$s = \frac{d \log_{10} r_m^{g/s}}{\omega^2 \, dt} \,. \tag{2}$$

It was found to be impossible to relate this effective sedimentation coefficient to a sedimenting species because the gel can be considered as a network of infinite molar mass. The motion of the gel interface was stated to be a viscous rather than an elastic type of flow as a gel showed little or no rapid recovery after the rotor has been decelerated. Johnson concluded [10] that the flow of a gel must involve continuous rupture and re-formation of the junction points and explained the sedimentation of the gel phase with this concept. Consequently, the equilibrium state occurring after the sedimentation of the gel phase could be considered from the kinetic point of view as an equal rate of rupture and reformation of the junction points. From the occurrence of an induction period, he concluded that the rupture of crosslinks might be a slow process. A point against the generalization of these

considerations is that it would be impossible for a gel with permanent crosslinks to sediment unless the permanent bonds are ruptured. But, it is known that such gels also sediment [11, 12, 26]. An alternative explanation is that the sedimentation of the gel can also take place as compression of the gel without rupture of crosslinks or bonds but with folding of the network chains and exclusion of solvent. This model seems to be more likely at least for chemically crosslinked gels as the energy to rupture chemical bonds is rather high. However, up to now none of these possibilities could be clearly verified experimentally. It might also be that no universal explanation of the sedimentation behavior of a gel exists.

Another subject of interest in this work was the effect of the variation of experimental parameters like the ionic strength, pH of the buffer and the temperature on the gel sedimentation. The term gel sedimentation means the sedimentation of the crosslinked polymer in the gel. The temperature (10°–25 °C) as well as a pH (6.5–7.8) and ionic strength (0.1–0.5) alteration caused little or no measurable change in the determined gel sedimentation rate. These statements were found to be only approximate due to a certain lack in the reproducibility of the experiments.

Considering equilibrium aspects of the gel sedimentation, Johnson stated [10] that the derived increase of the $\log_{10} r_m^{g/s}$ with the rotational speed agreed qualitatively with the Eq. (1) of Svedberg [4]. Considering the induction period which could be observed if the initial gel concentration was not very different from the average equilibrium concentration, it was stated from changes in the Schlieren traces of the gel phase during this period that internal structural changes of the gel occur, before a movement of the gel meniscus starts. The transition between $\log_{10} r_m^{g/s}$ vs. time curves with and without induction period was found to be not well defined. From an irregular line in the Schlieren pattern, Johnson [10] concluded that structural changes occur which require finite time to occur within this period. As alternative explanation the redistribution of solvent inside the sedimenting gel phase was given.

The next study by Johnson's laboratory treated the sedimentation behavior of several dilute gelatin gels under sedimentation velocity conditions [13]. It was reported that the same centerpiece had to be used for all experiments to ensure reproducibility. The variations in the results using different centerpieces or centerpiece materials for identical samples were up to 30%. This might have been an adhesion effect (as suspected in the study) which is very strong with gelatin. As no impregnation of the centerpieces or other preventive measures given later in this article were applied, the observed drastic effects are explainable.

As in the previous study [10] the separated soluble parts which occurred in significant amounts behaved as real solution giving a sedimentation coefficient which equals that of gelatin in solution. It was stated that the occurrence of solution components cannot be caused by the high pressures in the centrifuge cell as ΔV for the sol gel transition is negative [14]. Further evidence is provided by the observation that the same amount of solution component was observed over a range of centrifugal fields.

Three general characteristic features of a $\log_{10} r_m^{g/s}$ vs. time plot for a sedimenting gel were stated [13] which can vary widely in different gels (see Fig. 3): a) the induction period at the beginning of the experiment, b) a period with a maximum movement of the gel boundary where the slope is constant, and c) the end of the experiment where the slope is continuously decreasing to zero. It must be stated that the transition between these periods is continuous. It is important to note that the induction period is probably just due to the fact that the movement of the gel meniscus is treated as indicative for the sedimentation of the gel. In fact, sedimentation of the polymer in the gel occurs right after the application of an ultracentrifugal field which is expressed in the formation of a concentration gradient in the gel phase even if the meniscus does not sediment [73]. Hence the movement of the center of mass should be considered [1, 2] rather than that of the gel meniscus. In that case no induction period is expected. As this is not yet proved experimentally, the treatment of Johnson remains in this review, but with a note of caution.

Sedimentation coefficients for the gel [13] have been calculated on the basis of the maximum movement of $r_m^{g/s}$. These values are the effective sedimentation coefficients of the gel in the following and can be considered to be rather inaccurate as only a small time range of the gel sedimentation (constant sedimentation velocity) can be used to calculate the sedimentation coefficient of the gel. This disadvantage can probably be circumvented if the movement of the center of mass is used to calculate the sedimentation coefficient rather than that of the gel meniscus [1]. Looking at the effect of the gel maturation temperature and the run temperature on the sedimentation coefficients of the soluble component and the gel as well as the amount of soluble polymer, drastic effects were observed. Increase of the maturation temperature from 2° to 20 °C at 1 h maturation time increased the amount of the soluble component from 20% to 90%. The sedimentation coefficient of the soluble component remained nearly constant, whereas that of the gel was decreased by a factor of ≈ 3 from 14.5 S to 4.5 S. Below 20 °C the gel sedimentation was found to be insensitive to the run temperature for a few degrees below the setting temperature, whereas it was very dependent on it above 20 °C reflecting the breakdown of the gel structure up to complete gel melting. It was attempted to monitor the gelation in a sedimenting solution by decreasing the temperature from 23 °C (Melting

point) to 19 °C over 4.5 h. Needle-like sedimenting striations appeared in the Schlieren patterns after a time, which corresponded to aggregates leading to later gelation. Such aggregates have only been observed near the melting point or just before gelation. Their concentration is decreased drastically with the maturation time of the gel, much more than the concentration of the non aggregated solution component.

The maturation time of the gels was also found to influence the sedimentation behavior of the gel and the amount of solution component. On aging at 20 °C, there was a large decrease in the gel sedimentation coefficient during the first hour, whereas the changes with maturation times up to 3 months were small. In the entire period from a few minutes to 3 months the solution component was decreased from 40% to 25% for the system gelatin/water. Carrying out the same sort of experiment at 2 °C, the solution component (20% after 1 h) vanished after 44 h, whereas the sedimentation coefficient of the gel increased by a factor of 2.

By studying the effect of the initial gel concentration between 1 and 3% by wt. on the sedimentation behavior and the amount of soluble component, it was found that at 20 °C and an initial gel concentration of 1.5% by wt, material was still partly in solution, which was built into the network at 2% by wt. The effect was less pronounced at 18 °C and vanished at 2–5 °C. Some experiments on the effect of the ionic strength showed that deionized gels, somehow representing a structure of precipitated aggregates embedded in a weak network, sedimented much faster than a normal gelatin/water gel. When KCl ($c = 1$ mol/l) was added, the sedimentation was slower than that of the gel with water. This implies that the sedimentation velocity of the gel depends on its structure. Addition of KSCN (0.5 mol/l) to a 1% by wt. gelatin solution prevented the formation of aggregates and hence no gel was formed.

Separating the gelatin gel into the gel and the solution component by preparative centrifugation showed that the freeze-dried solution component was far more rapidly soluble than the freeze-dried gel fraction and gave no gel even at 2% by wt. At a concentration of 5% by wt. a gel was formed which showed that the gelatin molecules in the soluble fraction are at least partly able to form a network. In an experiment with a 2% by wt. gel [13] where the solution phase was removed three times from the ultracentrifuge cell and replaced by the same amount of water, no solution component separated from the sedimenting gel interface anymore, after the gel was allowed to mature for 40 h at 4 °C. This gives evidence for the conclusion that the low molecular solution component may be removed by such a procedure. Further evidence for this was found in an experiment where the sedimentation of the first of the three extracts was compared with a diluted solution of the remaining gel phase. The extract showed a considerable tail of slower sedimenting components, e.g. components with a lower molar mass.

As the sedimentation behavior of a gel was found to be dependent on its structure it was investigated if the sedimentation coefficient of the gel can be correlated with its rigidity. It was found that the rigidity of the gelatin gels seemed to be related with the amount of solution component but not with the sedimentation coefficient of the gel. It was also, pointed out [13] that the soluble parts do not directly contribute to the gel rigidity, but do so only indirectly by lowering the concentration of molecules which build up a network. Highly rigid gelatin gels had only 20% solution component, whereas gels with low rigidity had 40–50% solution component. This effect was even observed if the molar mass of the two types of gelatin molecules forming the gel was comparable.

Systematic sedimentation velocity experiments on diluted gels from commercial gelatins and gelatins from soluble collagens were described by Metcalfe [15]. This study contains many more results than could be included in the previous publication [13]. Metcalfe compared the sedimentation behavior of the gelatin gels over a wide range of physical conditions using mainly three quantitative measures: a) The maximum rate of gel sedimentation of the gel interface as defined above, b) the amount of solution component, and c) the sedimentation coefficient of the solution component. As the rotor was cooled by about 0.8 °C due to the adiabatic expansion of the rotor during the acceleration to 60 000 rpm, precise temperature control was not possible in all sedimentation velocity studies.

It could be shown that sedimentation studies are a sensitive method for the examination of changes in the gel structure and the interaction of the gel with other molecules. Although a qualitative relationship between the proportion of the solution component and the rigidity of the gels was found, no quantitative relation could be derived for this as it was concluded that mainly the changes within the gel itself, e.g., of its structure, are responsible for the rigidity changes. Also, no correlation was found between the gel rigidity and the maximum gel sedimentation rate which seemed to be more dependent on the gel structure. Sedimentation equilibrium of gelatin gels was found to be reached much slower than that of agar gels and was expected to be more complicated as the solution component in the gelatin case moves through the gel phase.

Reflecting upon the reproducibility of the experiments, considerable time-dependent effects after gelling through further crosslinking of the gelatin gel were found [15]. In contrast to the previous study [13], it was found that

adhesion of the weak gels to the ultracentrifuge centerpieces had no effect on the sedimentation coefficient of the gel whether the material of the centerpiece was changed, the surface was lubricated, the sector angle was altered or the gel was loosened from the cell walls and windows before the experiment. The more rigid gels were generally loosened from the cell walls and windows as a considerable adhesion took place. The gel sedimentation coefficient was found to be proportional to the applied centrifugal field at constant gel column length just as it was found for agar gels [10], whereas the induction period was inversely proportional to the field. The sedimentation coefficient of the gel was found proportional to the length of the gel column, independent of the sector angle of the centerpiece (e.g., the gel volume) and unchanged if a water layer was placed above the gel column or not. The independence of the sedimentation coefficient of the presence of a water layer on top of the gel column already showed that the total hydrostatic pressure has a negligible influence on the sedimentation behavior of the gel as it could be derived theoretically later [43]. But the dependence of the sedimentation coefficient of the gel on the column length hints at an error either in the definition of gel sedimentation or an experimental artifact because an independence of both quantities is expected as in the case of solutions. It seems likely that both the definition of the gel sedimentation coefficient s via the movement of the meniscus gel/sol as well as the experimental conditions caused the observed dependence on the column height. As shorter gel columns provide a shorter distance between the observed acceleration and deceleration of the meniscus gel/sol in the log $r_m^{g/s} r$ vs. time plot, it may well be that the maximum velocity of the meniscus was not yet reached before the sedimentation velocity was decreased, again due to the forces acting against the sedimentation of the gel. This explains the observed increase of the sedimentation coefficient with the column height. Another reason for the dependence of s on the gel column height could be the presence of a significant amount of soluble parts in the gelatin. It was found in sedimentation equilibrium experiments with dialyzed gelatin/water gels without soluble parts that the determined swelling pressure-concentration curves were independent of the filling height [38] as should be expected, whereas a dependence of the same results was found for gelatin/water gels containing soluble parts [42]. Whatever the precise reason for the dependence of s on the gel column length is, results from such measurements should be treated with care.

As reported in previous studies dealing with ultracentrifuge experiments with gels, Metcalfe [15] had problems to detect the concentration gradient inside the gel phase, especially in the more concentrated gels. Therefore it was attempted to monitor the concentration distribution inside the gel phase by placing a gel consisting of alternating dyed and undyed gelatin in the ultracentrifuge cell. This attempt was not successful as the dyed gelatin was distributed in irregular lumps throughout the gel column after the experiment implying a circulation of the polymer in the gel phase during the sedimentation. The density differences between dyed and undyed gel have been given as possible explanation as the applied dyestuff was known to promote gelation. Alternatively, this observation partly supports the explanation of Johnson – at least for the case of the physically crosslinked gelatin/water gel – that gel sedimentation occurs when crosslinks are ruptured [10].

Consequently, it was attempted to establish a large density gradient inside the gel phase by layering a 2% by wt. gelatin gel above a 4% by wt. gelatin gel. The sedimentation coefficient derived from the movement of the boundary between the two gels as well as that from the movement of the meniscus gel/solvent from the 2% by wt. gel agreed closely with the values obtained for the separate gels with the same column height. From this it could be deduced that the sedimentation rate of the boundary between the two different concentrated gels was approximately independent of the presence of the gel above it. A continuous density gradient was not established as expected initially.

When the effect of the initial gel concentration on the gel sedimentation coefficient was investigated in the range of 1.5–5% wt., a maximum of the sedimentation coefficient for 2% by wt. was found for 18 °C shifting down to 1.5% by wt. at 5 °C. A plot of log $1/s$ (s = sedimentation coefficient of the gel) against log c (c = average concentration in the gel at time t) yielded ranges of linear dependencies with a slope n for gelatin/water gels. Using data for dilute agar/water gels [10], a similar range of linear relationship was found for agar, restricted extensively by the equilibrium approach of the agar gels. However, it was stated that the maximum gel sedimentation rate alone is not sufficient to completely characterize the sedimentation of the gel as no relationship between this sedimentation rate and the induction period could be found. If the movement of the center of mass would be taken to calculate the sedimentation coefficient of the gel [1], it can be expected that no induction period is observed anymore, which shows that the definition of the gel sedimentation coefficient of the gel via the movement of the center of mass is the more general one.

In addition to the already published [13] results of the effect of the ionic strength on the gelatin gel sedimentation coefficient, the concentration of KCl in the range of 10^{-5} to 1 mol/l was found to have a dramatic effect on the sedimentation coefficient of the investigated 2% gel at 18 °C [15]. Increasing the KCl concentration for example

from 10^{-3} to 10^{-2} mol/l and hence the ionic strength, the sedimentation coefficient of the gel was decreased from nearly 60 S to 20 S. In contrast to the gel, the increase of the ionic strength led to an increased sedimentation coefficient of the solution component. The pH affected the gel sedimentation coefficient very strongly as well, giving a maximum near the isoelectric point of the gelatin.

The solution component was the subject of intensive study. It could be shown that the fraction of soluble material could be decreased significantly to negligible magnitude by decreasing the temperature of the ultracentrifuge experiments to 5 °C or lower. Sedimentation studies of these fractionated solution components suggested a molar mass of only 10000 g/mol in contrast to the 10-fold or higher value of the gelatin. This could explain the already outlined lack of gelling ability of such a solution component. Further work especially on the soluble parts from gelatin gels applying optical rotation measurements and amino acid analysis was reported by King [16].

Additional experiments were carried out to compare the sedimentation behavior of gels from acid and alkali processed gelatin, fractionated gelatin and gelatin from soluble collagen. The sedimentation coefficients for gels from soluble collagens matured at 1 h at 18 °C were found to agree with those of alkali processed commercial gelatins. But in contrast to the other gels investigated, the amount of solution component at 18 °C in gels from soluble collagens was found to be much lower (e.g. <15% by wt.) down to only 2.5% by wt. Under these conditions the amount of solution component from fractionated gelatins (the α & β components have been purified) was found not to be very much dependent upon the composition of the gelatin, e.g., the fraction. Comparing the sedimentation coefficients of the corresponding gels, a small increase of s was found for the α-fraction, whereas s was slightly decreased with respect to the unfractionated gelatin for the β-fraction.

The effect of heating was studied with a gel containing only a very small amount of solution component. The amount of solution component was found to increase slightly with each heating step due to the thermal degradation of the gelatin, whereas the sedimentation coefficient of the gels increased reflecting the weaker gel structure.

The period 1966–1970

Another study on the sedimentation behavior of gelatin gels was published in 1967 by the group of Johnson [17]. This publication dealt mainly with the results in [15], but included a more detailed consideration of the dependence of the gel sedimentation coefficient on the average gel concentration. When the column length of the gel $(r_b - r_m^{g/s})$ was introduced into this empirical equation a more detailed plot of $\log s + \log(1 + r_m^{g/s}/r_b)$ versus $\log(1 - (r_m^{g/s}/r_b)^2)$ could be introduced which gave a straight line with the slope $n + 1$. The parameter n was suspected to be somehow related to the gel structure.

Considering the application of Eq. (1) in this review on sedimentation velocity experiments with gels, it could be stated that this equation must apply more to equilibrium conditions than to the steady flow during the sedimentation velocity run. As the reason, it was given that under these conditions swelling is a slow process compared to the gel sedimentation.

