4

Analytical Ultracentrifuge Technologies for the Characterization of Biopolymer Gels and Microgels

HELMUT CÖLFEN

Max-Planck Institute of Colloids and Interfaces, Colloid Chemistry Department, Kantstrasse 55, D-14513, Teltow-Seehof, Federal Republic of Germany

Introduction

Gels and microgels are an important class of substances from both scientific and economical viewpoints. A variety of analytical techniques are available for their characterization but only very few researchers have been studying gels/microgels by means of analytical ultracentrifugation in spite of the fact that this technique is a powerful tool for the determination of thermodynamic, elastic and molecular parameters and structural properties of gels. This lack of popularity might be due to experimental difficulties concerning the detection of the polymer concentration in turbid gels, adhesion problems etc. Nevertheless the potential benefit of such experiments has over the years led to several significant investigations in this field. These have resulted in the introduction of a theoretical treatment of the sedimentation of even multicomponent gels and also an improved experimental approach which permits the characterization of a gel/solvent system in a limited concentration range in a single sedimentation equilibrium experiment. Also established is the gradient method which avoids many of the adhesion or detection problems researchers have struggled with before. For microgels that have been prepared and crosslinked in emulsions, an interesting rapid sedimentation velocity technique is now 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 of applications which may be possible in the future.

Since the introduction of a new generation of analytical ultracentrifuges namely the Optima XL-A by Beckman Instruments (Palo Alto, USA), a renaissance of analytical ultracentrifugation can be observed especially in the field of Biophysics/Biochemistry. No longer are time consuming photographic data aquisition techniques necessary anymore. Hence, it can be expected that analytical ultracentrifugation will play an important role in the characterization of biopolymer systems again. A feature of many biomolecules is self-association which can often lead to large aggregates or even gel/microgel formation. Especially if the gel/microgel quantity is small it is hard, if not impossible, to characterize their physical and structural properties by means of

Biotechnology and Genetic Engineering Reviews - Vol. 16, April 1999
0264–8725/99/15/87–140 $20.00 + $0.00 © Intercept Ltd, P.O. Box 716, Andover, Hampshire SP10 1YG, UK

common techniques. Analytical ultracentrifugation however needs only small sample amounts and can yield a large variety of information. This review will describe the capabilities of analytical ultracentrifuge technologies for the characterization of such systems.

The first experiments with gels in an ultracentrifugal field were reported in the early years of the technique by McBain and Stuewer (1936) and by the pioneers Svedberg and Pedersen (1940). For the following two decades however only two studies were published using the ultracentrifuge as a tool for the quantitative detection of microgels. In the 60's and 70's Johnson and coworkers carried out basic investigations on the behaviour of gels in the centrifugal field (Johnson and Metcalfe, 1963, 1967; Johnson, 1964, 1968, 1971, 1972; Johnson and King, 1968). After a further ten years of relative stagnation, researchers became interested once more in using the analytical ultracentrifuge as a tool for the characterization of gel properties. In these years the characterization of gels in the analytical ultracentrifuge has been advanced with regards to both theoretical and experimental aspects. Nowadays the ultracentrifuge can be more effective than any other method known for the characterization of gels. As many as seventy samples can be characterized simultaneously in terms of thermodynamic, elastic, molecular and structural parameters. Another great 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 is the case up to now, especially in the light of modern data aquisition and computer techniques. This review will cover the technical advances and applications that have been achieved so far and will look forward to what can be achieved in the future. More detailed aspects can be found in another article recently published by the author, to which the interested reader is referred (Cölfen, 1995). The present review will cover the following: a general part describing the basic principles of the technology; sedimentation velocity; sedimentation equilibrium; density gradient methodology; the gradient method, and as part of the conclusions will provide an estimate of the potential of this technology for the future.

General behaviour of a gel in an ultracentrifugal field

THEORETICAL CONSIDERATIONS

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 *Figure 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. This concentration gradient is considered in the so-called gradient method and the determination of the sedimentation coefficient via the movement of the centre of mass.

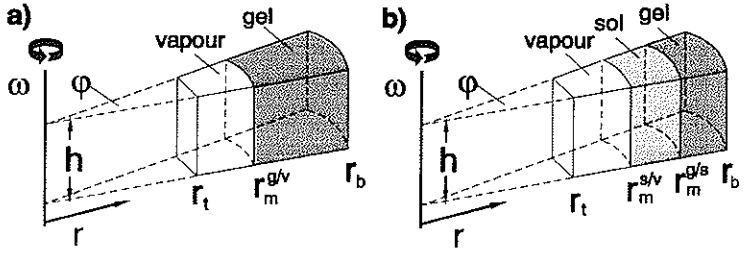


Figure 1. Gel in an ultracentrifuge cell. a) At the beginning of the experiment or at low speeds where no sedimentation of the gel phase occurs. b) At high speeds where the gel phase has sedimented establishing a sol phase. ω is the angular velocity, h the height of the ultracentrifuge cell, φ the sector 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/vapour, *s/v* = boundary solvent/vapour and *g/s* = gel/solvent. Redrawn from Cölfen (1993) and printed with kind permission of Dr Köster Verlag, 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/vapour. These processes are illustrated in *Figure 2*.