This work was followed by a further sedimentation velocity study on gelatin gels attempting to describe the sedimentation behavior of gels observed in the previous studies more quantitatively [18]. An important point was the attempt to measure the polymer concentration distribution inside the gel phase during sedimentation which normally could not be observed throughout the whole gel phase with any of the standard ultracentrifuge detection optics. As the attempts with layers of dyed and undyed gelatin failed [15], a gel was set up from completely dyed gelatin. When the optical densities of the photographic negatives recorded during the sedimentation of the gel were evaluated with a microdensitometer, it was found that the optical density and hence the polymer concentration was approximately constant throughout the gel. This is in contradiction to the prediction of Svedberg and Pedersen due to Eq. (1) which would lead to a concentration gradient inside the gel phase [4]. Further, with increasing time an increase in the optical density was recorded. This was stated to be due to the concentration increase of the gel as its volume is decreased during the sedimentation. But the concentration determination with optical densitometer readings of the Schlieren negatives must be full of artifacts as it can be expected because of the photographic evaluation process of the negatives. Their optical density depends strongly on the exposure time and the conditions of film development. Hence these results should be treated as rather erroneous especially because they could not be confirmed by later studies.

When the concentration dependence of the gel sedimentation coefficient was investigated at temperatures of 4°, 10° and 18 °C, it was found that a log s vs. log c gave a linear dependence above gel concentrations of 1.5% by wt. (experimentally determined sol/gel transition). With falling temperature the dependence of the gel sedimentation coefficient upon gel concentration increased. From this observation an empirical relation was deduced which was already indicated in the previous paper [17]:

$$s = k \frac{r_b - r_m^{g/s}}{c^n} . \tag{3}$$

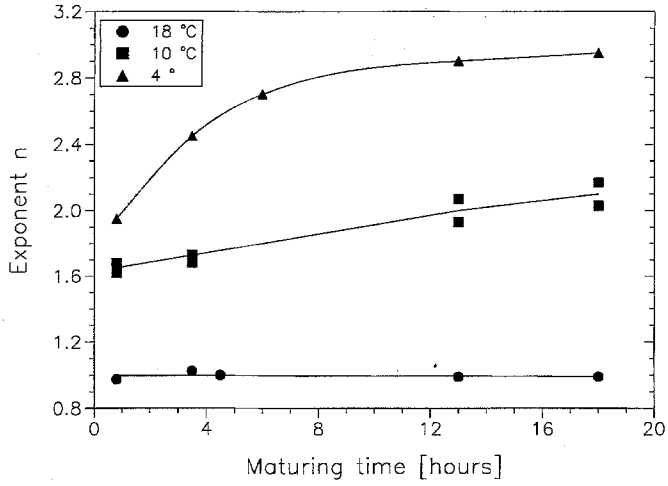

Fig. 4 Effect of temperature and maturation time on the value of n for aqueous lime processed ossein gelatin gels of 2% by wt. Figure redrawn from Johnson P, King RW.,"Sedimentation studies on gelatin gels"; J Photograph Sci 16 (1968) 82–88 with kind permission of the author and the Royal Photographic Society of Great Britain Devon, U.K.

Plots of $\log s + \log(1 + r_m^{g/s}/r_b)$ versus $\log (1 - (r_m^{g/s}/r_b)^2)$ yielded the empirical parameter n [17]. After it had been pointed out that the shape of the $\log r_m^{g/s}$ vs. time plot influences the derived n, some dependencies of n on the temperature and the maturation time were presented (See Fig. 4).

It was stated from this behavior that at temperatures below 18 °C n increases with decreasing temperature with the maturation time indicating drastic changes in the gel structure on aging. Furthermore it was pointed out that n was also dependent on the ionic strength and the pH of the solvent. An increase in the ionic strength caused a significant decrease of n in the isoelectric region at all considered temperatures. However, this effect was small or caused a slight increase in n at higher pH. If the pH was increased away from the isoelectric region, n decreased significantly. When urea, a structure disorganizing agent which hinders hydrogen bridge bonding, was added in increasing quantities, n fell rapidly down to zero.

From the experimental results it was concluded that the empirical parameter n gives an indication of the crosslinking degree and the gel structure. High values of n ($n = 3$) were found to represent extensive crosslinking, whereas a low value of $n = 1$ indicated a weakly crosslinked network (see Fig. 4). From the sedimentation behavior of highly asymmetric molecules with high effective volumes in dilute solution compared with that of weakly interacting systems, it was concluded that n was suitable for interpreteting solution sedimentation behavior as well.

In a study on the sedimentation of DNA, the anomalous rotor speed dependence of the sedimentation of T-2 bacteriophage DNA was found to be caused by a reversible pressure induced gel formation [19]. The gel network was observed to form at the cell bottom first (where the polymer concentration and thus the probability of network formation is the highest), expanding towards the top of the cell when certain speed and concentration thresholds were applied. It was deduced that pressure favored the gel formation.

In a more comprehensive article, Johnson summarized the basic findings so far derived on the sedimentation behavior of gels [20]. On the analysis of flow under the centrifugal field, an experiment was reported where the rotor was stopped after the gelatin gel was maximally compressed. Leaving this gel for 15 h at 18 °C, only very limited reswelling was observed. This gel showed a similar sedimentation behavior to the original one with the exception that no induction period was observed anymore. From this experiment it was concluded that the sedimentation of a gelatin gel must be regarded as irreversible. Recent results show that this behavior can be related to additional crosslinking of the gel by the soluble gelatin component during the gel sedimentation which leads to the formation of a gradient gel [72].

Some more detailed considerations as well as some further experimental results concerning the structural parameter n were described in this work. Addition of sodiumdodecylsulfate in small amounts < 0.01 mol/l surprisingly strengthened the gel reflected in an increasing n which was explained by an associating effect of this long chain molecule. Higher surfactant concentrations caused a steady decrease of n again. Chemically crosslinking of the gelatin with glutaraldehyde decreased n further from an already low level, although the number of crosslinks was obviously increased. The same behavior was found for almost completely covalently crosslinked 3% acrylamide gels which had an n value of nearly 0. These findings were explained in a way that n is low or zero for gels with a small number of covalent bonds and high for those with a large number of weak bonds. This interpretation suggests only some qualitative proportionality between n and the crosslinking density.

The period 1971–1980

In two closely related later papers, Johnson more closely considered the polymer concentration distribution inside the gel phase during sedimentation and at sedimentation equilibrium [21, 22]. For the example of agar, he pointed out that in the case of gels, the polymer is not removed from the system being deposited at the cell bottom as it

occurs during sedimentation velocity runs with solutions. For agar gels without significant amounts of soluble components he showed that after the experiment the compressed gel pad swelled to its original condition when in contact with the supernatant solvent in the ultracentrifuge cell. The sedimentation behavior observed for this swollen gel was identical to that in the initial experiment. This swelling behavior is a very important difference to the behavior of gelatin gels with significant amounts of soluble component as stated above and explained later [20, 72].

Discussions on the polymer concentration distribution inside the sedimenting gel and a comparison to a solution have already been published [10, 15, 18]. As did Svedberg and Metcalfe, Johnson stated that the gel concentration cannot be observed throughout the whole gel phase with the Schlieren optics of the ultracentrifuge.

Additional to the already reported dependence of the equilibrium column length on the applied centrifugal field [10], Johnson proved for agar gels that these equilibrium gel column lengths are real equilibrium values with the thermodynamic principle of path independence of an equilibrium. The same column length was reached independent of if this value was achieved by deswelling (approaching equilibrium from lower speeds) or swelling of the gel (approaching equilibrium from higher speeds).

As the optical detection system could not be applied to derive the polymer concentration inside the gel phase, a microtome was used to cut the gel column into thin slices after it was removed from the ultracentrifuge cell once the equilibrium had been reached. The gel density of agar was found to be linearly dependent on the polymer concentration as was derived for gelatin/water and κ-carrageenan/water gels by other workers as well [23]. Therefore the density of the gel slices as a measure of their concentration was determined in a density gradient column with benzene and carbon tetrachloride to avoid swelling of the gel. The plots of the determined agar concentrations vs. the radial position in the cell (represented by the slice number) at different stages of the experiment clearly showed a linear polymer concentration distribution in the gel phase at equilibrium although a plateau zone near the meniscus $r_m^{g/s}$ was observed which was considered as a possible artefact (see Fig. 5). This result is in disagreement with the constant polymer concentration in the gel phase which has been derived via scans of the local optical density of the Schlieren negatives before [18].

Combination of Svedbergs Eq. (1) [4] with that of Freundlich and Posnjak $\Pi_S = \Pi_0 \cdot c_2^k$ (see above) yielded a k-value of approximately 2 for agar from the sedimentation equilibrium concentration distribution. The Π_0 of $2.52 \cdot 10^5$ dynes/cm^2 was found to be in good agreement with one for gelatin of $2.7 \cdot 10^5$ dynes/cm^2 given by Freundlich [5]. However, it is known that the relation of Freundlich and Posnjak [5] is only empirical and not all workers found that this equation describes the concentration dependence of the swelling pressure (see for example [4]).

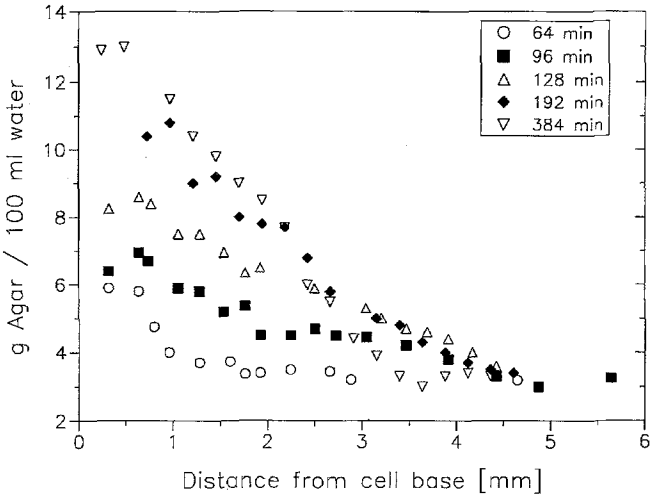

Fig. 5 The concentration distribution in the ultracentrifuge cell at different times of sedimentation for 3% by wt. washed oxoid ionagar in water at 23 150 rpm. Equilibrium was reached after 384 min. Redrawn from Johnson P., "Velocity and equilibrium aspects of the sedimentation of agar gels"; J Photograph Sci 19 (1971) 49–54 with kind permission of the author and the Royal Photographic Society of Great Britain, Devon, U.K.

The techniques for sedimentation analysis of gels presented above have been applied to the gel-like fraction of porcine gastric mucus in a mucus dispersion at pH 3.5 and approximately 20 °C [24]. The typical sigmodial plot of $\log_{10} r_m^{g/s}$ vs. time for gels was obtained for the whole mucus as well as for the mucus in which the supernatant fraction containing 3 other components had been removed by previous centrifugation. A structural parameter n of 2.5–2.6 (see Eq. (3)) was derived for a 2% by wt. mucus gel with the procedures described above, indicating a large amount of weak intermolecular interactions. This interaction was found to get stronger at higher concentrations with $n = 3.3$ for a 6.9% by wt mucus gel.

At a pH of 7.3 the proportion of the gelling component was found to be much smaller than at pH 3.5. The same effect up to a vanishing gel content was observed as expected when several structure disorganizing agents like 8 mol/l urea, 6 mol/l formamide, 6 mol/l guanidine hydrochloride, 10% triton X-100 and particularly 2% sodium deoxycholate with and without 5% mercaptoethanol were added.

DNA was found to form a liquid gel-like phase in a buffer with low ionic strength of Na^+ at neutral pH after a high centrifugal field was relaxed to a lower one [25]. This gel-like phase showed a negative concentration gradient with increasing radius. This is the opposite of what

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normally would be expected for the polymer concentration gradient inside a real gel phase. An explanation for this surprising behavior was given that a front with an extremely high concentration gradient is formed at the cell bottom at the high centrifugal field from which Na-f_d-DNA aggregates begin to diffuse back into the solvent layer producing the observed hypersharp interface upon field relaxation. The aggregation was found to be pressure independent, giving evidence that it is arising from steric interaction caused by Brownian motion of the DNA molecules. Compressibility calculations based on the height of the Schlieren patterns above the baseline pattern were performed. From this compressibility the partial specific volume inside the gel-like phase at different radii was calculated. It was found that the compressibility at the well defined interface gel-like phase-solution was much greater than at the bottom of the cell while the partial specific volume was less. The decreased partial specific volume and hence the increased density is consistent with the observed increased polymer concentration at the interface gel-solution. From the calculations a change in the conformational state of the gel-like material between the interface and the cell bottom was concluded. The formation of the gel-like phase was not observed, if Mg^{2+} ions were used in the buffer instead of Na^+.

W. Borchard was the first to apply the analytical ultracentrifuge as pressure generator for the determination of the swelling pressure and thermodynamic properties of a chemically crosslinked gel in a larger temperature range from 25 °–70 °C [26]. Polystyrene-cyclohexane gels with different crosslinking densities and removed soluble components have been investigated in two specially constructed types of ultracentrifuge cells (see Fig. 6).

Cell a) was not found to be reliable for the system investigated. Cell b) was based upon the principle that the precisely manufactured sinter metal plate – impermeable for the gel – was pressed upon the gel causing the pressure. As already performed by Johnson [21, 22], the equilibrium state was reached from lower as well as from higher rotational speeds to prove the equilibrium. Because the buoyancy term was found to be very small for the system investigated and hence no concentration gradient could be detected inside the gel phase, the polymer concentration in the gel was calculated from the shift of the sinter metal plate and the initial degree of swelling of the gel.

The calculated equilibrium pressure tensions were related to the corresponding volume fractions at different temperatures. The swelling pressure was the pressure needed to keep the gel volume constant at different temperatures with respect to an appropriately chosen reference temperature. The polymer volume fraction in the gel corresponding to the constant gel volume could be calculated assuming volume additivity. The intersections

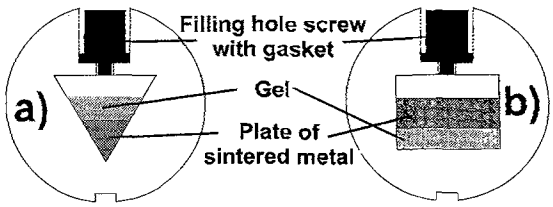

Fig. 6 Ultracentrifuge centerpieces a) for the determination of the sedimentation equilibrium and b) for the determination of the swelling pressure equilibrium. Redrawn from Borchard W., "Über das Quellungsverhalten von Polystyrol verschiedener Netzwerkdichte in Cyclohexan"; Progr Colloid Polym Sci 57 (1975) 39–47 with kind permission of the author and Steinkopff Verlag, Darmstadt, FRG

between the curve for the polymer volume fractions at different temperatures and the isotherms in the volume fraction vs. equilibrium pressure tension plots gave the swelling pressures at the different temperatures.

From the swelling pressures, the change in the chemical potential of the solvent $\Delta\mu_1$ and from that the differential entropy of dilution ΔS_1 and the differential dilution enthalpy ΔH_1 were calculated in good agreement with the values determined by other authors. It was found that the evaluation of the results with the statistical theories for swollen networks led to physically meaningless results giving some evidence that some of the basic assumptions of the statistical theories are not fulfilled anymore for the gels investigated.

An idealized theoretical treatment of an elastic swollen gel which is compressed by an ultracentrifugal field leading to an equilibrium degree of swelling was given by Bloomfield [27]. The model used and fitted to the experimental values was able to describe the degree of swelling of a gel under ultracentrifugal force satisfactorily, but assuming a distinct Flory–Huggins interaction parameter χ. Therefore, the applied model could not be used to describe the swelling behavior of the gel completely just from the experimental parameters and results. Assuming volume additivity of mixing and considering the free energy of the gel as a sum of ultracentrifugal, elastic and mixing terms, Bloomfield derived an expression containing the unknown Flory–Huggins interaction parameter χ and experimental parameters which depends upon the degree of swelling of the gel. For simplicity the gel has been assumed to be of rectangular shape. For the experimental values which had to be inserted into this equation, it was found that χ could not exceed the value of 0.5. This is physically meaningful as the gel is to be considered as a molecule with an infinite high molar mass which has to demix, if χ exceeds 0.5.

Assuming χ-parameters of 0, 0.25 and 0.5 to cover the whole possible range and using the experimental values for an experiment with casein/water in a preparative ultracentrifuge as well as those for the free swelling of casein/water

in absence of the ultracentrifugal field, Bloomfield derived three pairs of curves. From these plots he was able to determine the degree of swelling under the ultracentrifugal force from a given degree of free swelling. A basic finding from these considerations was that the effect of the ultracentrifugal field on the degree of swelling increased considerably with increasing χ. This is conclusive as a low χ represents high polymer-solvent interactions, whereas for the formation of a compressible network, a considerable amount of polymer-polymer interactions is required to build up the network junctions represented by a $\chi \cong 0.5$. The best agreement with data from intrinsic viscosity measurements for the casein micelles investigated was found for a $\chi \cong 0.5$. The same χ has been found in ultracentrifugal studies of gelatin/water gels [54].

The compression effect was observed to rise with increasing degree of swelling, increasing rotational speed and the increased gel column length which is to be expected. It must be noticed that the gel piece did not swell to its original amount anymore after the ultracentrifugal field was removed. As one explanation it was postulated that additional crosslinks have been formed during compression. The same was found for gelatin/water gels with soluble components which can act as crosslinking agents [72].

A sedimentation velocity method for the rapid characterization of gels was described by a Ukrainian group [28]. They investigated difco agar gels (matured for 24 h and 3 months) at 20 °C, pH 7 and the very low concentration of 0.15% using Schlieren optics. Considering the rapid sedimentation which took place already at the acceleration of the rotor, they derived basically the same results which were already presented in the ultracentrifuge papers by Johnson's group (see for example [10, 13, 17]) before without citing them. But the interpretation differs. During the induction period at the beginning of the experiment, a destruction of the gel structure was assumed to occur as discussed by Johnson (The notes of caution with the induction period discussed above for Johnson's papers must be applied here as well). The constant sedimentation velocity at the following period is reported to represent that of the aggregates which form a gel again later induced by the increased concentration and the hydrostatic pressure leading to a decrease in the sedimentation velocity until the sedimentation equilibrium is reached. This argument would not explain the equilibrium reported for the sedimentation of agar gels [21, 22], because it is most unlikely that the original network structure would be built up from the sedimenting aggregates again which must be postulated by the equilibrium nature of this process. Furthermore the figures in this article show the sedimentation of a phase with a defined phase boundary and not the typical Schlieren peak or striations to be expected if the sedimenting species would consist of aggregates at certain times. The investigated agar gels contained soluble parts which were found to sediment individually.

Overall, the results presented [28] are only of qualitative nature and outline the use of sedimentation velocity experiments with gels. To derive quantitative results, it was considered as necessary to accelerate the rotor with a well defined characteristic. Furthermore the construction of specialized centerpieces was considered to be necessary for future applications without pointing out requirements these centerpieces have to meet.

The period 1981–1990

The gel-like phase formed by DNA molecules which was observed earlier [25] was found to be formed in sedimentation equilibrium as well [29]. Monitoring the polymer concentration with the absorption optical system, an abrupt increase in the absorbance (being relatively insensitive to the wavelength) was found near the cell bottom. This optical discontinuity was interpreted as a boundary between the DNA solution and the light-scattering gel-like phase. The gel-like phase was classified to be liquid crystalline. Its formation was found to be reversible. The phase transition was suggested to be of the first order. The relation between the DNA volume fraction, including the electrostatic radius, at the transition point and the effective asymmetry of the molecules as a function of the salt concentration was found to be in approximate agreement with various theoretical treatments. Application of the scaled particle theory to the gel phase at fixed particle lengths yielded an effective hard core radius for the DNA in agreement with values reported in the literature. The scaled particle theory is an alternative approach to the Flory-Huggins theory used by other groups [44, 54, 60, 68] to derive molecular parameters from swelling pressure-concentration curves.

A gel-like phase which has been formed from calf thymus DNA in NaCl solution at a high rotational speed of 48 000 rpm in an ultracentrifuge cell has been studied by Richard at various lower speeds between 4800 and 30 000 rpm [30]. The so-called gel-like phase formed cannot be considered as a separate phase, as indicated in Fig. 7. If a gel phase were present, a meniscus (sharp discontinuity) between the gel and the solution must occur like that between air and the solution phase. Usually, a discontinuity of the concentration occurs at such a boundary which is also represented by a discontinuity in the Schlieren pattern or in the interference fringes. All these characteristics of a phase boundary cannot be observed here. So it is likely that this gel-like phase is a microgel or, better, a dispersion of swollen particles

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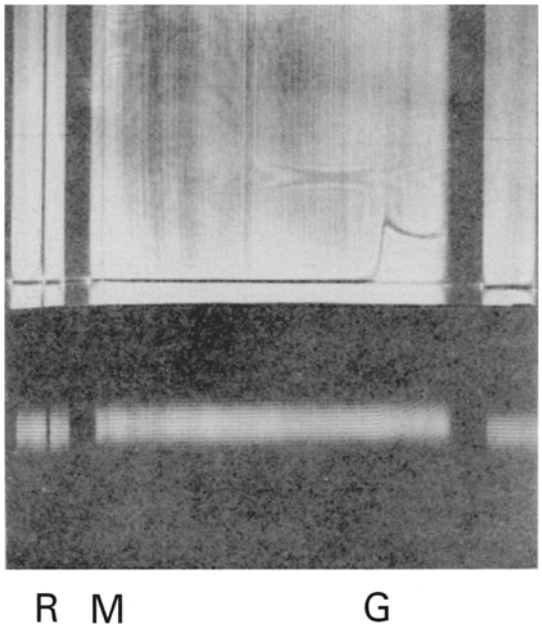

Fig. 7 Schlieren (top) and interference (bottom) patterns for a calf thymus DNA gel under pressure at equilibrium [30]. Temperature 20 °C, rotor speed 10 000 rpm, Schlieren bar angle 70°. The reference mark (*R*) is that point from which distances are measured on the photograph, (*M*) is the solvent-air meniscus and (*G*) is the top of the gel like phase column. Reproduced from Richard AJ., "Centrifugal field relaxation and ionic strength effects on calf thymus DNA gels"; Biopolymers 22 (1983) 935–943 with kind permission of the author and John Wiley and Sons, Inc., New York, USA

which shows solution characteristics [6]. Similar equilibrium Schlieren patterns to those of Richard – although less pronounced – were observed by Scholte for the solutions of polystyrene in toluene [31].

However, the column height of the gel-like phase was found to be strongly dependent on the rotational speed and on the NaCl concentration. The strong dependence on the salt concentration was explained as a reflection of the changes in the electrostatic persistence length of the DNA and the electrostatic interactions between the charged macromolecules as a function of the ionic strength of the solvent. Equilibrium values for the column height of the gel-like phase could be obtained reproducibly within 24–60 h. The strong speed dependence of the column height of the gel-like phase indicates that this phase might consist of a microgel because this behavior was found also for very small gel particles [35], as will be presented later in this review.

The radially dependent concentration of the DNA was calculated from a volume and mass balance in combination with the observed interference fringe shifts. The application of the interference optical system was only possible because of the low gel concentrations of only up to 0.6% by wt.. At high gel concentrations only the Schlieren optics can yield reasonable results as found recently by other authors and shown in Fig. 11 [65].

After the determination of the polymer concentration in the gel, the swelling pressure of the gel was calculated with Eq. (1) [4]. A constant buoyancy factor for each ionic strength was assumed for the different applied rotational speeds. Afterwards the relation of Freundlich and Posnjak [5] mentioned before was applied and found to fit the data well with $k = 2.6$. However, the linear fit of the plot of $\log \Pi_S$ against $\log c_2$ only worked well for all investigated polymer concentrations for the lowest rotational speeds of 4800 and 13 000 rpm and for all applied rotational speeds up to 30 000 rpm in the range of about 0.4–0.6% by wt. polymer in the gel. At lower polymer concentrations and at the higher rotational speeds considerable deviations from the Freundlich and Posnjak equation can be observed which have not been discussed beside the hints that these deviations might be caused by diffusion effects. But it is obvious that diffusion effects should be relatively stronger at low speeds than at high speeds. Furthermore, diffusion effects are most unlikely because it is referred to equilibrium states. So this explanation of the deviations found especially at the higher speeds is somewhat inconclusive. As a further result, Richard found in this work that with $MgCl_2$ as electrolyte the gel-like phase did not form.

Trohalaki et al. [32] found a turbid phase when centrifuging DNA in NaCl solutions at a high concentration of 65 mg/ml. Using uv-absorption optics, they found a sharp phase boundary in the equilibrium pattern. Within this phase a second transition was suggested. This turbid phase scattered light so strongly that the DNA concentration could not be determined anymore. This agrees with recent findings on a κ-carrageenan/water gel [65]. Using solution columns of about 12 mm, the time to reach equilibrium was found to be 1 week, a typical value for gels under these conditions [38, 42, 56, 68]). This is a further hint that the turbid phase was a gel. It is interesting to note that it was not possible to achieve re-equilibration on descending from higher to lower speeds within reasonable times of 3 weeks. This can either be explained by a very slow re-equilibration especially of high concentrated gels or by an irreversible process. Such irreversible process is found for gels containing soluble parts [51, 68, 72]. The concentration distribution in the turbid phase was estimated taking the light scattering into account. Afterwards the reduced osmotic pressure was calculated for each phase by analogy to [29]. The scaled particle theory was found to describe the properties of DNA appropriately in the phases observed.

In another study, Richard investigated the effect of alkali metal ions and the multivalent cations spermidine and spermine on the swelling pressures of the gel-like

phase formed by calf thymus DNA [33]. The swelling pressures have been determined as described in the previous study [30]. A rather linear concentration of $\log \Pi_S$ on $\log c_2$ was found for LiCl, NaCl, KCl, RbCl and CsCl according to the relation of Freundlich and Posnjak given above [5]. From these linear relations, negative values for k were calculated between -0.7 and -0.8 in contrast to those reported in previous studies and the order of magnitude between 2 and 5 tabulated by Freundlich for different systems [5, 30]. The k value for CsCl was found to be different to the common one for all other salts. This difference was explained by an interaction of CsCl with DNA shielding the charges of the DNA more efficiently than the other alkali chlorides because of its size.

It should be noticed that Richard reported a DNA gel in 0.1 mol/l CsCl to be so concentrated that the fringes of the ultracentrifuge Rayleigh interference optics were lost. This agrees with the findings for gels presented in Fig. 11 [65].

Upon addition of spermidine, a reduction of the swelling pressure was observed with increasing polycation concentration indicating the collapse of the DNA network structure. Further increase of the spermidine concentration resulted in hindrance of the gel formation. Similar behavior was observed for spermine with the difference that the swelling pressure increased at very low spermine concentrations.

The finding that DNA forms a gel-like phase during sedimentation equilibrium experiments [29] was considered more closely for a high molecular weight calf thymus DNA of $10 \cdot 10^6$ g/mol by Richard [34]. It was reported that the time needed to reach the equilibrium at 20 °C was unusually high with 72 h with respect to a solution. This could be confirmed by all equilibrium studies on gels reviewed later here (see for example [38, 68]).

The scaled particle theory [29] was applied to the gel-like phase using plots of the reduced swelling pressure (in analogy to the reduced osmotic pressure of a solution) vs. polymer concentration. When the monomer molar mass of the DNA was given, nonlinear curve fitting yielded values for the effective particle length and radius in good agreement with those derived by other techniques. In principle it should also be possible to calculate the primary chain molar mass as well by applying the scaled particle theory with nonlinear curve fitting. The calculated curves for the reduced swelling pressure vs. polymer concentration, assuming the effective particle lengths and radii derived by the regression, were in very good agreement with the experimentally derived points, indicating that the scaled particle theory is valid and gives reasonable results.

It was found that gels were formed with increasing NaCl concentration in the buffer reflected by a decreasing effective particle length due to the increased crosslinking density of the network. At salt concentrations higher than 0.5 mol/l NaCl, the interference fringes in the gel-like phase became undetectable, giving further evidence for a more concentrated gel. Increasing concentration of $MgCl_2$ in a solution with constant NaCl concentration yielded also the decrease in the effective particle length being observed for NaCl alone. But in this case the decrease in the effective particle length was explained as the result of increased charge shielding by the Mg^{2+} which led to greater flexibility of the helical chain.

The data for the gels in the NaCl solution could be explained with the concept of supercoiling of the linear DNA. This concept could not be applied for the $MgCl_2$ solution anymore as the divalent counterions were suspected to condense onto the DNA in a shell rather than leading to DNA supercoiling.

The breakdown of the DNA network upon increasing spermine concentration observed in a previous study [33] was monitored by the effective particle length. As expected for a structural network breakdown, the particle length was found to increase although the particle radius remained nearly constant. But above a limiting spermine concentration the effective particle length decreased and then increased again. At higher concentration an aggregation process was observed which might explain the later increase in the effective length. However, the evidence for the structural network breakdown could not be clearly confirmed by these data.

Lange [35] introduced the analytical ultracentrifuge for the determination of the degree of swelling and crosslinking of even extremely small gel quantities in a dispersion containing small swollen particles. Lange placed the gel with an excess of swelling agent in the ultracentrifuge cell and centrifuged at 20 000 rpm for 1 or 2 h to compress the gel with a smaller specific volume than the swelling agent at the cell bottom (see Fig. 8).

He, then reduced the speed as much as possible (1000 rpm) to allow the gel to swell to its maximum degree of swelling. The centrifugal field at 1000 rpm is assumed to be so low that no deswelling of the gel due to the generated swelling pressure occurs. The swelling equilibrium was reached after only 1 h. These conditions might differ from system to system. From the position of the boundary gel/solvent which could be observed with Schlieren, absorption or Rayleigh interference optics, he could calculate the volume of the swollen gel from the known dimensions of the cell. As the volume of the polymer in the gel was known from its mass and partial specific volume, the degree of swelling could be calculated by dividing the volume of the swollen gel through the polymer volume.

These calculations can only be made if the gel contains only crosslinked molecules, the polymer is distributed

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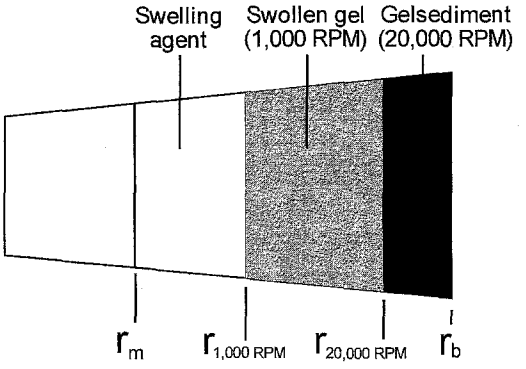

Fig. 8 Sector-shaped ultracentrifuge cell (single sector) for determination of the swelling degree of small amounts of polymer gel (schematic) [35]. r is the distance from the rotor axis with the indices m = meniscus of the swelling agent and b = cell bottom. The indices 1000 rpm and 20000 rpm refer to the gel boundary at 1000 or 20000 rpm. Redrawn from Lange H., "Determination of the degree of swelling and crosslinking of extremely small polymer gel quantities by analytical ultracentrifugation"; Colloid Polym Sci 264 (1986) 488–493 with kind permission of the author and Steinkopff Verlag, Darmstadt, FRG

largely homogeneously in the gel, and no substantial swelling agent occlusions occur. These assumptions were found to be fulfilled for the investigated crosslinked polybutadienes, polychloropropenes and powdered polyurethane foams. But a note of caution needs to be expressed if microgels are suspected to contain soluble polymer [74]. This soluble fraction must be extracted prior to the swelling experiment.

The calculation of the volume of the swollen gel via the phase volume in the cell might be questioned at least for rather monodisperse spherical particles which cannot be packed without gaps. Hence the volume is not completely filled with the swollen gel. This would then lead to the calculation of a wrong swelling degree.

From the degree of swelling the average degree of polymerization p_c and the molar mass M_c of an elastically effective network chain between two crosslinks could be determined for the polybutadiene and polychloropropene gels according to the theory of Flory and Rehner [36] under simplifying assumptions. The method described is a rapid and effective way to derive the above-mentioned parameters of gels. Unfortunately, it is restricted to uncharged polymer gels without soluble parts or with completely extractable soluble parts which means mainly chemically crosslinked gels. Furthermore, occlusions of solvent might be a problem as well as the calculation of an erroneous swelling degree caused by gaps in the packing of spherical particles.

The first improvements in the experimental set-up for sedimentation equilibrium experiments with gels were published by Holtus et. al. using a Beckman model E ultracentrifuge [37]. A more detailed description of all the following results can be found in another publication [38]. Some of these improvements were already reported for the analytical ultracentrifugation of solutions [39, 40]. The mercury light source of the Schlieren optical system was replaced by a He-Ne laser combined with a beam expander which consisted of two cylindrical lenses producing the virtual light source slit in analogy to an optical arrangement described by Klodwig and Mächtle [40]. The light source could be modulated with an acousto-optical modulator (AOM) driven by a multiplexer which was able to set freely defined time delays to a trigger impulse. Theoretically, this multiplexer was able to resolve 0.3° sectors on the spinning rotor. As the whole modulation system has been further improved in later studies, it is described there in detail [64, 65].

The photographic detection system of the ultracentrifuge was replaced by a computer and video camera based image display system. Hence, the Schlieren patterns of the gels could be accessed directly avoiding the process of evaluating the photos. But the relevant contours in the Schlieren patterns had to be redrawn on a graphics tablet so that despite the easier access of the data in the Schlieren pattern via computer software, no real improvement was made as the redrawing of the patterns was tedious and time-consuming, as much so as the original photographic evaluation. Such a system would not be suitable for a sedimentation velocity experiment with quickly sedimenting molecules and hence the application range of the ultracentrifuge was limited. Furthermore, the documentation of the experiments was rather unsatisfactory as only those parts of the Schlieren pattern have been stored which were found to be of importance and hence have been redrawn. These disadvantages were due to the application of a semiautomatic image analysis system. Nevertheless, for the application to the equilibrium ultracentrifugation of gels, this image analysis system proved to be useful.

The temperature in the ultracentrifuge cells was measured with a specially designed temperature-measuring cell based upon the temperature dependence of the refractive index [70]. Replacing the refrigeration system of the ultracentrifuge by a cryostat ensured a more sensitive temperature control. The original vacuum system was found to not be useable at the extremely long run durations of up to 3 weeks as the diffusion pump oil started to degrade during a run. Therefore, the original vacuum system was replaced by a modern high power vacuum pump yielding a sufficient vacuum for the equilibrium experiments without the diffusion pump.

As the equilibrium experiments with gelatin/water gels using long gel columns of about 10 mm needed 3 weeks, including the proof of equilibria, a six-place rotor was used to increase the efficiency of the experiments. To allow an

individual monitoring of each sample, a multiplexer triggered by a reflection signal from the rotor code ring was introduced. The cells were selected in a way that a number corresponding to an angle with a fixed position on the rotor code ring was selected. With this angle, delay time intervals to the triggering impulse were derived and an output impulse was generated after this time interval. The multiplexer controlled an acousto-optical modulator (Bragg cell) which was used to modulate the laser light beam (see also [39,40]). Whenever the selected cell passed the optical channel, the laser light was allowed to illuminate the Schlieren optics and hence produced an image. The entire modulation system was found to resolve the two 2° sectors of a double sector cell at all rotor speeds. The theoretically resolvable sector angle was even lower with 0.3°.