At the beginning of the experiment (with the angular rotor velocity $\omega = 0$), the polymer concentration in the gel is constant. Increase in the rotor speed ω_1 after a time t leads to a decrease in the polymer concentration, c_2 at the meniscus between gel and vapour (see *Figure 2*). At a critical angular velocity ω_2 the polymer concentration drops 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

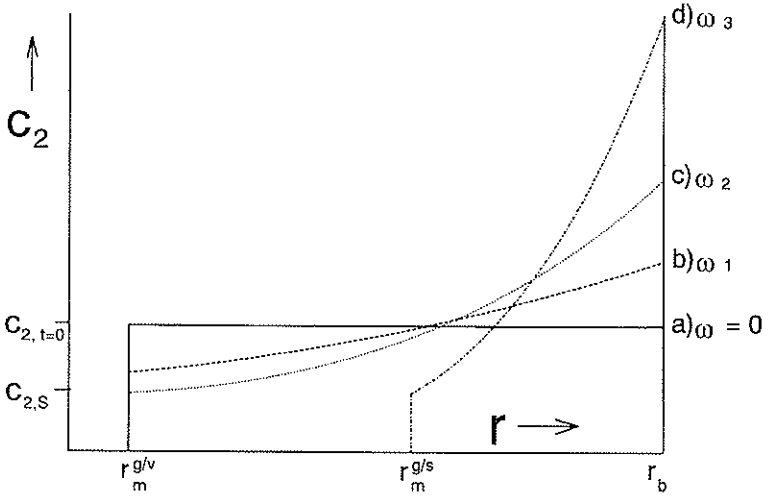


Figure 2. Radial dependence of the local polymer concentration in the gel c_2 at different angular velocities ω_i . . $c_{2,s}$ = concentration of the maximum swollen gel. Redrawn from Cölfen (1993) and printed with kind permission of Dr Köster Verlag, Berlin, FRG.

(ω_3). This corresponds to case b) of *Figure 1*. The sol phase might consist of pure solvent as well as of a solution of non-gelling material. The gel phase sediments until an equilibrium is reached. From the equilibrium states of both, case a) and case b) in *Figure 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 centre of mass has to be considered rather than that of the meniscus gel/sol (Steensgard *et al.*, 1992; Borchard and Hinsken, 1997; Hinsken, 1998).

PRACTICAL PROBLEMS

Adhesion

Adhesion of the gel to the cell walls of the ultracentrifuge cell is a very significant problem when bulk gels are to be investigated. This becomes evident for the example of the system gelatin/water – the prototype of a gelling system – and also a good glue. Without any precautions, the gels stick to the centrepiece walls and windows so that elastical forces upon movement of the gel phase will distort the experimental results. One elegant way of avoiding this would be to prepare such systems in the form of a microgel and then look at their properties but this provides great practical problems of synthesis in many cases. Ways of minimising interactions between the centrepiece and window material and the gel have however been investigated.

The first person to look at the possibility of how to minimize adhesion to get reproducible results was Johnson. With Metcalfe (Johnson and Metcalfe, 1963) he reported that the same centrepiece had to be used for all sedimentation velocity experiments with gelatin/water gels to ensure reproducibility. The range of variation in the results using different centrepieces or centrepiece materials for identical samples was as high as 30%. Such errors can be minimized by impregnation of the centrepiece walls and even the windows with a thin film of highly viscous silicon oil resp. the impregnation of the centrepiece walls with a Teflon spray (Holtus, 1990; Cölfen, 1993). Other approaches consider even the construction of centrepieces and windows made from a material which shows minimized adhesion. One example are polycarbonate centrepieces and polymethylmethacrylate windows for experiments with gelatin/water (Cölfen, 1993). However, such materials reduce the maximum applicable speed significantly (40000 rpm for polycarbonate, 20000 rpm for polymethylmethacrylate acid, PMMA).

Optical detection

The choice of the optical system for a successful detection of concentration gradients inside a gel phase is very restricted as polymer concentrations are usually far above 1% (10 mg/ml) by weight and can reach concentrations of 40% (400 mg/ml) and beyond.

Whereas much effort has been spent on the development of very sensitive optical detection systems (e.g. fluorescence optics) for solution analysis down to the picomolar range of biopolymer quantities (important for example when very strong heterologeous interactions are the subject of interest), nothing has been directed at improving optical detection at very high concentrations. The only straightforward solution to the problem is the reduction of the optical pathlength from 12 mm to 6 or even 3 mm. However, shorter pathlengths are expected to cause extensive adhesion problems as in this case, the surface to volume ratio of the gel in the ultracentrifuge cell has already significantly been increased. However, even in such cases the optical signal is much higher than what some optical systems can cope with. This means that for example the Rayleigh interference optical system has no chance of providing optical records of polymer concentration inside a gel phase due to the far too high intensity differences between the interfering light beams. The ultraviolet/visible (UV/VIS) absorption optical detection system is unfortunately also not applicable due to optical saturation by the significant turbidity/light scattering: genuine UV/VIS absorption by proteins at these concentrations is in any case usually far above the linearity limit of the Lambert-Beer law and thus, the optical signal can more or less only be used at best to follow the sedimentation of the gel phase: this is also the case with Rayleigh optics. The same limitations can be expected if the gradient method is applied since even the initial gel concentrations are far above the detection limit.

Turbidity detection (which has proved to be very useful for the examination of dispersions) also unfortunately cannot be applied for gel bulk phases as in this case, the turbidity cannot be corrected for the influence of the particle size (MIE scattering). Furthermore, it is a detection system which detects time dependent changes rather than radial concentration distributions which are essential for the investigation of gels.