An equation based on irreversible thermodynamics formally similar to the equation of Svedberg [4] (Eq. (1)) was given to calculate the swelling pressure of the isotropic binary gel in dependence of the radius. This equation contained the locally dependent partial density of the polymer ρ_2 as concentration variable. As this quantity was not directly accessible if the polymer concentration gradient could not be detected in the whole gel phase, an approximation had to be used relating ρ_2 to the locally dependent density of the gel assuming volume additivity of mixing. The locally dependent gel density could not be derived directly from the Schlieren patterns as the polymer concentration gradient could not be observed throughout the whole gel phase for the investigated gelatin/water gels. This problem was already described by other authors [4, 10, 15, 18]. With other workers, Holtus suspected turbidity of the gel to be causing this effect. But this would not explain the observation that the dark zone near the cell bottom in the Schlieren patterns is very well defined (at large ranges of initial polymer concentrations being investigated) and not continuous as would be expected for a turbidity increase with the polymer concentration. As a consequence of the fact that the concentration gradient could not be followed completely, the equilibrium polymer concentration distribution in the gel phase was calculated via a mass balance assuming a linear polymer concentration gradient in the gel phase. From this locally dependent polymer concentration, the locally dependent gel density was derived applying an experimentally determined linear relationship between the polymer concentration in the mass fraction scale and the gel density in the concentration range investigated. The introduction of the mass fraction as concentration variable for the polymer concentration is advantageous in the case of gels as, for example, pure gelatin is in the glassy state [41] and hence the volume of pure gelatin in the gel state cannot be determined, which would be necessary to calculate the volume fraction.

Overall, the ultracentrifuge was applied here as a pressure generator, generating radially increasing pressure inside the gel phase which led to a locally dependent deswelling of the gel and finally to a continuous swelling pressure equilibrium. With this method, the continuous dependence of the swelling pressure of a gel upon the polymer concentration could be determined in a larger concentration interval. This is a very important advantage as no other method for the determination of the swelling pressure with this capability exists. Furthermore, the swelling pressure curves of up to five samples could be obtained simultaneously under exactly the same experimental conditions, which is a further very important advantage.

This new method was tested on dialyzed gelatin/water gels in the concentration range of 2–8% by wt. without any detectable soluble components. It could be shown that the equilibria could be reached from higher and lower speeds as introduced as a proof for equilibria of agar gels by Johnson [21, 22]. The induction period reported by Johnson for sedimentation velocity experiments [10] was found in this study as well during the approach to equilibrium when the rotor speed has been selected too low.

The determined dependence of the swelling pressure on the polymer concentration (named swelling pressure curve in the following text) suffered from an error. It was assumed that all gels were initially swollen to their maximum degree of swelling and hence the concentration at the meniscus $r_m^{g/s}$ had to be the initial polymer concentration. Gelatin/water gels are able to swell much more as corresponds to their initial concentration. Therefore, the concentration of the maximum swollen gel (to be determined separately) had to be known for the applied mass balance, rather than the initial concentration of the gel. Although the errors in the swelling pressure curves caused by this wrong assumption are significant [68], the general order of magnitude of the swelling pressure curves as well as their relation to each other is not basically changed.

It was found that the swelling pressure curves – although the swelling pressure equilibrium could be proved – intersected for gelatin gels with initial concentrations lower than 7% by wt. at 20 °C (example given in Fig. 13). At 10 °C, all swelling pressure curves in the investigated concentration range intersected. This finding was in contrast to the Flory–Huggins theory of a homogeneously swollen gel with a concentration independent interaction parameter. It was concluded that the lower concentrated gelatin networks must be inhomogeneous. Furthermore, the swelling pressure curves showed a significant dependence upon the gelation time of the gel (3 days and 7 days) although the selected times were large and the gelatin gel should be considered as matured in both cases. Nevertheless, the swelling pressure curves for the longer maturation times were steeper, indicating an increased crosslinking

density with respect to the shorter matured gel. These findings are in qualitative agreement with observations on the empirical parameter n by other workers [18]. In this study n was increasing with the maturing time, indicating an increased crosslinking upon maturing in the time interval up to 20 h. This increase was more pronounced at 4 ° and it vanishes at 18 °C.

As an alternative explanation for the time dependence of the swelling pressure curves for gelatin/water gels, a stiffening of the chains due to further helication with time and hence an altered stress-strain behavior was given by Holtus [38]. Furthermore, an effect of the gelation temperature on the swelling pressure curves was observed as it could be expected due to the changes in the network crosslinking density. These results showed again that swelling pressure curves may be used as sensitive measure of structural changes in the gel network as it could already be demonstrated by the application of the scaled particle theory [29, 34]. The reproducibility of the swelling pressure curves was found to be good. A clear influence of the gelling conditions on the gel structure and hence on the swelling pressure curves could be proved.

An attempt was made to calculate the concentration independent Flory–Huggins interaction parameter χ and the crosslinking density from the swelling pressure curves. It gave physically meaningless negative crosslinking densities. This showed that at least the model with a concentration independent interaction parameter could not be applied.

The period 1991–1993

This situation was improved in a following study [42]. As this work is rather difficult to access, only those findings are reviewed here which have not later been published elsewhere. In this study it was found that the substitution expression for the partial density of the polymer in the equation for the swelling pressure given by Holtus [38] leads to larger errors for higher concentrated gels (e.g. >10% by wt.). The introduction of a correction term could improve the situation. An experimental improvement, a temperature-controlled device was introduced to maintain constant gelling conditions of the gels as found to be essential in [38]. Furthermore, some impregnation techniques were reported to minimize the adhesion of the investigated gelatin/water gels at the cell walls and windows of the ultracentrifuge cells.

For gelatin/water gels, it could be shown that the presence of soluble parts which are not incorporated into the polymer network caused various problems in the determination of the swelling pressure curves. Even the filling volume of the ultracentrifuge cell and hence the length of the gel column was of detectable influence in these cases similar to the findings reported by Johnson's group (see for example [15]). An attempt to correlate the point where the Schlieren optical system failed to detect the concentration gradient in the gel phase with a critical polymer concentration was unsuccessful. The nature of this effect still remained unknown.

In 1991, a trilogy of papers was published treating the swelling pressure equilibria of swollen crosslinked systems in an ultracentrifugal field in detail. The first paper dealt with theoretical considerations for a binary gel [43]. After pointing out the general behavior of a sedimenting gel and the nature of the swelling pressure equilibrium, irreversible thermodynamics has been applied to derive the following equation for the swelling pressure of a binary gel:

$$\Pi_S = \omega^2 \int_{r_m^{g/s}}^{r} \frac{\rho_2}{\rho_1 \tilde{V}_1}(1 - \tilde{V}_2\rho) r dr, \tag{4}$$

where ρ is the gel density, ρ_1 the partial density of the solvent and ρ_2 the partial density of the polymer. All densities depend on the radial distance r. Basic assumptions for the application of this equation were:

- The deformation of the binary gel leads to a continuous isothermal equilibrium,
- The gels remains isotropic during the deformation (which is certainly valid for small deformations),
- Gel and solvent are incompressible, e.g., the partial specific volume is pressure- and location independent,
- Volume additivity of the pure components,
- In the equilibrium case, the swelling equilibrium is reached at the meniscus gel/sol ($r_m^{g/s}$).

This equation has been used already in earlier works [37, 38]. As the theoretical considerations are given in more detail in this work, they have not been mentioned before in this review. A new but very important assumption in this study is the last of those given above. The gels were not considered as swollen to a maximum extent at their initial concentrations, but only at the meniscus gel/sol. This means that the concentration at this meniscus is not the initial gel concentration as assumed before, but that of the maximum swollen gel which needs to be determined separately.

Equation (4) is not only formally similar to Eq. (1) of Svedberg; it could be shown that Eq. (1) is a special case of Eq. (4) for highly swollen gels which is expressed in the term "generalized Svedberg–Pedersen equation". The application of Eq. (4) for gels, where the concentration gradient in the gel cannot be detected in the whole gel phase suffers from the required knowledge of the locally dependent polymer concentration which is not experimentally accessible in these cases. Therefore, Eq. (4) has been rear-

ranged substituting the partial specific volume of the solvent $\tilde{V}_1$ in the gel by the much easier accessible specific volume of the pure solvent $\tilde{V}_{01}$ which is fulfilled for not too concentrated gels. The resulting equation now only contained parameters which could be derived experimentally:

$$\Pi_S = \omega^2 \int_{r_m^{g/s}}^{r} \left(\rho - \frac{1}{\tilde{V}_{01}}\right) r dr \,. \tag{5}$$

Considering the influence of the hydrostatic pressure on the gel concentrations, e.g., an effect of the hydrostatic pressure on the obtained swelling pressures, an expression was derived to calculate the polymer concentration in the gel at the meniscus gel/sol ($r_m^{g/s}$) under the influence of the hydrostatic pressure of the solvent column. The effect was estimated to be small in agreement with earlier findings.

The second part of the trilogy deals with the determination of molecular parameters from the swelling pressure curves [44]. Applying Flory–Huggins statistical theory of polymer solutions and the theory of rubber elasticity to the swelling of a nonelectrolyte polymer system, a modified semi-empirical Flory–Huggins equation was obtained [45,46]. Substituting the difference in the chemical potentials of the solvent $\Delta\mu_1$ by $-V_1\Pi_S$ with V_1 = partial molar volume of the solvent, an equation has been derived which semiempirically relates the swelling pressure of the gel at a known concentration to molecular parameters of the gel:

$$-\frac{\Pi_S V_1}{RT} = \underbrace{\ln(1-w_2) + w_2 + \chi_{w,o} w_2^2 + \chi_{w,1} w_2^3}_{\text{Mixing Term}} + \underbrace{C_w w_2^{1/3}}_{\text{Network Term,}} \tag{6}$$

with w_2 = polymer concentration in the mass fraction scale (index 0 refers to the initial concentration), χ_w = linearly concentration dependent interaction parameter $\chi_w = \chi_{w,o} + \chi_{w,1} w_2$ which is an apparent value due to the semiempirical nature of the Flory–Huggins equation applied, C_w = apparent network constant (explained in more detail in the original article). Both, the interaction parameter and network constant are not explicitly stated as apparent values in the following to avoid repetitions, although a strict consideration would require this.

More than the interaction parameters of the polymer-solvent system can be derived. The network constant C_w allows to calculate the elastic modulus, the shear modulus and the molar mass of the network chains if the functionality of the crosslinking points is known. C_w and both terms of χ_w could be derived by means of a nonlinear iteration procedure from the swelling pressure curves. This iteration delivers good results as the swelling pressure curves, which can be calculated with the three derived parameters, coincide well with the observed ones. The fitted parameters are unique, which was tested using different starting values which could even be physically meaningless for the systems gelatin/water and κ-carrageenan/water [42].

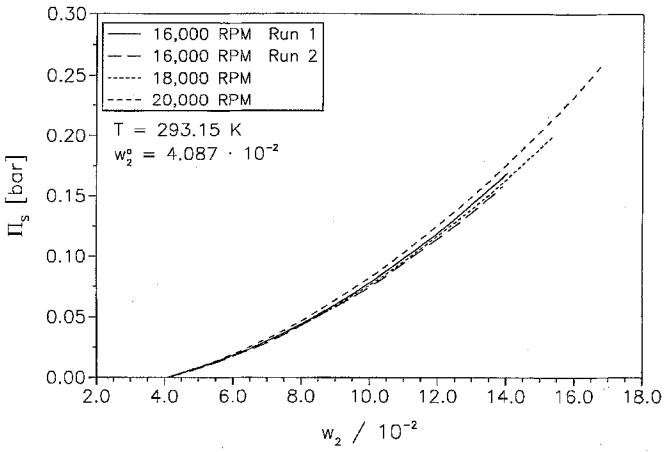

Fig. 9 Swelling pressure Π_S of the system gelatin/water vs. concentration of gelatin for different rotational speeds. w_2 is the weight fraction of the polymer. Reproduced from Holtus G, Cölfen H, Borchard W., "Swelling pressure equilibrium of swollen crosslinked systems in an external field. II"; Progr Colloid Polym Sci 86 (1991) 92–101 with kind permission of Steinkopff Verlag, Darmstadt, FRG

The generalized Svedberg–Pedersen equation for a binary system (Eq. (5)) as well as the modified Flory–Huggins equation Eq. (6) were applied to ultracentrifuge experiments with a photographic gelatin in the concentration range of 2–10% by wt.. The sample was dialyzed to meet the assumption of a binary system as close as possible. In order to enable the calculation of the swelling pressure curves via a mass balance which is given in the third part of the trilogy [51], the maximum degree of swelling of the physically crosslinked networks had to be determined separately.

A basic conclusion from the generalized Svedberg–Pedersen equation is that the swelling pressure of a gel must be constant at a certain polymer concentration, independent of the applied rotational speed. This is clearly fulfilled for the investigated dialyzed gelatin/water gels as shown in Fig. 9.

Furthermore, it could be shown that the interaction parameter and the network constant derived from the swelling pressure curves by means of a nonlinear iteration procedure described the swelling pressure curves very well and could hence be treated as reliable although the semiempirical Eq. (6) used to derive these parameters is only valid for a non-electrolyte. Charge effects have not been suppressed by adding salt additional to that amount resulting from the gelatin manufacturing process.

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The interaction parameters derived for the gelatin/water gels gave further insight into the gel structure. From the concentration dependencies of both terms of the linearly concentration dependent interaction parameter, it could be derived that the degree of branching increased with increasing polymer concentration of the gels. Extrapolation of the two linearly concentration dependent parts of the interaction parameter to zero concentration would even allow the estimation of the interaction parameter of a completely uncrosslinked and unbranched polymer chain, a quantity which is difficult to measure directly because of the self-association of the gelatin. Both parts of χ_w were found to be temperature independent at 10° and 20 °C.

The χ_w-parameters which have been calculated for the initial gelatin gel concentration were found to be nearly constant between 10° and 20 °C and 2 and 10% by wt. The value derived of 0.497 was found to be very close to 0.5. Hence the gelatin/water gels must be very close to a miscibility gap as a gel is a system with an infinite molar mass which should demix at $\chi_w > 0.5$ if the influence of the network term in Eq. (6) could be neglected. Furthermore, the χ_w-parameter of 0.497 was in very good aggreement with results derived for gelatin solutions with osmosis and light scattering in the range of 0.491–0.499 [47–49]. The network constant C_w at 10 °C was found to be nearly twice as large as that at 20 °C. This finding could be confirmed by measurements of the complex shear modulus. Overall, the agreement with the results of other independent methods shows that the ultracentrifuge can be used not only to derive the swelling pressure curves of gels, but also some of their molecular parameters. It is remarkable that such good agreement with the results of other methods could be achieved, although one of the basic assumptions for the application of the generalized Svedberg–Pedersen equation is that the system is binary which is not strictly fulfilled. The investigated system is clearly no nonelectrolyte system as assumed in the modified Flory–Huggins Eq. (6) and no additional salt has been added. A possible explanation for the good agreement of the results is that gelatin can contain salt/ash from the manufacturing process which might shield the charges of the polyelectrolyte. But although the results agree nicely with those from other techniques, the applied Flory–Huggins equation can only be semi-empirical.

From the network constant, the number average molar mass of the chains between the crosslinks could be calculated assuming a certain functionality and endlinked chains. For gelatin/water it was found that the network chains had a higher molar mass than the primary chain for some gels. This could only be possible, if gelatin molecules associate before crosslinking. This explanation is likely as it is known that gelatin is a self-associating system, even if the molecules are so small that they cannot form a gel [50]. A crosslinking of these associated chains would furthermore lead to the observed branched networks.

As gelatin/water gels are in the state of a rubber under the applied conditions, the Young and shear modulus of the gels could be calculated assuming a poisson ratio of 0.5. The derived values were found to be about 10 times lower than the storage shear modulus at 1 Hz obtained with a torsion oscillation viscometer. This difference was explained by the frequency dependence of the complex shear modulus.

The question arose as to whether the Flory–Huggins theory could still be applied for the highly branched gelatin/water gels. For a gel concentration above 6% by wt. this was considered to be no problem (coil overlap), whereas at lower concentrations the local polymer densities in the network might be different. The latter case would explain intersecting swelling pressure curves for different concentrated gels as reported before [38, 42].

The third part of this trilogy dealt with remaining unsolved problems of the ultracentrifugation of gelatin/water and κ-carrageenan/water. These problems were mainly caused by the presence of soluble parts in the gel which was assumed to be a binary mixture [51]. After a description of the sedimentation of a gel in an ultracentrifugal field, a mass balance based on the cell geometry and the equilibrium position of the meniscus gel/sol ($r_m^{g/s}$) is given which enables to calculate the polymer distribution in the ultracentrifuge cell if the concentration gradient is not visible throughout the gel phase. An assumption for this mass balance was a linear concentration gradient which has been proved before [21, 22, 38]. Also, the relation between the gel density and the polymer concentration had to be determined as well as the maximum degree of swelling of the gel.

For the example of a gelatin/water, it was shown that the swelling pressure curves no longer superimpose for different rotational speeds (see Fig. 9) once soluble parts are present in the gel. This hints at the occurrence of an irreversible process. But in this study it was supposed that true equilibria have not been reached, which was caused by the presence of soluble polymer material. A rough estimation of the time to reach an equilibrium state was given. The influence of the soluble parts was supposed to increase with increasing rotational speed. Overall the swelling pressure equilibrium of the gel was thought to be accompanied by a sedimentation–diffusion equilibrium of the soluble parts. This assumption neglected the self-association of the soluble parts which implies an association of soluble parts to network chains if certain concentration limits would be exceeded [50]. The altered solvent activity caused by the superposition of the gradient of the soluble parts was given as explanation for the observed deswelling of the gel at increased rotational speeds.