Hence, the only optical system which can be applied with success is the Schlieren optics: for a comparison of the optical traces for the Rayleigh interference-, Schlieren- and UV/VIS-optics, see *Figure 10* and (Cölfen, 1995). Even this system however does not detect the whole concentration gradient in every case. This holds especially for sedimentation velocity experiments, where more or less the only detectable time dependent trace is the position of the gel/sol meniscus. Therefore, assumptions about the concentration gradient or other independent local concentration gradient measurements (microtome cuts of the gel at sedimentation equilibrium with subsequent concentration determinations, etc.) have to be made. A big step forward towards a quantitative optical detection of concentration gradients has however been the low speed equilibrium gradient method (*Figure 1a*). Furthermore, sophisticated modifications like the ultrasensitive Schlieren optics (Cölfen and Borchard, 1994c) could be reversed by simply exchanging some lenses to give a very insensitive modification suitable for this very special application. Unfortunately, nobody has tried this out so far as the only commercial analytical ultracentrifuge, the Beckman XL-I does not even have the capability for a Schlieren detection, although there is the prospect of an on-line facility in the future (see, Clewlow *et al.*, 1997). However, even from the simple detection of the equilibrium position of the meniscus gel/sol (detectable by all common ultracentrifuge optical systems), a lot of information is still available.

Sedimentation velocity techniques

BULK GELS

Historically, the first investigations of gel systems used sedimentation velocity technology. However, throughout the years, it turned out that although sedimentation velocity proved to be the method of choice for microgels, *sedimentation equilibrium* proved much more effective to characterize bulk gels, and we will consider this later. The first pioneering investigations of gels in an ultracentrifugal field were reported by McBain and Stuewer (1936) on agar gels. 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*. By means of this simple device it could be shown that low concentration agar gels in the range of 0.31–1.6% by wt. and short maturation times reached swelling pressure equilibrium. When the movement of the gel/sol meniscus ($r_m^{g/s}$) was plotted against the time of sedimentation for different concentrated agar gels, the sedimentation rate of the gel was found to be constant in the beginning of the experiment but then decreasing to zero with time. A simple formula for the calculation of the swelling pressure was provided (McBain and Stuewer, 1936) which differs from that derived from thermodynamic considerations. The concentration dependence of the swelling pressure 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 behaviour of solutions. The gel concentration was assumed to be constant at different radial positions in equilibrium. This proved to be not true, as was shown in many of the later works. Theoretically it was stated that the sedimentation velocity of a gel cannot be constant. It was suggested (McBain and Stuewer, 1936) that the sedimentation rate 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.

Svedberg was the other early worker who investigated the behaviour of gels in an ultracentrifugal field (see Svedberg and Pedersen, 1940). He also found that a gel shows a different type of behaviour from solutions in the ultracentrifuge. The latter show a constant sedimentation rate, independent of the column height whereas this is not the case for gels. This provided the verification of the considerations of (McBain and Stuewer, 1936). Furthermore, Svedberg had already stated that two cases have to be distinguished in 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 *Figure 1*. Svedberg also derived an equation to represent the so-called 'hydrostatic partial pressure' of a gel which is the swelling pressure at equilibrium (Svedberg and Pedersen, 1940):

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

with Π_S = swelling pressure, r = distance from the axis of rotation with the indices m for meniscus and g/s for the gel/solvent boundary, c_2 = polymer concentration (usually expressed as partial density of the polymer in the gel in g/ml), ω = angular velocity, $\tilde{v}_2$ = 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 presented later in this review (Borchard, 1991) for binary and highly swollen gels. 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. As McBain and Stuewer before, Svedberg found for agar gels that the swelling pressure of the gel is roughly proportional to the polymer concentration in the gel.

The first basic study to be published on the behaviour of a macroscopic gel in the ultracentrifugal field came from P. Johnson in 1964 (Johnson, 1964). 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 minutes for the agar gels. The concentration of the gels was determined to a low degree of accuracy by compressing the gel at 60,000 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 was able to demonstrate some important properties of biopolymer gels (Johnson, 1964): he was able to show that a gel shows typical velocity characteristics in a limited range. He found a sharp gel-solution interface which was sedimenting in the direction of the applied field. Again, the sedimentation behaviour of a gel was found to be fundamentally different from that of a solution (see also Svedberg and Pedersen, 1940). In a sedimenting solution boundary the concentration is continuously decreased due to the radial dilution caused by 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. 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.

Johnson then tried to relate the initial slope of the $\log_{10} r_m^{gls}$ plot to the square of the rotational speed (Johnson, 1964) to calculate a sedimentation coefficient of the gel phase but found only a little range where this could be accomplished. In contrast to a solution, the plot of $\log_{10} r_m^{gls}$ against time was not linear for a gel but showing 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 *Figure 3*). This equilibrium value was dependent on the rotational speed and the initial gel concentration.

Furthermore an 'induction' period at the beginning of an experiment was described where no sedimentation of the gel phase occured 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 $\log_{10} r_m^{gls}$ plots vs. time (linear portion between rise and decrease of the slope, (see *Figure 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.

Nowadays, it is known that the movement of the *centre of mass* has to be considered (Borchard and Hinsken, 1997). Nevertheless Johnson used this plot to define the

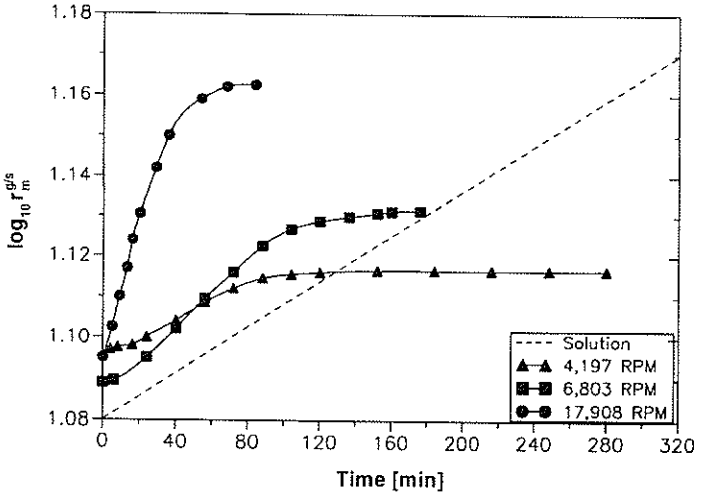


Figure 3. Plots of $\log_{10} r_m^{g/s}$ against time from gel sedimentation diagrams for 0.5% difco-agar gel at various speeds. The dashed line represents a schematic diagram for a solution. Modified figure from Johnson (1964) printed with kind permission of the author and the Royal Society, London, U. K.