It was observed that the intersection of the swelling pressure curves described before [38, 42, 44] could be altered by changing the rotational speed. In conclusion, it was pointed out that extreme care is necessary in interpreting intersecting swelling pressure concentration curves. The quantity of soluble parts present in the gelatin gels was found to be independent of the gelation time. The finding was explained by the low molar mass of the soluble parts and their lack of gelling ability. This could be proved in a later study [50]. Despite the obvious problems with the presence of soluble parts, it could be shown that the reproducibility of the experiments was good even under such circumstances.

For the system κ-carrageenan/water equilibria could be proved by the method used by Johnson and Holtus [21, 22, 38], although soluble parts could be detected. It was stated that the soluble parts did not influence the swelling pressure equilibria of the gel in that case. This finding supports the view that the phenomena described for gelatin/water before are not only caused by a superposition of the swelling pressure equilibrium of the gel with a sedimentation-diffusion equilibrium of the soluble parts. This is a further evidence for an irreversible process like the change of the crosslinking density of gelatin/water gels upon increase of the rotational speed (see also [20]). Discontinuous steps of the refractive index gradient have been observed in the Schlieren patterns of the sol phase formed by deswelling of the κ-carrageenan gel. This was explained by the formation of aggregates and microparticles which are not able to move into the gel network. These aggregates could be formed by those carrageenan types which are generally not able to gel.

The degree of swelling of only partially crosslinked gels can be determined with a procedure introduced by Müller [52]. He measured the distribution of the sedimentation coefficients (*s*-distribution) of individually suspended latex particles in a thermodynamically good solvent at least at two different rotational speeds. At the low speed (2000 rpm) the *s*-distribution for the swollen crosslinked particles was determined, whereas at a higher speed (e.g., 40 000 rpm) the corresponding distribution of the soluble polymer could be obtained. The derived *s*-distributions yielded information about the portions of dissolved and crosslinked polymer, the degree of branching of the soluble polymer, and the degree of swelling Q [35] due to the following equation:

$$Q = \frac{f \cdot d_{T,K}^2}{s_{\text{swollen}}} \cdot \frac{\rho_{T,K} - \rho_0}{18 \cdot \eta}, \tag{7}$$

with s = sedimentation coefficient, $d_{T,K}$ = diameter of the compact, unswollen particles, $\rho_{T,K}$ = density of the compact, unswollen particles, ρ_0 = density of the dispersion medium and η = viscosity of the diluted dispersion. f is a factor according to $m_r = f \cdot m$ where the mass of the particle m_r reduced by the soluble part is related to the mass m of the particle consisting of soluble and insoluble parts. f could directly be obtained with the interference optics applied, whereas the diameter $d_{T,K}$ of the unswollen particle had to be determined first in a separate experiment, e.g., via turbidity measurements in an analytical ultracentrifuge. In Eq. (7) it is assumed that the hydrodynamic diameter of the particle is not affected by the leaching process of the particles in the dispersing medium. A further approximation has been made by neglecting the concentration dependence of s when using Eq. (7) with the sedimentation coefficient at 5 g/l instead of that at zero polymer concentration. From the degree of swelling the polymerization degree p_c and the molar mass of the elastically effective network chains between the crosslinks M_c is available applying the Flory–Rehner theory [36].

The method was tested with styrene-butadiene latices from a batch process in cyclohexane as solvent. The derived results are given in Fig. 10.

It can be seen that the particle size does not increase anymore after a conversion of 70% has been reached, whereas the crosslinking takes place indicated by a decreasing degree of swelling instead of the former branching of the molecules at low conversion degrees. With increasing conversion the *s*-distribution becomes bimodal at 83% conversion indicating the presence of crosslinked (high *s*-values) and soluble (low *s*-values) polymer. This makes clear that the solution component has a much lower *s*-value than the gel. The *s*-distributions could also be used to detect small differences in latex stabilities.

Although the method described might bear some inaccuracies caused by neglect of the concentration dependence of the sedimentation coefficients, it enables a rapid characterization of latices by the degree of swelling, the distribution of crosslinking and branching and the portions of soluble and crosslinked polymer.

Mächtle looked at the behavior of styrene/acrylonitrile-copolymer (SAN) grafted onto polybutylacrylate particles (PBA) in a tetrahydrofuran-diiodomethane density gradient to study, if the grafted molecules were completely bound covalently or not [53] in analogy to the studies of Shaskoua and van Holde [6] and Shaskoua and Beaman [7]. About 20% of the SAN was found not to be covalently bound to PBS. With a sedimentation velocity run, the sedimentation coefficient and the molar mass of the dissolved SAN was determined as well as its concentration via integration of the Schlieren peak.

In a publication of Cölfen and Borchard [54], the findings of the previous paper trilogy have been presented in a comprehensive way adding some new results which will be presented here only. It could be shown that a

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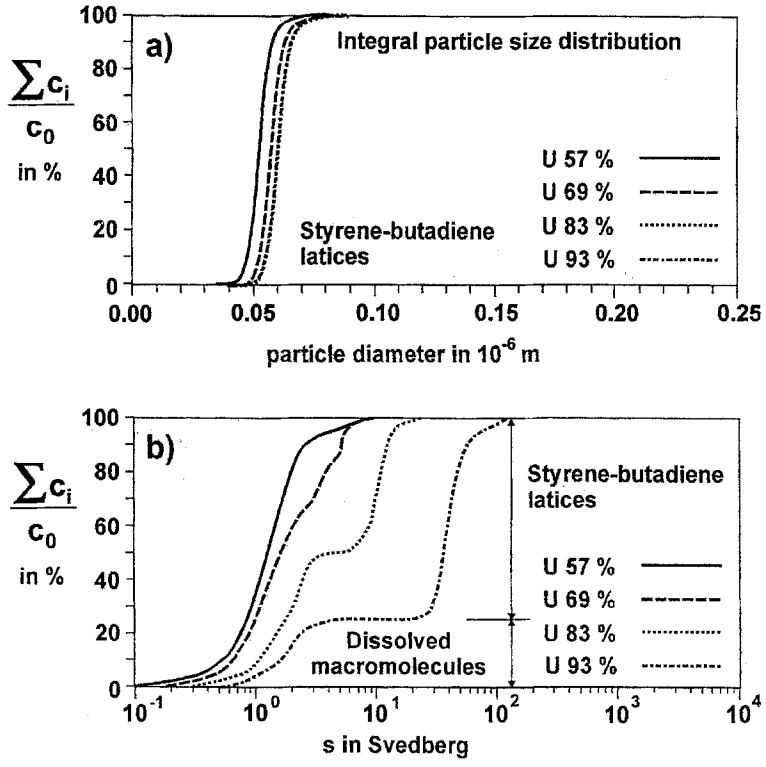

Fig. 10 Particle size distribution and s-distribution of latices of the same polymerization process with increasing conversion U. s_{50} for the latices after 83% conversion is 10.5 Svedbergs, whereas it is 40 Svedbergs after 93% conversion. The corresponding Q^*-values (Q^* is the degree of swelling derived with Eq. (7) and the sedimentation coefficients at 5 g/l [52] instead of infinite dilution) are 3000 for $U = 83\%$ and 200 for $U = 93\%$. $\Sigma c_i/c_0$ reflects the sedimentation coefficient distribution. Redrawn from Müller HG, Schmidt A, Kranz D., "Determination of the degree of swelling and crosslinking of latex particles by Analytical Ultracentrifugation", Progr Colloid Polym Sci 86 (1991) 70–75 with kind permission of the author and Steinkopff Verlag, Darmstadt

linearly concentration dependent χ_w-parameter in Eq. (6) is sufficient for the description of the polymer-solvent interactions of the system gelatin/water. Higher χ_w-terms proved to be of negligible magnitude.

A more detailed consideration of the previously reported gelatin network formation of associated chains was given. Taking the overlap region (assumed to be similar to that of collagen) of two associating or crosslinking molecules into account, the average functionality of the network chains could be estimated. For a 3% by wt. gelatin gel at 20 °C an average functionality of 2.9 was derived which clearly shows the network formation of associated polymer chains. It was pointed out that this functionality <3 clearly shows that not all chains can be endlinked to the network which leads to the already observed highly branched structure of gelatin gels [44]. A model of the gelatin gel network was given, taking these special findings into account.

As further reason for the previously reported intersection of the swelling pressure curves for gelatin/water gels (see Fig. 13), the concentration dependence of the χ_w-parameter was given. This concentration dependence of χ_w can be due to the fact that a large concentration range is covered in the experiments. However, a clear reason for the intersection could still not be given due to the lack of model calculations with varying χ_w-parameters.

The concentration dependence of the static shear modulus calculated from the network constant C_w has been compared with the directly measured real part of the complex shear modulus at 1 Hz [55]. Both concentration dependencies have been found to be of a linear type in the double logarithmic scale. For different gelatin/water gels, it could be shown that at low gel concentration, the real part of the complex shear modulus is about 10 times higher than the static value from the ultracentrifuge measurements. This discrepancy was found to decrease at increasing polymer concentration due to the decreasing viscous properties of the gel. Therefore it should, in principle, be possible to determine the intersection point between the lines in the double logarithmic plot for the static shear modulus (from ultracentrifuge measurements) and the dynamic shear modulus (real part of the complex shear modulus determined in a torsional oscillation experiment). This point should define the gel concentration at which all viscous properties of the gel disappear. As the gel enters the glassy state, this characteristic point is the glass transition.

After the procedure to determine molecular, thermodynamic and elastic properties of gels from sedimentation equilibria had been established, another study was carried out investigating the sedimentation equilibria of κ-carrageenan/water gels more closely as it had been done in

previous studies [56]. These results were partly published [60]. The gels have been investigated in the concentration range between 1 and 5% by wt. due to the high turbidities of the gels and the restricted carrageenan solubility. Again, equilibria could be proved by reaching them from different rotational speeds [21, 22, 38, 42, 51]. However, this equilibrium proof was only possible if the higher speed was applied for shorter times than that needed to reach a new equilibrium. In the latter case the superposition of the sedimentation-diffusion equilibrium of the soluble parts (0.3–0.6 mg/ml as shown later [60]) with the swelling pressure equilibrium of the gel was given as an explanation for the failure of the proof of equilibrium. This is inconclusive unless the soluble parts equilibrate very slowly. The superposition of the two equilibria should be present at any speed and be reversible if the soluble parts can move freely in the network as it is assumed unless the equilibrium processes involved are very slow and beyond the time scale of the experiments. Instead, an irreversible process seems to take place as observed in other studies [20, 51].

A linear relationship was found between the initial gel concentration and the concentration in the maximum swollen κ-carrageenan/water gel. This relation could be supported by theoretical considerations under certain assumptions and was also found for different gelatin/water gels [68].

Hinsken [56] found so-called "double peaks" in the Schlieren patterns of the sol phase as reported before [51] and interpreted them in terms of association of the κ-carrageenan. The results were found in qualitative agreement with the Gilbert theory for reversible associating polymers [57, 58]. The question was raised of why a self-association of the soluble parts could be observed although they were not incorporated into the network, suggesting as the answer the presence of different non-gelling carrageenan components which are still able to associate.

As for gelatin/water an intersection of the swelling pressure concentration curves below certain initial polymer concentrations in the gel was observed (see Fig. 13 for the gelatin example). The Flory–Huggins interaction parameter was found to be linearly concentration dependent in analogy to the system gelatin/water. But in contrast to that system, χ_w was found to be around 0.44, increasing very slightly with the polymer concentration. From that it was concluded that water is a good solvent for κ-carrageenan/water. Via the linear concentration dependence of both χ_w-terms in Eq. (6) the crosslinking and branching degree was found to increase with the polymer concentration.

A linear concentration dependence was supposed for the network constant C_w in the investigated small concentration range. Extrapolation to a vanishing C_w yielded a value of 0.99% by wt. which was identical with the critical concentration of gel formation. This result was seen as further evidence for the quality of the parameters derived from sedimentation equilibria of gels. Later investigations on the same dataset showed [60] that this dependence was in fact nearly quadratic and finally led to a good agreement with the predictions of de Gennes following the C^* theorem [59].

With a rather high assumed molar mass of about 500 000 g/mol for the primary chain of κ-carrageenan, the number of crosslinking points per polymer chain could be estimated to be at least five, probably much higher. This result is clearly different from that for gelatin gels where for some gels less than two crosslinking points have been observed per chain [54].

The determined static shear moduli for κ-carrageenan in the double logarithmic plot showed a linear concentration dependence in analogy to the results derived by another author for the system gelatin/water at low polymer concentrations (summarized in [54]). The slope of both lines was 2.26 which was in good agreement with the value of 2.25 predicted theoretically by the scaling theory of De Gennes [59]. However, the curves published by other authors for higher concentrated gelatin/water gels as summarized in [54] should have been taken into account as well. From this, the slope of 2.25 seems to be the exception, independent of the measuring method. Furthermore, it could be established in previous studies that the shear modulus determined with the ultracentrifuge is a static value due to its equilibrium nature whereas, for example, in torsional oscillation experiments dynamic values are obtained. For a given system, the dynamic values were always higher (for the system gelatin/water at low polymer concentrations even by factor 10 [54]) due to the viscous parts of the dynamic shear modulus. In a creep experiment, it could be stated that the shear modulus decreased by factor 2 already after 24 h experiment duration [54]. Furthermore, it is known that the slope of the regression lines in double logarithmic shear modulus vs. polymer concentration plots are always higher for the static shear moduli than they are for the dynamic ones for concentrations beyond the glass transition [68].

Taking all this into account it seems not appropriate to compare a concentration dependence of a static shear modulus for κ-carrageenan/water with a dependence of a dynamic value for the system gelatin/water. Although these curves are nearly parallel and even have nearly the same ordinate, and further, the slope implies that both systems can be described by the scaling theory of De Gennes [59], the other results described for gelatin/water must be taken into account as well [54]. Other curves – partly based on twice as many measuring points for gelatin/water [68] – starting still in the diluted range

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considered by Hinsken and extending to much higher polymer concentrations, showed a linear dependence with comparable error intervals as well, but a slope of 2.94 rather than 2.26. This would certainly allow the conclusion that, for this case, the C^* theorem of de Gennes cannot be applied.

From the good agreement of the experimental results with the C^*-theorem of de Gennes [59] in the investigated concentration range, Hinsken concluded that the influence of the soluble parts on the swelling pressure equilibria is negligible for κ-carrageenan/water. Most of the primary chains have been assumed to become a network chain, although the amount of soluble parts has not been quantified in this study. In a later publication [60], the amount of soluble parts was determined to be $<0.056\%$ by wt., which certainly justifies this conclusion.

The period 1994 up to 1995

The results given before by Hinsken have partly been published in more detail in another paper [60]. Although κ-carrageenan is a polyelectrolyte, no ionic term has been added to the calculation of the swelling pressure from the sedimentation equilibria via the generalized Svedberg–Pedersen Eq. (5) because no additional electrolyte was added. The potassium ions were considered to be fixed to the sulphate groups of the network. Therefore it was assumed that the presence of ionic groups is described by effective values of χ_w and C_w in the modified semi-empirical Flory–Huggins Eq. (6). However, charge effects might be expected. The Flory–Huggins theory was considered as semi-empirical, not only because of the polyelectrolyte sample, but also because the conformational details of the network chains and the structure of the junction points of the network are unknown.

As in the previous study [56], it was concluded that if the same swelling pressure equilibrium cannot be reached from lower and higher speeds anymore, soluble parts are responsible. But this path dependence was only observed if the swelling pressure equilibrium at the higher rotational speed was reached before the speed was decreased again to check the equilibrium approach at the initially selected speed. It was argued that soluble parts superimpose the swelling pressure equilibrium of the gel with their sedimentation–diffusion equilibrium. Back diffusion of the soluble parts was considered to be very slow, which causes an additional deswelling of the gel with respect to the state reached from lower speeds. As the soluble parts are not characterized yet, this interpretation may be doubtful as it is not clear if the soluble parts are low molecular which would not explain their sedimentation at the applied relatively low speeds or they are polymers and just not able to gel (for example other non-gelling carrageenan types). In the latter case they might at least be able to associate under the conditions of the κ-carrageenan network formation restricting their free mobility in the network which has been postulated.

As further reason for a very slow back-diffusion of the soluble parts, the reversible association of the soluble parts to the gel network as well as a self association of the soluble parts is presented without experimental proof of the suspected self-association of soluble parts as given in [72] for gelatin/water. It was argued that the soluble parts would then be trapped by their association reactions and back-diffusion should be decreased considerably.

As third possibility of the observed path dependence reaching the equilibrium, partial crystallinity of the junction points was considered. The crystalline junction points were assumed to be surrounded by a highly amorphous phase and not the pure solvent which should cause anisotropy of the gels. The soluble parts were considered to be trapped in the crystal junction points leading to a gradient of the crosslinking density depending on the radius. The last possibility discussed in terms of a crosslinking density gradient by trapped soluble parts is similar to that found as explanation for irreversible structural changes in gelatin/water gels [72], but with the difference that the junction points are treated to be crystals for κ-carrageenan here.

Other given possibilities to explain the path dependence were the high deformations of the gels leading to an unstable network structure with regrouping of junction points and the prevention of reswelling of the gel by friction due to a high gel strength.

These given explanations differ from each other and no experimental evidence could be given yet to support one of these or even another explanation for the observed path dependence. In addition, the amount of the soluble parts (0.3–0.6 mg/ml) was stated to be of negligible magnitude as the results were found to be in excellent agreement with the scaling theory of de Gennes [59]. This is somewhat contradictory from the discussion given above; a clear influence of the soluble parts must be expected unless only the alteration of the network structure by the centrifugal force or a prevention of swelling by the strength of the gel are responsible for the observed path dependence of the swelling pressure equilibria.

Certainly, further experiments are needed to clarify the role of the soluble parts on the swelling pressure equilibria or even the network structure of κ-carrageenan. It would be particularly interesting to investigate the long-term behavior (e.g., if the original equilibrium state can be reached, even after months), the self-association of soluble parts (although some evidence for a reversible self-association has been given) and their behavior during sedimentation of the gel which is accessible in principle via stained

soluble parts. Such investigations led to a deeper understanding of the sedimentation behavior of gelatin/water gels [50, 72] which finally resulted in the introduction of the gradient method [73]. This method has already been suggested as a potential approach to avoid the irreversible structural changes in gelatin networks [72].

For speeds below a critical value, reversible swelling pressure equilibria could be proved by coinciding swelling pressure-concentration curves for κ-carrageenan/water [44, 72]. The theoretical dependence of the concentration of the maximum swollen gel on the initial gel concentration already given in [56] was treated in more detail. It was even considered that for κ-carrageenan the chains are in the partially helicated state prior to gelation rather than random coils and the influence of partial helication on the quadratic mean end-to-end distance of the chains was discussed. This is a much more realistic treatment of the terms in the network constant C_w in Eq. (6) than that used before and gives another argument for the semi-empirical character of the modified Flory–Huggins equation (Eq. (6)) at least for the system κ-carrageenan/water and all other systems where the polymer chains at least partly helicate prior to gelation.

The intersection of the swelling pressure curves [56] (compare also Fig. 13 for the gelatin case) was supposed to be caused by a concentration dependence of the interaction parameter. However, simulation calculations using the presented datasets to investigate the influence of the concentration dependence of the interaction parameter on the swelling pressure-concentration curves were not performed, although such calculations help to understand a similar behavior for the system gelatin/water [68].

When the good agreement with the C^*-theorem of de Gennes (already discussed above for ref. [56]) was considered, it was concluded that the exponent of 2.25 in the double logarithmic shear modulus vs. concentration plot is not only restricted to chemically crosslinked gels but is in a certain sense universal. This statement should be considered with extreme care as no discussion was included about why all other results for gelatin/water gels (except the presented) – independent of static or dynamic shear moduli for gelatin/water – clearly disagreed with an exponent of 2.25 [54]. The static shear moduli yielded too high slopes compared to a value of 2.25, whereas the slope for the dynamic values was too low. As the concentrations in these investigations were partly as low as those investigated by Hinsken for κ-carrageenan, and further, the concentration dependence was considered over a much higher concentration interval, the concentration differences certainly cannot account for the differences in the scaling exponent. So it cannot be concluded without doubt from these results that an exponent of 2.25 as predicted by the C^*-theorem must be obtained for low polymer concentrations.

Although soluble parts were detected for the systems gelatin/water and κ-carrageenan/water, it was not possible to take their influence onto the swelling pressure equilibria into account yet because the theory for the sedimentation of a gel was only derived for a binary system [43]. For these reasons the general theory has been extended to ternary systems treating a gel with one inert soluble component [61]. Basically the same approach as for the binary system [43] was used, applying irreversible thermodynamics. The difference to the binary system was made in distinguishing between the solution and the gel phase taking into account that the gel phase consists of three components (crosslinked polymer, soluble polymer, and solvent), whereas the solution phase only consists of solvent and soluble component. Finally, equations were obtained for the change of the specific chemical potential of each component in each of the two phases.

This general solution allowed several cases to be distinguished. The case that the soluble component is so highly molecular that it does not enter the gel phase was discussed as well as that of a free mobility of the soluble component within the network. The latter case would lead to an additional deswelling of the gel according to the well known deswelling method of Boyer and Spencer [62]. Experimentally, it becomes obvious that already the ternary gel is difficult to handle as the radial concentration distribution of the soluble component as well as that of the crosslinked polymer have to be determined independently. Hence the superposition of the swelling-pressure equilibrium of a gel with a sedimentation–diffusion equilibrium of soluble parts could not yet been fully quantified experimentally.

The heterogeneous swelling equilibrium was described with a ternary state diagram [61] to illustrate the situation if different amounts of an inert soluble component are added to the elastic binary mixture. Furthermore, it was discussed what happens if an ultracentrifugal field is applied to such a ternary mixture. It was pointed out that the gel phase needs to move towards the cell bottom due to mass conservation. Furthermore, the addition of a soluble component to a binary gel has been discussed in terms of mass conservation. It was stressed that the total amount of soluble component in the gel can be determined by dialyzing the gel. But this implies different assumptions: The gel is insoluble at the considered conditions and the soluble component is completely inert to the network and can thus be dialyzed, which can be a very slow process. The latter assumption is at least not fulfilled for physically crosslinked gelatin/water gels [50, 72].

The amount of the soluble component in the solution phase was suggested to be obtainable via the application of

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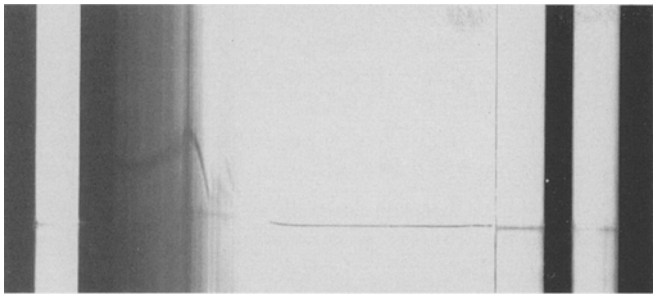
the interference optics. Here, it must be taken into account that meniscus depletion methods (see for example [63]) for the determination of the fringe shift at the meniscus solution/vapor cannot be applied in every case because 1) this would decrease the accuracy of the evaluation for the gel due to the decrease of the gel column height unless additional experimental time is invested for the overspeeding after the equilibrium run, and 2) the soluble parts may be of such low molecular mass that the meniscus depletion does not work anymore [50]. The suggestion to stain the soluble component selectively with an UV-marker to determine its sedimentation equilibrium concentration profile is difficult to realize, although possible [72]. It becomes quite obvious that already the ternary system, which considers the soluble components with their molar mass distribution as one single component is difficult to handle experimentally due to the limitations of the present optical ultracentrifuge detection systems.

In 1994, two papers were published which were dedicated to the development of a more effective experimental set-up for the hitherto very time-consuming sedimentation equilibrium experiments with gels [64, 65]. In the first part, the basic improved instrumentation applied to the Beckman Model E was described. To use multi-place rotors, a modulation system based on an acousto-optical modulator (AOM) was improved to modulate the light of a continuous He-Ne laser. This system was partly based on that which has already been described by Holtus [37, 38]. The trigger impulse for the multiplexer was derived by picking up a reflective area on the rotor code ring with a reflection light gate. After generation of a rectangularly shaped impulse from the signal of the reflection light gate in an impulse transformer, a multiplexer generates a time-delayed output signal with respect to the input signal. The multiplexer TTL-output (TTL = transistor transister logic) is converted into an RF-signal (RF = radio frequency) in the driver for the acousto optical modulator (AOM). With this system, it is possible to deviate the laser light beam into the optical channel as soon as the desired cell passes by. To ensure, that only the refracted light gets into the optical channel, a set of three blinds is used, which are also applied for optical alignment procedures. By means of a modified sophisticated Schlieren optical system [65] which generates a very small band of light illuminating the cell, it is possible to resolve even a 0.3 °C sector on the spinning rotor at its maximum speed of 60 000 rpm. This is the most important prerequisite for the application of multichannel centerpieces. However, the application of an AOM has disadvantages concerning the reduction of the light intensity. These disadvantages have been reported and it was concluded that modern high power laser diodes with about 100 mW at 670 nm are the best light-source for modulation. An alternative would be to apply the conventional 10–30 mW 670 nm laser diodes and to apply a camera which is either extremely light sensitive or able to integrate the input signal up to a sufficient intensity.

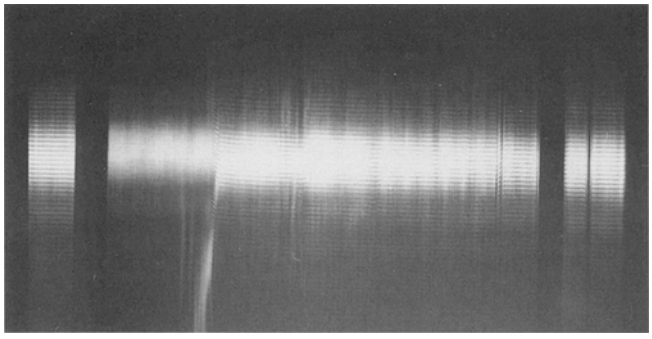
To increase the sample capacity for one experiment, an eight-hole rotor of a Heraeus AZ 9100 ultracentrifuge was modified for use in the Beckman Model E. A further optimization concerned the photographic system for the Schlieren optics which was replaced by a fully automatic picture digitization system based on a video camera in analogy to developments for solutions [40, 66]. Not only are the Schlieren patterns digitized in a high resolution, but they are also converted into a high-contrast binary picture which needs less disk space. Some considerations were made concerning the number of gray scales of the picture, its resolution and the amount of required disk space, as well as space-reduction techniques. After the storage of the binary picture, it is converted to a highly packed file format and evaluated by a fully automatical evaluation program. In case of poor picture quality or if desired for other reasons, the evaluation can be done manually as well. By means of a fully macro-driven spreadsheet program, the swelling pressure-concentration curves are evaluated, followed by a non-linear numerical iteration according to Eq. (6). Finally, the molar mass of the network chains, the static shear modulus and other quantities obtainable from the network constant C_w are determined with a fully macro-driven spreadsheet. The whole software package requires only a little user input and performs most of the evaluations fully automatically.

The second paper of this trilogy dealt with several technical improvements for an efficiency increase of the sedimentation equilibrium experiments [65]. At first, the parts of the modulation system were described including the photo pick-up, the impulse transformer and a newly designed programmable multiplexer. It was pointed out that the Schlieren optical system is the detection system of choice if concentrated and turbid gels need to be investigated (see Fig. 11(a)). It was shown that no fringes can be detected in the gel phase if Rayleigh interference optics was used due to the intensity differences of the interfering light beams (Fig. 11(b)). The high turbidity of the gels also restricted detection with the uv-absorption optics due to the significant light-scattering phenomena (Fig. 11(c)).

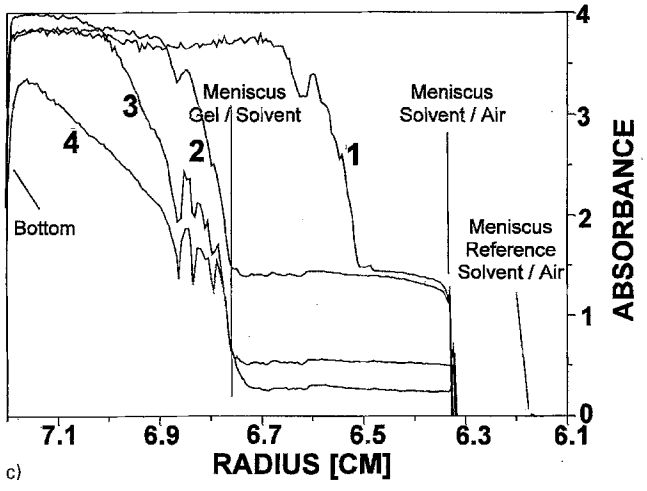
Modifications of the original Schlieren optical system of the model E ultracentrifuge were described in detail. These modifications were necessary because only a very small band of light is needed to illuminate the measuring cell if multiplace rotors are to be used with multi-channel centerpieces (resolution 0.3 ° on the spinning rotor). This high resolution was achieved by focusing the light onto the measuring cell in one plane whereas it had to remain parallel in the perpendicular plane. It was stressed that the



a)



b)



c)

Fig. 11 A sedimenting κ-carrageenan/water gel (2% by wt. at 20 °C, 20 000 rpm after at least 1 day) observed with different optical detection systems. a) Schlieren optics (The original Schlieren optics of the Beckman Model E with the mercury lamp has been used. The light intensity in the gel phase can be increased much if a laser is applied as lightsource), b) Rayleigh interference optics with a He-Ne Laser as light source and water as reference solvent, c) Beckman Optima XL-A uv-absorption optics at three different wavelengths (curves 1 and 2 = 230 nm, curve 3 = 280 nm, curve 4 = 350 nm). The absorbance is defined as $\log(I_0/I)$ with I = Intensity here. The time between the scans of the different wavelengths was 18 min (99 averages). Water was used as reference solvent. KELF centerpieces were used to avoid adhesion of the gel. All pictures do not correspond to the state of sedimentation equilibrium. Reproduced from Cölfen H, Borchard W., "A modified experimental set-up for sedimentation equilibrium experiments with gels. Part 2: Technical developments"; Anal Biochem 219 (1994) 321–334 with kind permission of Academic Press, Inc., Orlando, Florida, USA

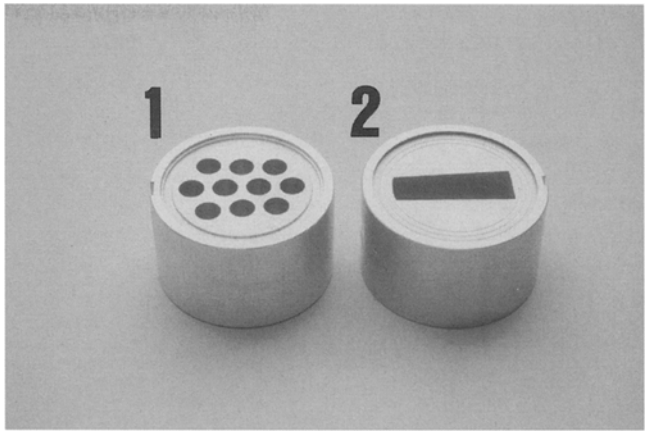


Fig. 12 Ten-hole short column multichannel centerpiece (1) and a conventional 4° monosector centerpiece (2)

Schlieren optical system is a complex one-especially the modified one-which has different properties in two perpendicular planes. Hence the optical pathway was split to give one for each plane, making the system more understandable.

An important increase in the efficiency of the ultracentrifugal experiments was achieved by the introduction of ten-hole short column multichannel centerpieces (see Fig. 12). They have the same circular sample chambers as the eight-hole short column centerpieces designed for solutions by Yphantis [67], but their radial arrangement and their diameters are different. Together with the application of the eight-hole rotor, a simultaneous investigation of up to 70 samples in one equilibrium experiment was possible for the first time. The centerpieces were specially designed for Schlieren optics and allow the sedimentation equilibrium of the gel to be reached in 2 days instead of 1 week as before for the 1 cm gel columns. The restricted choice of materials for the centerpieces for experiments with gelatin/water gels was discussed. Polycarbonate was found to be the best material for the centerpieces for this particular system, whereas polymethylmethacrylate (PMMA) was used for manufacturing the cell windows. PMMA was tested to be applicable up to 20 000 rpm, whereas the polycarbonate centerpieces could be used up to 40 000 rpm. It was pointed out that the benefit of multichannel centerpieces for experiments with solutions could be even greater. Several centerpiece designs with up to 44 channels per centerpiece have been introduced for use with Rayleigh and uv-absorption optics as well. Again it was pointed out that a programmable multiplexer as well as modified optics allowing a highly resolved location of light on the spinning rotor are prerequisites for the application of these centerpieces.

It was found that even with polycarbonate centerpieces and PMMA windows, the adhesion of gelatin at the cell walls of the centerpieces and at the cell windows could not be completely inhibited, especially not for higher concentrated gels. As this adhesion becomes obvious in a broadening of the meniscus gel/sol, the experimental accuracy, especially in the case of short gel columns, is decreased. Therefore, a simple correction was presented to locate the meniscus in case of its broadening. Assuming a parabolic shaped meniscus, the real meniscus (that is the meniscus position if it would be infinitessimaly small) was found to be 1/3 of the total broadness of the meniscus added to the end which is located nearer to the cell bottom. The benefit of this simple correction could be demonstrated for gelatin/water gels.

If the new 10-channel centerpieces with their circular sample chambers are used, the mass balance for sector-shaped sample chambers [51] – which has to be used as soon as the polymer concentration gradient cannot be detected in the whole gel phase – is different for the circular sample channels due to the different sample channel geometry. Therefore, a volume calculation for the various phases in the ultracentrifuge cell was presented in order to enable the use of the mass balance for the new centerpieces as well.