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. Upon variation of the experimental conditions which are known to effect the gel structure like the ionic strength, pH of the buffer and the temperature on the gel sedimentation, it was found that 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 which is against all expectations. It was found to be impossible to relate the 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 (Johnson, 1964) 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. An argument 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. Nevertheless, it is known that such gels also sediment (Borchard, 1975a; Cölfen and Harding, 1994). 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. Nevertheless, up to now none of these possibilities could be clearly verified experimentally.

Johnson (1964) also 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 for the case of the gelatin as deduced from the extensive spreading of the Schlieren peak. By 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 by allowing a gel to mature but still very significant soluble portions remained after long times.

Considering equilibrium aspects of the gel sedimentation, Johnson (1964) also stated that the derived increase of the $\log_{10} r_m^{g/s}$ with the rotational speed agreed qualitatively with the equation (1) of Svedberg (Svedberg and Pedersen, 1940). Considering the induction period which could be observed if the initial gel concentration was not very different from the average equilibrium concentration, Johnson also concluded from changes in the Schlieren traces of the gel phase during this period, that internal structural changes of the gel occur before any movement of the gel meniscus commences. As is now known today, this process is simply the locally dependent deswelling of the gel.

A further study from Johnson's laboratory treated the sedimentation behaviour of several dilute gelatin gels under sedimentation velocity conditions (Johnson and Metcalfe, 1963). As in the previous study (see Johnson, 1964) the separated soluble parts which occured in significant amounts behaved as real solution giving a sedimentation coefficient which equals that of gelatin in solution. It was stated that the occurence of solution components cannot be caused by the high pressures in the centrifuge cell as ΔV for the sol gel transition is negative (Flory and Garrett, 1958). 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 (Johnson and Metcalfe, 1963) which can vary widely in different gels (see *Figure 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 (Hinsken and Borchard, 1995).

Sedimentation coefficients for the gel have been calculated on the basis of the maximum movement of $r_m^{g/s}$ (Johnson and Metcalfe, 1963). These values are subsequently referred to as the *effective sedimentation coefficients* of the gel in the text that follows 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 be circumvented if the movement of the centre of mass is used to calculate the sedimentation coefficient rather than that of the gel meniscus (Hinsken and Borchard, 1995). 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. An attempt was made 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 behaviour 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 three 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. If the same experiment was performed at a temperature of 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 behaviour 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, representing somehow 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 rate 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 (Johnson and Metcalfe, 1963) 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 behaviour 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. Nevertheless, it was pointed out (Johnson and Metcalfe, 1963) that the soluble parts do not directly contribute to the gel rigidity but 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 (1965). This study contains far more results than could be included in the previous publication (Johnson and Metcalfe, 1963). Metcalfe compared the sedimentation behaviour 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.

It could thus 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. Reflecting upon the reproducibility of the experiments, considerable time dependent effects after gelling through further crosslinking of the gelatin gel were found (Metcalfe, 1965). In contrast to the previous study (Johnson and Metcalfe, 1963), it was found that adhesion of the weak gels to the ultracentrifuge centrepieces had no effect on the sedimentation coefficient of the gel whether the material of the centrepiece 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. Nevertheless, the more rigid gels were loosened generally 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 (Johnson, 1964), whereas the induction period was inversely proportional to the field. The sedimentation coefficient of the gel was found to be proportional to the length of the gel column, independent of the sector angle of the centrepiece (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 from the presence of a water layer on top of the gel column had already demonstrated that the total hydrostatic pressure has a negligible influence on the sedimentation behaviour of

the gel, as was also shown theoretically later (Borchard, 1991). However, the observed dependence of the sedimentation coefficient of the gel on the column length indicates an error in the definition of gel sedimentation as the sedimentation of a gel has to be treated as a movement of its *centre of mass* rather than a movement of the meniscus gel/sol (Borchard and Hinsken, 1997). Therefore, all results based on the effective sedimentation coefficient from the movement of the meniscus gel/sol have to be treated with care, especially as the reported gel sedimentation coefficients are generally of the same order of magnitude of soluble polymers and not a factor of 10 lower, which has been found recently (Borchard and Hinsken, 1997).

As had been reported in many previous studies dealing with ultracentrifuge experiments with gels, Metcalfe (1965) had problems in detecting 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 (labelled) 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 (Johnson, 1964). It is also likely that adhesion of the gel lead to the observed rupture of the gel.

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 has not been established as expected, initially due to the view of rupture and reformation of the network upon sedimentation.

When the effect of the initial gel concentration on the gel sedimentation coefficient was investigated in the range of 1.5–5% by 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 (Johnson, 1964), a similar range of linear relationship was found for agar, restricted extensively by the equilbrium approach of the agar gels. Nevertheless, 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 centre of mass is taken to calculate the sedimentation coefficient of the gel (Borchard and Hinsken, 1997), no induction period is observed anymore which shows that the definition of the gel sedimentation coefficient of the gel *via* the movement of the centre of mass is more correct.

In addition to the already published (Johnson and Metcalfe, 1963) 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 2% gel investigated at 18°C (Metcalfe, 1965). 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 10,000 g/mol in contrast to the tenfold 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, has been reported by King (1967).

Further experiments were carried out to compare the sedimentation behaviour 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 weight. Under these conditions the amount of solution component from fractionated gelatins (the α and 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.