A comprehensive description of the modifications of the experimental ultracentrifuge setup for gels [64, 65] as well as experimental results [44, 50, 51, 54] was given by Cölfen [68]. The theory of the sedimentation of a gel derived by Borchard for binary [43] and ternary gels [61] was extended to a N-component system at constant temperature and pressure. The change of the specific chemical potential of the component i (1 = solvent, 2 = crosslinked polymer, 3-N = non-gelling components) $\Delta\tilde{\mu}_i$ is given by:

$$[\Delta\tilde{\mu}_i(c_1, c_2, c_3, \ldots, c_{N-1})]_{T,P} = \omega^2 \int_{r=r_m^{g/s}}^{r} (1 - \tilde{V}_i \rho) r dr \; ;$$

$$i = 1,2,3,\ldots,N \; . \qquad (8)$$

This equation is the generalized Svedberg–Pedersen equation for an N-component system. Up to now, the relation between the difference of the osmotic pressure and the change of the chemical potential is only defined for the binary case which certainly restricts the application of Eq. (8). But even if it were defined for N-component systems, a better treatment of a multicomponent gel would not be possible because it is still not experimentally possible to detect the radial concentration gradients of all individual components with the present analytical ultracentrifuge detection optics. The approach to determine the individual concentration gradients of all components in a ternary gel by extracting the soluble component, staining it, and then performing an ultracentrifuge experiment with the combined application of Schlieren (sum gradient) and uv-absorption optics (stained soluble component) is already very difficult to realize. Furthermore it has to be taken into account that a gel will deswell if it is in contact with a solution instead of a pure solvent. Therefore the approach in Eq. (8) is of theoretical nature in the general form. However, it is the most general description of the sedimentation equilibrium of a gel available up to now, containing all cases treated before.

The other possible approach of using wavelength scans at different radii for multicomponent mixtures with at least some different chromophores as applied by Schuck for multicomponent mixtures in solution [69] is also difficult and maybe impossible for protein gels with very high absorbances of the crosslinked polymer due to its high concentration.

By applying an improved counterbalance cell based on that described in [70] which contains optical components to measure the temperature dependence of the refractive index, it was possible to provide an on-line calibration procedure for the Schlieren optics beside a precise temperature measurement with 0.1 °C accuracy [68, 71]. This is a very important point for the launch of on-line evaluations of Schlieren patterns which proved to be much more complicated than the Rayleigh interference or the uv-absorption patterns. Such on-line calibration of the Schlieren optical system is the necessary precursor for on-line measurements with gels as it will be required by an effective application of the gradient method to sedimentation velocity experiments with gels [73].

It could be shown experimentally that a significant error in the determination of the swelling pressure curves occurs if the gel is considered to be swollen to its maximum degree of swelling at its initial concentration as assumed by Holtus [38]. Therefore a precise measurement of the polymer concentration in the maximum swollen gel had to be introduced, which proved to be quite difficult in the case of dissolving samples as gelatin/water at 20 °C.

As reported before (see for example [37]), the swelling pressure curves for gelatin/water gels intersected beyond certain limits of the initial gel concentration (see Fig. 13).

Some variations of the two terms of the concentration dependent χ_w-parameter and the network constant C_w in Eq. (6) for a given swelling pressure curve showed that initially intersecting swelling pressure curves did not intersect anymore if both terms of the χ_w-parameter were taken as constant. This shows that the concentration dependence of the χ_w-parameter is responsible for the intersection of the swelling pressure curves for gelatin/water.

As gelatin gels containing soluble parts cause intense experimental difficulties [51] to derive correct swelling pressure curves, a first attempt was reported to investigate the behavior of stained soluble parts inside the gel phase during a sedimentation equilibrium experiment. Soluble

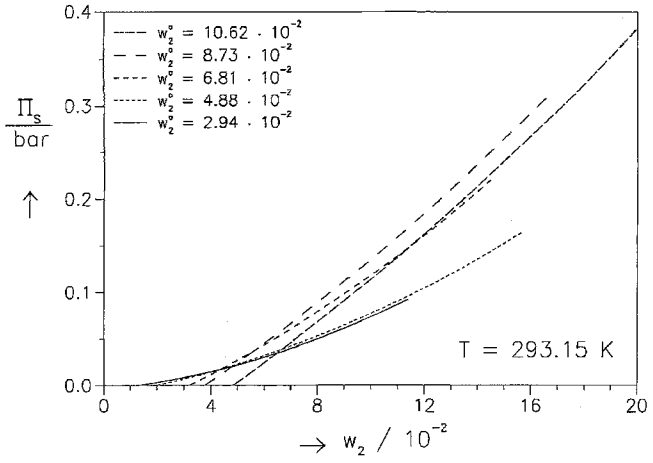


Fig. 13 Swelling pressure curves for the system gelatin/water. Π_S = swelling pressure and w_2 = polymer concentration in the mass fraction scale with the index 0 for the initial gel concentration. Figure reproduced from Cölfen H., "Bestimmung der thermodynamischen und elastischen Eigenschaften von Gelen mit Hilfe von Sedimentationsgleichgewichten in einer Analytischen Ultrazentrifuge am Beispiel des Systems Gelatine/Wasser"; Verlag Köster Berlin (1994) with kind permission of Dr. Köster, Berlin, FRG

parts have been extracted from gelatin/water gels as described in [50] and stained with fluoresceinisothiocyanate. The distribution of the soluble parts during a sedimentation equilibrium experiment could be observed using uv-absorption optics. It was found that the gradient of the soluble parts did not reach its supposed equilibrium if the rotational speed was lowered from a higher one, not even after months. However, it was still concluded that the swelling pressure equilibrium of the gel was superimposed by a sedimentation–diffusion equilibrium of the soluble parts leading to additional deswelling of the gel although this would be a reversible and not the in fact observed irreversible process. Therefore it was concluded that the soluble parts (which have been stained) could not have a significant influence on the swelling pressure equilibria of the gel due to their observed low concentration of about 2.5 mg/ml. The fact that a gel which contains soluble parts does not swell up to its original degree of swelling once it has been exposed to a higher rotational speed was interpreted to be caused by higher molecular weight soluble parts (e.g., soluble parts with molecular weights above 4000 g/mol which are normally only weakly attached to the gel network and hence would be soluble in an excess of solvent). These soluble parts should have a very low back-diffusion so that their concentration gradient leads to the observed overall deswelling of the gel.

This interpretation of an irreversible process – that a gel containing soluble parts does not swell up to its original degree of swelling once it has been exposed to a higher rotational speed – cannot be uphold from the present point of view. A sedimentation–diffusion equilibrium of the soluble parts must be (by definition) a reversible process as well as the swelling pressure equilibrium of the gel. Hence, it is not understandable why no sign of the back diffusion of soluble parts could be observed, not even after months if the ultracentrifugal field has been lowered by magnitudes. Even for very slow diffusion processes which might occur in gels, at least a minimal movement of the soluble molecules leading to an alteration of their concentration gradient should be detectable on a time scale of months. As this is not the case, the influence of the soluble parts on the gelatin/water gel structure must be irreversible.

It was tried to use the set of thermodynamic parameters derived from the swelling-pressure equilibrium curves to predict stability limits for gels [68]. This was done by extrapolating the regression functions for $\chi_{w,0}$ $\chi_{w,1}$ and C_w (derived from Eq. (6)) dependent on the polymer concentration to the whole concentration range. It was found that these regression functions could only be applied within the range of gel concentrations which have been experimentally observed.

With the extrapolation functions for the radial prediction of the thermodynamic parameters an attempt was made to find out the nature of the dark zone in the Schlieren pattern of gels, especially gelatin/water. A correlation was found between the mixing and the network term in Eq. (6) for the investigated gelatin/water gels at that radial position where the dark zone in the Schlieren pattern starts. A closer investigation of the change of these terms with the polymer concentration showed that both terms are equal at the meniscus gel/sol. But with increasing polymer concentration (i.e., with increasing radius inside the gel phase), the mixing term increases much faster than the network term [36]. As the χ_w-parameters for this concentration region are higher than 0.5, a demixing of the gel with its infinite high molar mass should occur if only the mixing term is considered. But the network term of the chemical potential seems to prevent this. At a certain ratio between network and mixing term, which has been found constant for different concentrated gelatin gels, it seems that the network term cannot prevent a demixing anymore. At exactly this location, the start of the dark zone in the Schlieren pattern is observed. Due to this interpretation, the dark zone in the Schlieren pattern should be caused by a demixed gel. This demixing of gelatin gels could not yet be proved by other methods and must hence be treated with caution as several other possibilities can be responsible for the dark zone as well. However, it was reported that during the sedimentation of 18.25% by wt. gelatin gel at 10 °C, suddenly a dark zone in the Schlieren pattern was formed within the gel phase. At this zone,

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a discontinuity of the polymer concentration gradient inside the gel phase was observed. This behavior was explained by a demixing of the gel into two gels with the same crosslinking density but a different distribution of the network chains [68]. This could lead to a light refraction which cannot be detected by the optics anymore. Nevertheless, this gel would still remain clear as it could be observed after the ultracentrifuge experiment in contrast to a demixing solution.

It could be shown that the swelling pressure-concentration curves which are derived from ultracentrifuge experiments are very reproducible [68]. Furthermore, it was demonstrated that the application of the circular sample channels in the newly designed ten-hole centerpieces yields the same results as those derived in sector-shaped cells. This justified the application of the ten-hole centerpieces. Summarizing the modified ultracentrifugal technique [64, 65] for the investigation of gels, it was pointed out that no other method is able to characterize 70 samples (e.g., a complete gel/solvent system) in only one experiment which lasts a few days. Such measurements require highly sophisticated devices for the experimental set-up and the data aquisition which are quite expensive. As further advantage of the ultracentrifuge investigation of gels, the continuous equilibrium which can be determined by the selection of the rotational speed was pointed out. As a rather large concentration range is covered within the gel phase, unstable regions could be detected. With the set of thermodynamic parameters derived from Eq. (6), the prediction of these regions is in principal possible.

It was pointed out that it should be possible to apply the generalized Svedberg–Pedersen equation to the solution phase as well as yielding the concentration dependence of the osmotic pressure which could be used to determine M_n of the soluble parts as well as their second osmotic virial coefficient. As the greatest present disadvantage of the technique the necessity of supplemental measurements like the determination of the maximum degree of swelling or the determination of the concentration dependence of the gel densities was stated. The present limitations of the ultracentrifuge technique were seen in the restriction to clear gels due to the optical detection systems of the ultracentrifuge. The application of a mass balance for the calculation of the non detectable polymer concentration inside the gel phase was suggested. Furthermore it was stressed that gels cannot be investigated if the adhesion cannot be successfully suppressed. Also, gels with a vanishing buoyancy term (e.g., $(1 - \bar{V}_2\rho) = 0$) are not accessible due to the lack of sedimentation. A further limitation was seen in the fact that it is not possible to cover the complete concentration range in the gel phase in the ultracentrifugal field. The reason for this is the limitation of the applicable swelling pressure by the maximum rotational speed of the ultracentrifuge.

The influence of soluble parts on the swelling-pressure-concentration curves, e.g., the entire network structure of the gel was the subject of a further study [72]. An alkaline treated gelatin of $M_n = 68\,000$ g/mol containing soluble parts with a M_w of 2900 g/mol was investigated. It could be shown that these soluble parts possess no gelling abilities, but are at least partly able to associate/aggregate to the network and form new crosslinks upon compression. For such a gel, it was found that the swelling-pressure concentration curves for the same gel at different rotational speeds did not coincide as is required as equilibrium proof [44]. It was found that the slope of the swelling pressure curves decreased with increasing rotational speed after a certain critical speed limit has been exceeded, hinting at an increase in the crosslinking density and assuming that the semiempirical Flory–Huggins theory for homogeneous networks could be applied. However, the maximum swelling pressure at the cell bottom was constant for a given rotational speed, as would be expected. It was stated that the process observed is irreversible because even after 9 weeks the gel did not swell up to its original degree of swelling at a certain rotational speed, once the speed had been increased. It was pointed out that the initial interpretation of this phenomenon as a superposition of a sedimentation–diffusion equilibrium of the soluble parts with the swelling pressure equilibrium of the gel leading to additional deswelling [68] cannot be supported because this would be a reversible process as discussed above. If an irreversible process takes place, it is no longer possible to compare the swelling pressure curves nor any of the parameters derived by application of Eq. (6) because they correspond to different network structures of the gel. But the apparent thermodynamic parameters derived for such a case hint at the increase of the crosslinking density upon increase of the rotational speed.

Still it could be shown that it is possible to prove swelling pressure equilibria by means of coinciding swelling pressure curves if the network structure is kept constant. This can be maintained by reaching the equilibrium at a certain speed and then awaiting the equilibrium at a lower one, where the gel network structure is still the same but different from the network structure at the initial gel concentration as soon as a critical value of the rotational speed is exceeded. Also, it could be shown that the reproducibility of the swelling pressure curves is good despite the alterations of the network structure after an increase of the rotational speed [72]. This gives evidence for a good reproducibility of the network structure even if the crosslinking density has been changed by additional crosslinking of soluble parts leading to a gradient gel.

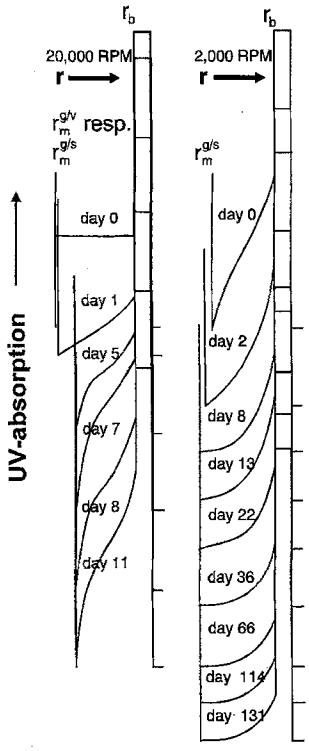

Fig. 14 uv-absorption as a function of radial displacement from the center of rotation r for a sedimentation equilibrium experiment with an 8.5% by wt. gelatin/water gel. The gel contains 0.26% by wt. (related to the solution) FITC-stained soluble parts (M_w= 2900 g/mol). The filling height of the ultracentrifuge cell was 1 cm. The index b refers to the cell bottom, m to the meniscus with the phase boundaries explained in Fig. 1. The scanning wavelength was 390 nm. The left series of traces shows the deswelling of the gel after the rotational speed of 20 000 rpm is applied whereas the right series shows the swelling of the previously compressed gel at a lower speed. Figure reproduced from Cölfen H., "Bestimmung der thermodynamischen und elastischen Eigenschaften von Gelen mit Hilfe von Sedimentationsgleichgewichten in einer Analytischen Ultrazentrifuge am Beispiel des Systems Gelatine/Wasser"; Verlag Köster Berlin (1994) with kind permission of Dr. Köster, Berlin, FRG

As the irreversible process upon increase of the rotational speed is observed only if the gel contains soluble parts (compare for example to [38]), the irreversibility must be related to this low molecular weight component. Therefore the results of the investigation of the concentration distribution of fluoresceinisothiocyanate stained soluble parts in gelatin/water gels at different rotational speeds [68] have been reconsidered (Fig. 14).

It can be seen that the swelling pressure equilibrium of the gel at 20 000 rpm (constant $r_m^{g/s}$) is reached after approximately 7 days and this agrees with previous experiments [42, 44]. From the changes in the gradient of the soluble parts (M_w = 2900 g/mol) it was concluded that they must be associated or aggregated to the gelatin network, as the soluble parts are not only self-associating, but further are similar to the gelatin in their chemical composition [50]. The formation of the observed steep gradient cannot be explained by the sedimentation of a low molecular weight species at moderate rotational speeds. Further evidence for the association of the soluble parts to the gelatin network can be given by the observation that the concentration gradient of the soluble parts still becomes steeper after the swelling pressure equilibrium of the gel has been reached. As the association is favored at higher polymer concentrations, it should preferably take place at the cell bottom where the polymer concentration is the highest. This is precisely what is observed.

When the rotational speed is decreased to 2000 rpm (just to maintain the observation of the processes by uv-absorption optics in a sufficiently stable running rotor), a speed where the gel should swell up to its original degree of swelling, the concentration gradient of the soluble parts should vanish. But this is not observed [72]. The swelling pressure equilibrium of the gel is roughly reached after 8 days, but the gel is not swollen to its original degree of swelling anymore, indicating irreversible changes in the network structure. The concentration gradient of the soluble parts is also still significant. Slowly it becomes flatter due to the slow back-diffusion of uncrosslinked soluble parts, until it remains constant after 114 days. It is obvious that this concentration gradient cannot correspond to the sedimentation–diffusion equilibrium of a species with M_w = 2900 g/mol at 2000 rpm, even not when self-association occurs as the association constant was found to be small [50].

The interpretation of this phenomenon was that soluble parts, which partly associate or even aggregate preferably at the higher gel concentrations of the deswollen gel near the cell bottom, form new crosslinks with neighbor polymer network chains and hence lead to the formation of a gradient gel [72]. If such an altered gel swells again at a lower rotational speed, this gel should not swell uniformly in an excess of solvent after such an ultracentrifuge experiment. This could be proved when a sector-shaped gel piece swelled to a nearly rectangular shaped one [72]. Hence, the crosslinking density at the meniscus gel/sol must be lower (leading to the uptake of more solvent) than that at the cell bottom, as was predicted. The observed increase of the crosslinking density leading to a gradient gel must be closely related to the soluble parts present in the gelatin gels leading to the already described gradient gel [72].

It was pointed out that the additional crosslinking does not allow further investigation of the original gel structure [72]. As this is the aim of the ultracentrifuge experiments, future investigations of gels with soluble parts causing additional crosslinking have to be carried out at rotational speeds below the critical value without

the sedimentation of the gel phase. Under such conditions, the crosslinking of associated/aggregated soluble parts is unlikely due to the much smaller concentration gradients inside the gel (see also below).

In a recent paper, Hinsken and Borchard presented a new approach to evaluate sedimentation equilibrium experiments with gels [73]. This so-called "gradient method" evaluates the local polymer concentration via the detectable concentration gradient in the Schlieren pattern. As this is not possible when high rotational speeds are applied because a dark zone occurs near the cell bottom [4, 10, 15, 18, 38, 68], low speeds are used so that no sedimentation of the macroscopic gel phase occurs (see case a in Fig. 1). The local slopes of the swelling pressure curves are calculated using a differentiated form of the generalized Svedberg–Pedersen equation (Eqs. (4) and (5)) for a binary system [43].