Another study on the sedimentation behaviour of gelatin gels was published in 1967 by the Johnson group (Johnson and Metcalfe, 1967). This publication dealt mainly with the results in Metcalfe's PhD Dissertation (Metcalfe, 1965) 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 ill-defined nature of the sedimentation coefficient via the movement of $r_m^{g/s}$, the parameter n can only be a qualitative and empirical quantity.

Considering the application of Equation (1) in this review to the 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 an explanation it can be said that under these conditions swelling is a slow process compared to gel sedimentation.

This work was followed by a further sedimentation velocity study on gelatin gels attempting to describe the sedimentation behaviour of gels observed in the previous studies more quantitatively (Johnson and King, 1968). 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 (Metcalfe, 1965), a gel was set up from completely dyed gelatin. When the optical density 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 (Equation (1)) which would lead to a concentration gradient inside the gel phase (Svedberg and Pedersen, 1940) as well as with numerous experimental findings obtained later. 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. The constant polymer concentration in the gel phase must be an artifact of the optical densitometer readings of the Schlieren negatives or an effect of a too high concentration of the dyestuff far above the linearity range of Lambert-Beer's law.

When the concentration dependence of the gel sedimentation coefficient was investigated at temperatures of 4, 10 and 18°C, it was found that 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 (Johnson and Metcalfe, 1967):

$$s = \mathrm{k}\,\frac{r_b - r_m^{g/s}}{c^n} \tag{2}$$

Plots of $\log s + \log(1 + r_m^{gls}/r_b)$ versus $\log(1 - (r_m^{gls}/r_b)^2)$ yielded the empirical parameter n (Johnson and Metcalfe, 1967). 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 *Figure 4*).

It was stated from this behaviour 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 causing 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 disruption agent which breaks hydrogen 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

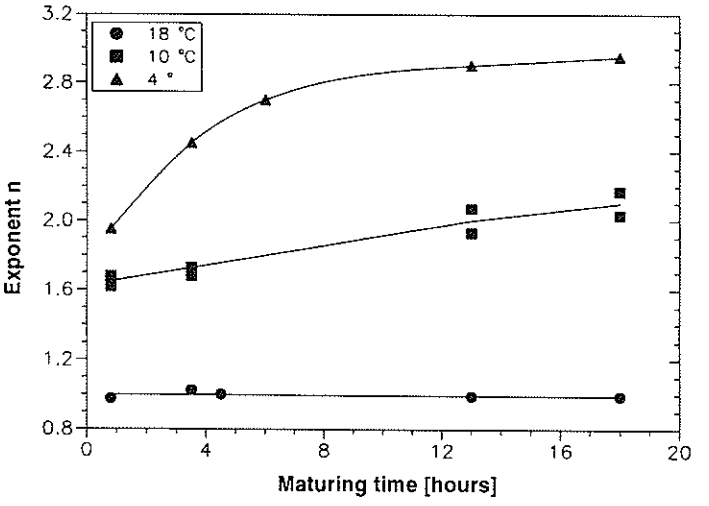


Figure 4. Effect of temperature and maturation time on the value of n for aqueous lime processed ossein gelatin gels of 2% by weight. Figure redrawn from Johnson and King (1968) with kind permission of the author and the Royal Photographic Society of Great Britain, Devon, UK.

a weakly crosslinked network (see *Figure 4*). From the sedimentation behaviour 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 interpreting solution sedimentation behaviour as well.

In a more comprehensive article, Johnson (1968) summarized the basic findings so far derived on the sedimentation behaviour of gels. 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 limitited reswelling was observed. This gel showed similar sedimentation behaviour 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 although numerous later results show the reversibility. Recent results show that the reported irreversible behaviour 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 (Cölfen and Borchard, 1994; 1995).

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 suprisingly 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. Chemical crosslinking of the gelatin with glutardialdehyde decreased n further from an already low level, although the number of crosslinks was obviously increased. The same behaviour 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.

In two closely related latter papers, Johnson (1971, 1972) considered the polymer concentration distribution inside the gel phase during sedimentation and at sedimentation equilibrium more closely. 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 behaviour observed for this swollen gel was identical to that in the initial experiment. This swelling behaviour is a very important difference to the behaviour of gelatin gels with significant amounts of soluble component as stated above and explained later (Johnson, 1968; Cölfen and Borchard, 1995).

The techniques for sedimentation velocity 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 (Johnson and Rainsford, 1972). 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 Equation 2) 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 weight of 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.

A similar sedimentation velocity method to those presented above for the rapid characterization of gels was described by a Ukrainian group (Babskij and S'edin, 1977). 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 Johnson, 1964; Johnson and Metcalfe, 1963; 1967) 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 in the early papers. (The notes of caution with the induction period discussed above for Johnson's papers must be applied here as well). The constant sedimentation rate for 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 situation reported for the sedimentation of agar gels (Johnson, 1971; 1972), 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 (Babskij and S'edin, 1977) 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 centrepieces was considered to be necessary for future applications without pointing out requirements these centrepieces have to meet.

After these studies, sedimentation velocity experiments ceased to be performed (or at least appeared to be) for the characterization of gels for more than 20 years as since the early 70's, the power of sedimentation equilibrium experiments with gels was recognized as a more powerful alternative procedure.

The sedimentation velocity of a gel was subject of a recent paper by Borchard and Hinsken (1997). In contrast to the treatment so far that the movement of the meniscus gel/sol must be used for the calculation of the sedimentation coefficient, they could show that the movement of the center of mass has to be considered. In contrast to a solution, where a relative motion of the polymer to the solvent occurs, for a gel, a relative movement of all components to each other takes place. Thus for an n-component system, $n-1$ independent fluxes have to be considered resulting in a relevant flux density under sedimentation velocity conditions. From irreversible thermodynamics, an alternative definition of the sedimentation coefficient s was derived for a binary system and early stages of sedimentation ($t \rightarrow 0$):

$$s = \frac{\alpha}{\rho_2}(1-\tilde{v}_2\rho) \qquad \text{with } \alpha = \frac{\rho_2 v_2}{(1-\tilde{v}_2\rho)\omega^2 r}$$

$$\text{equivalent to } s = \frac{dr_s/dt}{\omega^2 r} = \frac{\ln(r_s/r_{s,0})}{\omega^2 t} \tag{3}$$

where α is a phenomenological coefficient, v_2 = the relative velocity of the polymer and r_s is the radial position of the center of mass defined by:

$$r_s = \frac{\int_{r_m}^{r_b} \rho_2(r) r^2 dr}{\int_{r_m}^{r_b} \rho_2(r) r\, dr} \frac{\sin\varphi}{\text{arc}\varphi}, \text{ resp. } r_{s,0} \text{ at } t = 0 \text{ accessible by extrapolation.}$$

With such a treatment, induction periods are no longer observed at the beginning of the experiment because the changes of the polymer concentration gradients have already been taken into account, even if sedimentation of the meniscus gel/sol has still not occurred. This is an important improvement as it enables the determination of sedimentation coefficients of gels even at low rotational speeds, where no sedimentation of the meniscus occurs and thus adhesion and detection problems are minimized.

For longer experimental times, the equilibrium is approached, thus no relative flux density occurs anymore, meaning that the flux by sedimentation has to be balanced by that of diffusion in analogy to the solution case:

$$\underbrace{\alpha(1-\tilde{v}_2\rho)\omega^2 r}_{\text{Sedimentation}} = \underbrace{\alpha\left(\frac{\partial\tilde{\mu}_2}{\partial\rho_2}\right)_{T,P}\left(\frac{\partial\rho_2}{\partial r}\right)}_{\text{Diffusion}} \tag{4}$$

Thus, by measurement of the polymer concentration gradient inside the gel phase $\rho_2(r)$ at sedimentation equilibrium, the diffusion coefficient of the gel D can be obtained by Equation (5), if s and α are already known from the early experimental stages via Equation (3)

$$D = \alpha\left(\frac{\partial\tilde{\mu}_2}{\partial\rho_2}\right)_{T,P} = \frac{s\omega_i^2 r_s \rho_2}{(d\rho_2 / dr)} \tag{5}$$

where $\tilde{\mu}_2$ = specific chemical potential of the polymer.

For a 4.5% by wt. κ-carrageenan/water gel at 10°C, s was found to be 0.10–0.14 S and D to be 3.4–3.8 10^{-10} cm^2/s. Thus, s is found lower by a factor of 10, than that for the soluble polymer whereas D is even 3 orders of magnitude lower. Due to these extremely low transport quantities of a gel, the equilibration times are very long, as has already been reported before.

The definition of s via the movement of the center of mass may for the first time provide a quantitative analysis of quite rapidly obtainable sedimentation velocity data in terms of gel structures (following the early attempts by Johnson and coworkers who used however an inappropriately defined s). However, the method is restricted to completely transparent and thus not too concentrated gels. Furthermore, the Schlieren gradients are rather broad which might cause inaccuracies in the concentration determination. This however could well be circumvented by the use of on-line Schlieren optics (Clewlow *et. al.*, 1997) and should not be regarded as a major problem of the method.

MICROGELS

The analytical ultracentrifuge had, by 1958, already been used for the determination of the amount of microgel formation resulting from emulsion polymerisations (Shaskoua and van Holde, 1958). The microgels studied consisted of styrene crosslinked with divinylbenzene, methyl acrylate crosslinked with divinylbenzene and acrylonitrile crosslinked with methylene-bisacrylamide. Afterwards, styrene and acrylonitrile had been 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 could be observed 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. Afterwards 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 (Shaskoua and van Holde, 1958) yielding 26% of linear polymer. This therefore established the credentials of the ultracentrifuge as a quantitative method for analyzing linear polymer/microgel mixtures.

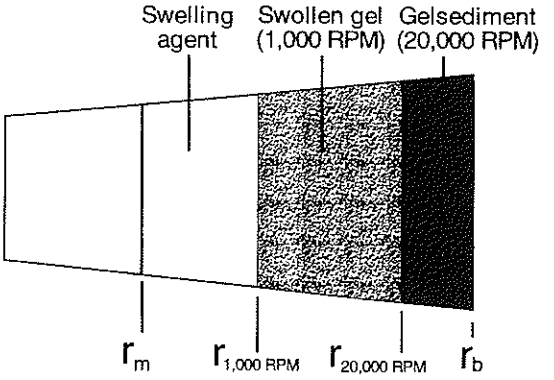


Figure 5. Sector shaped ultracentrifuge cell (single sector) for determination of the swelling of small amounts of polymer gel (schematic). r is the distance from the rotor axis with the indices m = meniscus of the swelling agent and b = cell bottom. The indices 1,000 rpm and 20,000 rpm refer to the gel boundary at rotor speeds of 1,000 or 20,000 rpm. Redrawn from Lange (1986) with kind permission of Steinkopff Verlag, Darmstadt, FRG.

In another study this method has been applied to characterize the crosslinking efficiency of an emulsion polymerization Shaskoua and Beaman (1958). The components for the polymerization of a microgel described in Shaskoua and van Holde (1958) 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 (Shaskoua and van Holde, 1958).

Lange (1986) 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 one or two hours to compress the gel with a smaller specific volume than the swelling agent at the cell bottom (see *Figure 5*).