As the concentration range between the meniscus gel/vapor and the cell bottom is much smaller ($<33.5\%$ concentration change from the initial gel concentration for the carrageenan example) than for the method used before, only a small part of the swelling pressure-concentration curve can be determined. But in contrast to those derived via a mass balance assuming a linear concentration gradient before, these swelling pressure curves are calculated for the real concentration gradients, whatever they may be. A further significant advantage of the gradient method is that irreversible changes in the network which were observed for gelatin/water [72] are not likely to occur at the selected low speeds beyond the critical speed where irreversible changes of the network structure are observed. Hence the network can be treated as unaltered so that all thermodynamic, molecular and structural parameters derived from the swelling pressure-concentration curves are corresponding to the original network and are not apparent ones [72]. Therefore it was suggested to use the gradient method as a test for statistical theories which describe the gelation.

By means of the path independence reaching an equilibrium, equilibria could be proved by constant equilibrium Schlieren gradients inside the gel phase (within the accuracy of the measurement) independent of if they have been reached from higher or lower rotational speeds.

The presented Schlieren patterns were evaluated quantitatively by integration beyond the Schlieren curve. As the gels were investigated in a monosector cell, no baseline is present, which is normally required for the integration. Additionally, the Schlieren gradients are very broad so that the concentration determination from these pictures can be inaccurate. The polymer concentration in the gel was approximately found to be a linear function of the radius although the gradients in the evaluated Schlieren patterns were bending upwards in the region of the cell bottom. This reflects a potential error of the gradient method: the quantitative evaluation of broad Schlieren gradients.

However, the evaluated polymer concentration gradients were used to calculate $(d\Pi_S/d\rho_2)$ which is the change of the swelling pressure with the polymer concentration. These values were plotted against the polymer concentration yielding linear functions for all evaluated Schlieren patterns. But the curves corresponding to the Schlieren patterns stated to be the equilibrium Schlieren pictures before did not coincide, which has to be expected for coinciding swelling pressure-concentration curves indicating an equilibrium. Instead curves for an equilibrium at 8000 and 6000 rpm were found to be similar. These results indicate the present inaccuracies of the gradient method.

As no further experiments, especially some using double-sector cells (baseline), were performed in this study, it cannot be estimated what difficulties or limitations arise when the gradient method is applied even with good quality Schlieren patterns with baselines and if the promising advantages expected for its application can be confirmed. The potential error source, the concentration determination from the integration beyond the rather broad gradients in the Schlieren patterns, especially at low phase plate angles might still be a problem. But as long as no extremely low gel concentrations are used, the Schlieren optics is the only realistic choice for a concentration determination in the gel phase as both the Rayleigh interference and the uv-absorption optics fail [65] (see also Fig. 11).

To derive a complete swelling pressure-concentration curve for a gel with a certain network structure, it was suggested to construct it from the parts of the swelling pressure curves derived for gels with different initial concentration but the same network structure. Such gels can only be prepared by drying a given gel to different concentrations. A proof that the network structure is not altered upon drying is not given, so that it is not yet clear if a swelling pressure-concentration curve can be constructed from different parts. Nevertheless the small concentration range of a swelling pressure-concentration curve derived with the gradient method should already be sufficient to calculate thermodynamical or structural parameters using the Flory–Huggins or scaled particle theories.

A study on the characterization of microgel properties itself using analytical ultracentrifugation came from Mächtle et al. [74]. The methods used were similar to those already described before for the quantitative detection of the amounts of microgel and uncrosslinked polymer, namely sedimentation velocity and density gradient centrifugation [6, 7, 9, 53]. The successful application of a simple step-by-step crosslinking theory of primary linear macromolecules [75] as well as the good agreement with results from light scattering makes this study an interesting

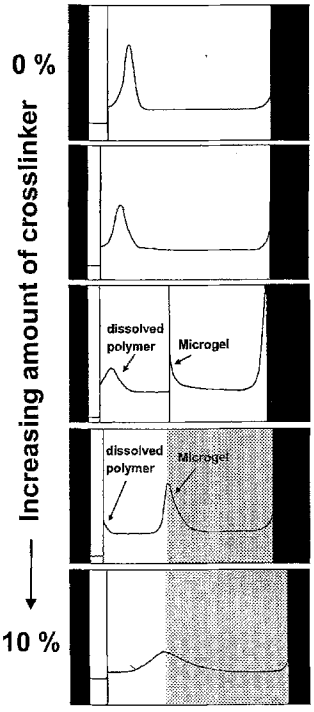

Fig. 15 Schematical Schlieren-photos of sedimentation runs in an analytical ultracentrifuge of five microgel dispersions with different concentrations of the crosslinker. The photos show slow Schlieren-peaks of macromolecules and fast Schlieren peaks of microgel particles. Redrawn from Mächtle W., Ley G., Streib J., "Studies of microgel formation in aqueous and organic solvents by light scattering and analytical ultracentrifuge"; Progr Colloid Polym Sci 99 (1995) in press with kind permission of the author and Steinkopff Verlag, Darmstadt, FRG

alternative to the characterization of gel properties with sedimentation equilibrium experiments as it is shown that rapid sedimentation velocity experiments with microgels already can yield important thermodynamic and structural information of the microgels [74] (see also [52]).

In the experimentally well grounded paper [74], 14 nearly monodisperse aqueous poly-*n*-butylmethacrylate (PBMA) dispersions were prepared by emulsion polymerization with different amounts of methallylmethacrylate (MAMA) between 0 and 10% by wt. These particles were first precisely characterized with respect to particle size distributions, diffusion coefficient, sedimentation coefficients and particle densities using analytical ultracentrifugation and light scattering. Then the particles were transferred into tetrahydrofurane (THF), a good solvent for PBMA to allow swelling of the crosslinked molecules and dissolution of all non-crosslinked ones.

The partial specific volume of the non-crosslinked sample was determined via density measurements in both solvents and was assumed to be constant for different amounts of crosslinker up to 10%. Strictly, this is not correct as the partial specific volume depends on the structure of the material which is changed here. But for this particular investigation, these changes which might lead to significant errors in the determination of the molecular weight are of minor importance as the emphasis of the study/discussion was not put on the molecular weights which would have then more advantageously and precisely been determined by sedimentation equilibrium experiments. However, the molecular weights determined with the Svedberg equation from sedimentation velocity data were consistant and reasonable for both the gel and the solution component. Hence the differences in the partial specific volume of the different crosslinked microgels must be negligible.

The different crosslinked particles have been investigated in a density gradient as well as in aqueous and THF dispersion/solution. In the aqueous dispersion, the density of the particles was found to be nearly constant, whereas in THF dispersion/solution a transition between the dissolved completely uncrosslinked molecules and the totally crosslinked microgel was observed. As it could be shown that the density gradient technique is able to resolve small structural differences between linear, branched and crosslinked molecules [9], from not more than two bands in the density gradient, it seems to be the case that linear molecules can coexist with the microgel without a significant amount of the transitional state, the branched large molecule in this example. From the density gradient experiments, the change from 0.1 to 0.2% of the crosslinker MAMA increased the microgel amount from 5 to 50% (compare also Fig. 15).

The sedimentation behavior of the different THF solutions/dispersions has been systematically investigated at different concentrations. In dependence of the amount of crosslinking agent, the transition between uncrosslinked soluble polymer and microgel formation could clearly be observed. In agreement with the density gradient technique the transition was between 0.1 and 0.2% of crosslinking agent. It was tried to calculate the critical amount of crosslinker by applying a theory of Flory for the stepwise crosslinking of a macroscopic gel phase [75], but this yielded a value of only 0.02% of crosslinker. This difference was explained by a lower reactivity of MAMA in copolymerization as well as by side reactions.

Figure 15 is a good example for the transition between a pure solution and a microgel to see that the Schlieren pattern of a microgel has more solution characteristics (Schlieren peak) rather than the characteristics of a separate gel phase (compare for example Fig. 15 with Fig. 11 and especially Fig. 7 in light of the differences).

In Fig. 15 it can be seen that at low crosslinking agent amounts, pure solution behavior is observed. But if the sedimentation coefficients of the solution components are compared for 0, 0.1 and 0.2% of MAMA, a steady increase can be observed hinting at the presence of branched polymer next to the linear uncrosslinked molecules. Although these transition states are not detected with density gradient experiments (due to the low overall concentration of a species with constant density), evidence for their presence can be seen in the increase of the solution peak sedimentation coefficient s with increasing degree of MAMA. If the

sedimentation velocity of the microgel is compared for different degrees of MAMA, a clear increase of the crosslinking density resulting in a decrease in the swelling ratio of the microgels and hence in increasing s-values is observed.

If the bottom region of the cell is observed in the microgel, it can be seen that according to the sample and experimental conditions, a more or less broad dark zone is observed which restricts the observation of the complete polymer concentration gradient, as is well known from experiments with bulk gel phases [4, 10, 15, 18], but is much less pronounced. As the investigation of the sedimentation behavior of a microgel is a sedimentation velocity technique rather than a sedimentation equilibrium method established for bulk gels, the region near the cell bottom is of no interest because only the relative movement of the Schlieren peak or the area under the peak is of interest for sedimentation velocity experiments rather than the observation of the complete concentration gradient.

The amount of microgel and dissolved polymer for the different ratios was determined by integration of the Schlieren peaks of both the solution and the microgel. The results from both components agree well and show that the transition between linear and crosslinked polymer occurs for crosslinker amounts of 0.1–0.5% and is continuous. This agrees with the results from light scattering. If the molar masses determined with the AUC (Svedberg relation) for different crosslinker ratios are compared with those from light scattering and the theoretically expected ones on the basis of the amounts of microgel and soluble polymer with their molar masses, the AUC results do not show the continuous increase of the molar mass expected theoretically and confirmed by light scattering. This showed that the amount of branched molecules must be negligible so that only microgel and linear polymer are present. The molar masses for both the soluble polymer and the microgel are constant, as can be expected because in the AUC the fractionated components are considered and not their mixture.

From the sedimentation coefficient of the microgel, the volume swelling ratio Q was calculated from the particle diameters applying Stokes law. The values were in good agreement with those from light scattering. The swelling ratio decreases strongly with the amount of crosslinker $<1\%$ from more than 30 to 5. For crosslinker contents $>1\%$ the swelling ratio is still decreasing. Even for 10% crosslinker, the microgel is weakly swollen. From the swelling ratios of the microgels, the Flory–Huggins interaction parameter χ was calculated to be 0.44 applying the Flory–Huggins theory for the crosslinking of a macroscopic gel [75]. This value and its good agreement with the results of other authors show THF is a good solvent for the microgel and furthermore, that the Flory–Huggins theory for macroscopic gels can be applied to microgels down to 60 nm as well.

From the concentration dependence of the sedimentation coefficients for the different crosslinked samples, it could be derived that only the microgel crosslinked with 10% of MAMA behaves as hard sphere, e.g., shows no concentration dependence of s. The concentration dependence was more pronounced, the lower the crosslinking density showing a systematical dependence. From that, a soft transition from the linear polymer coil via a "hairy ball" to the crosslinked hard sphere with increasing crosslinker amount could be concluded.

Outlook to the future

Due to the rapidly increasing technical possibilities, especially in the computer and electronics sector, it is expected that the efficiency of ultracentrifuge experiments with gels can be further increased tremendously enabling different on-line techniques. This will certainly enable new applications. Some trends can already be seen.

It became clear that the investigation of gel properties via a sedimenting gel meniscus, although exclusively applied in the past, has several disadvantages. There are adhesion problems, effects of the hydrostatic pressure of the sol column (although small) and anisotropic deformation in the ultracentrifuge cell which is not yet exactly theoretically treated although the general thermodynamics of anisotropic deformation has been treated by Borchard [11], but has not yet been applied to the ultracentrifugation of gels. Further problems arise from the failure of the Schlieren optics in the region of the cell bottom and, most importantly, additional crosslinking of the gel by soluble components. All these problems can be avoided if the rotational speed is chosen low enough that no sedimentation of the macroscopic gel phase occurs. In such a case, the polymer concentration gradient can be detected in the whole gel phase, the hydrostatic pressure of a sol column has no effect because no sol is present, the concentration gradients in the gel are so moderate that the concentration limit for additional crosslinking via soluble components is not exceeded and the deformations are so small that the gel can be treated as isotropic. Therefore it is expected that future work will be carried out investigating the swelling pressure equilibrium in a single gel phase where no sol phase has been introduced yet [73]. The disadvantage that a smaller polymer concentration range is covered by the swelling pressure curves here is not important for deriving the thermodynamic and elastic properties of the gel. Only if the stability of a gel system should be investigated, the two phase (gel + sol) method is more advantageous as the polymer concentration range

covered is much larger. If it is possible to relate the empirical parameter n of Johnson [17] with the structure of the gel, obtainable via sedimentation equilibrium experiments, it might be desired in special cases to run sedimentation velocity experiments with gels, if the above-mentioned disadvantages of the two phase method can either be avoided or suppressed. This would be the most rapid structural characterization of gels with the analytical ultracentrifuge.

Another rapid and precise characterization of gels based on sedimentation velocity experiments is achieved by studying the microgel properties rather than that of the macroscopic gel phase with all its difficulties and pitfalls [6, 7, 9, 35, 52, 53, 74]. As this characterization method avoids main disadvantages of the investigation of a bulk gel phase, namely anisotropic deformation and adhesion, the calculation of thermodynamic parameters from the swelling behavior might have future potential for gel characterization although the ultracentrifugal investigation of microgels was up to now more or less applied to studies of the efficiency of a grafting reaction [6, 7, 53]. As long as parameters like the Flory–Huggins interaction parameter or the molar mass of the crosslinked chains are desired, a sedimentation velocity run with microgels should be the method of choice. For this, a significant potential of applications is seen for microgel particles prepared by chemical crosslinking in emulsions (the wide field of polymer dispersions). For most physically crosslinked gels, this method cannot be applied due to the dissolution in an excess of solvent.

For physically crosslinked gels, the investigation of the bulk gel phase is still the method of choice because it is very often desired to study the properties of gels which will dissolve in an excess of solvent. For these gels, the "gradient method" described in [73] has the advantage that it produces swelling pressure curves via the exact radial polymer concentration and not via a mass balance assuming a certain concentration gradient. As the polymer concentration is detectable throughout the whole gel phase, the "gradient method" is suitable for on-line techniques which have recently been adapted for the Schlieren optics by the introduction of an online optical calibration method [68]. As both the hardware and software for the picture digitization is already so advanced that the digitization and picture evaluation of Schlieren patterns should be possible in real time if either sufficient contrast in the Schlieren patterns can be maintained by using a knife edge or it is evaluated via the Fresnel fringes in Schlieren patterns [76], exact kinetic studies of the gel sedimentation are for the first time possible. Up to now, such a real time Schlieren pattern evaluation system was not viable, because the corresponding software needed to be completely written by the user.

An application of a real time optical system can also be seen in monitoring the kinetics of swelling of latices or microgels. If the gels are highly compressed and deswollen at a high speed (say 60 000 rpm), the kinetics of swelling could be readily monitored as soon as the speed is lowered to 1000 rpm to allow swelling of the particles.

Another interesting application is the investigation of the diffusion of small protein molecules (which is detectable via the uv-absorption optics) in polysaccharide gels which are invisible for this optical system as long as the gel concentration is so low that light scattering of the gel does not disturb the observation of the protein. This would be an interesting development from similar measurements of protein phenomena through aqueous two-phase systems [77, 78]. This application may yield important information especially for the food industry if one thinks of flavor release or related fields. It should be possible to create a synthetic boundary in the gel phase in analogy to the solution case. The diffusion of the protein should take place in reasonable time intervals.

If the scaled particle theory could be used in conjunction with the thermodynamic and elastic parameters obtainable from the swelling pressure curves via the modified Flory–Huggins equation (6), it could be expected that the gel structure could be much better characterized than by each of these methods performed alone. As both methods work with a nonlinear numerical iteration of the swelling pressure curves, complimentary information is expected.

To summarize, the analytical ultracentrifuge could very well play an important role in future characterization of gels in the following fields:

- Simultaneous structural, thermodynamic and elastic characterization of a large number of gels (up to 70 at present) which is not possible by any other method known up to now.
- Rapid characterization of microgels and monitoring of the efficiency of crosslinking or grafting reactions.
- Kinetic swelling studies as well as kinetic studies of the gel sedimentation applying real time detection systems.
- Diffusion studies of proteins in polysaccharide gels.
- Stability investigations of gel systems.

It is likely that many more applications will follow if more researchers become interested in the analytical ultracentrifugation of gels as this is still a wide field with probably significant unexplored potential.

Acknowledgements The author thanks Dr. P. Johnson (Cambridge, UK), Prof. Dr. W. Borchard (Duisburg, FRG) and Dr. Steven Harding (Nottingham, UK) for the careful proofreading of this manuscript and useful suggestions. Dr. Paley Johnson is furthermore acknowledged for making available to the authors PhD-theses from members of his former working group. Dr. V. Itschenskij (Adlershof, FRG) is

acknowledged for translation of reference [28]. Furthermore, I thank Prof. Dr. W. Borchard and Dipl. Chem. H. Hinsken (Duisburg, FRG) and Dr. W. Mächtle (BASF AG, Ludwigshafen, FRG) for providing their unpublished material. In addition, all authors and editors are acknowledged for their kind permission to reproduce the figures used in this review.

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