Afterwards he reduced the speed as much as possible (1,000 rpm) to allow the gel to swell to its maximum degree of swelling. The centrifugal field at 1,000 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 one hour. 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 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. Nevertheless, a note of caution needs to be expressed if microgels are suspected to contain soluble polymer (Mächtle *et al.*, 1995). This soluble fraction must be extracted prior to the swelling experiment.

The assumption of no substantial swelling agent occlusions may be questioned at least for rather monodisperse spherical particles which cannot be packed without solvent occlusions. Nevertheless, it can be expected that in such a case the determined degree of swelling after speed reduction is also constant (and independent of the former applied speed). Therefore, such a criterion used in this study cannot be taken to prove no solvent occlusions in every case.

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 (1943) 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.

The degree of swelling of only partially crosslinked microgels can be determined with a procedure introduced by Müller (see Müller *et al.*, 1991). 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 (2,000 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 (Lange, 1986) due to the following equation:

$$Q = \frac{f\, d_{T,K}^2}{s_{swollen}} \frac{\rho_{T,K} - \rho_0}{18\ \eta} \tag{6}$$

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 Equation (6) 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 Equation (6) 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 (1943) theory.

The method was tested with styrene-butadiene latices from a batch process in cyclohexane as solvent. The results derived are presented in *Figure 6*.

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

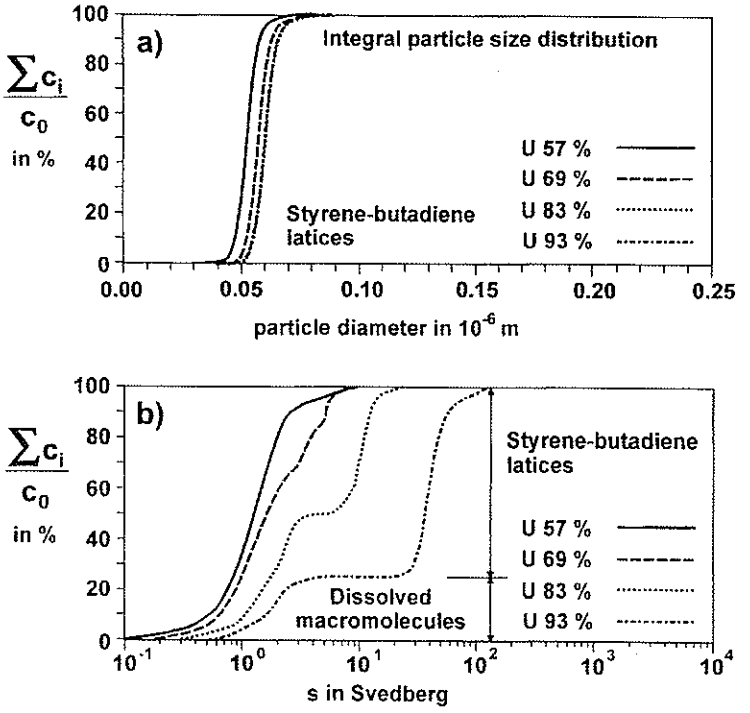


Figure 6. Particle size distribution and *s*-distribution of latices of the same polymerization process with increasing conversion *U*. s_{50} for the latices after 83% conversion is 10.5 Svedbergs whereas it is 40 Svedbergs after 93% conversion. The corresponding *Q*-values (*Q* is the degree of swelling derived using eq. (6) and the sedimentation coefficients at 5 g/l (Müller *et al.*, 1991) are 3,000 for $U = 83\%$ and 200 for $U = 93\%$. $\Sigma c_i/c_0$ reflects the sedimentation coefficient distribution. Redrawn from Müller *et al.* (1991) with kind permission of Steinkopff Verlag, Darmstadt.

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 it 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 the 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.

A study on the characterization of microgel properties itself using analytical ultracentrifugation was published by Mächtle and coworkers (Mächtle *et al.*, 1995). 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 (Shaskoua and van Holde, 1958; Shaskoua and Beaman, 1958; Buchdahl *et al.*, 1963; Mächtle, 1992). The successful application of a simple step by step crosslinking theory of primary linear macromolecules as well as the agreement with results from light scattering makes this study an interesting alternative to the characterization of bulk 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 (Mächtle *et al.*, 1995). However, this requires the feature that microgels of the same network structure as the bulk gels of interest can be prepared.

In the experimentally well grounded paper (Mächtle *et al.*, 1995), 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 degrees of crosslinker up to 10%. Strictly, this cannot be 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 been more advantageously and precisely determined by sedimentation equilibrium experiments. Nevertheless, the molecular weights determined with the Svedberg equation from sedimentation velocity data were consistent 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 as in 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 (Buchdahl *et al.*, 1963), from the observation of no more than two bands in the density gradient it seems to be the case that linear molecules can co-exist with the microgel without a significant amount in 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 *Figure 7*).

The sedimentation behaviour 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. An attempt was made to calculate the critical amount of crosslinker by applying a theory of Flory for the stepwise crosslinking of a macroscopic gel phase 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 7 is a good example for the transition between a pure solution and a microgel

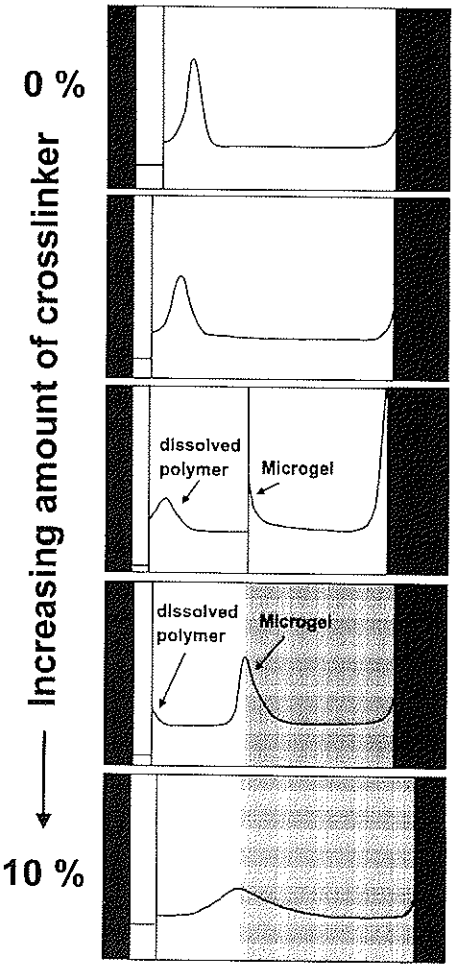


Figure 7. Schematical Schlieren-photos of sedimentation runs in an analytical ultracentrifuge for five microgel dispersions with different concentrations of the crosslinker. The photos show slow Schlieren-peaks of macromolecules and fast Schlieren peaks of microgel particles. Redrawn from Mächtle *et al.* (1995) with kind permission of Steinkopff Verlag, Darmstadt, FRG.

for showing 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 *Figure 7* with *Figure 10*).

In *Figure 7* it can be seen that at low amounts of crosslinking agent, pure solution behaviour 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 by 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 rate 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: hence increasing *s*-values are observed.

If the bottom region of the cell is observed for microgel samples, 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 well known from experiments with bulk gel phases (Svedberg and Pedersen, 1940; Johnson, 1964; Metcalfe, 1965; Johnson and King, 1968) but much less pronounced. This observation supports the view that the dark zone near the cell bottom is caused by light deviated out of the optical system. This problem should be possible to solve by use of a Schlieren optical system with additional focussing lenses. However, as the investigation of the sedimentation behaviour 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 analytical ultracentrifuge (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 results do not show the continuous increase of the molar mass expected theorectically 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 it can be expected because in the analytical ultracentrifuge (AUC), the fractionated components are considered and not their mixture. Thus, the continuous increase of the molar mass observed with light scattering is caused by the increased amount of microgel with increasing crosslinker density and thus an increase of the detected average molar mass.

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 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. This value and its consensus with the results of other authors show that 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 deduced 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.

Sedimentation equilibrium techniques

BULK GELS

The basis for the application of sedimentation equilibrium techniques for the characterization of gels had already been supplied by the observations of the sedimentation velocity characteristics by Johnson's group at Cambridge (see above). The most important finding in that respect was the demonstration that true equilibria are obtained (Johnson, 1971; 1972). 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 independently if this value was achieved by deswelling (approaching equilibrium from lower speeds) or swelling of the gel (approaching equilibrium from higher speeds).

Discussions on the polymer concentration distribution inside the sedimenting gel and the comparison with a solution had already been published before (Johnson, 1964; Metcalfe, 1965; Johnson and King, 1968). As Svedberg and Metcalfe, Johnson stated that the gel concentration cannot be observed throughout the whole gel phase with the Schlieren optics of the ultracentrifuge. 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 (Cölfen and Borchard, 1994d).

Combination of Svedbergs Equation (1) (Svedberg and Pedersen, 1940) with that of Freundlich and Posnjak $\Pi_s = \Pi_0 \cdot c_2^k$ 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 agreement with one for gelatin of $2.7 \cdot 10^5$ dynes/cm^2 given by Freundlich. Nevertheless, it is known that the relation of Freundlich and Posnjak is only empirical and not all workers found that this equation describes the concentration behaviour of the swelling pressure (see for example Svedberg and Pedersen, 1940). This is also valid for some later studies which although not cited here can be found in Cölfen (1995).

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 (Borchard, 1975a). Polystyrene-cyclohexane gels with different crosslinking densities and removed soluble components have been investigated in two specially constructed types of ultracentrifuge cells. Here a sintered metal plate was placed onto the gel so that this plate caused the pressure. The equilibrium state was reached from lower as well as from higher rotational speeds. 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 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 investigated gels.

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 (1976). 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 behaviour 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 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 (1976) 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 (Borchard and Cölfen, 1992).

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 (Cölfen and Borchard, 1995).

The first improvements in the experimental set-up for sedimentation equilibrium experiments with gels were published by Holtus and Borchard using a Beckman Model E ultracentrifuge (Holtus and Borchard, 1989; Holtus, 1990). These improvements concern the modification of the Schlieren optical system to an off-line data capture system with a modulable laser light source to enable the application of 6-hole rotors due to the extreme experimental durations of about *three weeks*. Further improvements were made with the temperature measurement and control system and the vacuum system.

An equation based on irreversible thermodynamics formally similar to the equation of Svedberg (Svedberg and Pedersen, 1940) (Equation (1) above) was provided to facilitate the calculation of the swelling pressure of the isotropic binary gel as a function of the radial displacement from the centre of the rotor. This equation contained the locally dependent partial density of the polymer $\rho_2(r)$ as concentration variable. As this quantity was not directly accessible if the polymer concentration gradient could not be detected in the whole gel phase (see also Svedberg and Pedersen, 1940; Johnson, 1964; Metcalfe, 1965; Johnson and King, 1968), an approximation had to be used relating $\rho_2(r)$ to the locally dependent density of the gel assuming volume additivity of mixing and applying a mass balance assuming a linear polymer concentration gradient in the gel phase. This procedure required the determination of the dependence of the gel density on the polymer concentration – a very tedious and time consuming measurement (Cölfen and Borchard, 1994d). Holtus suspected turbidity of the gel causing the optical detection problems. 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 it has to be expected for a continuous turbidity increase with the polymer concentration. Overall, the ultracentrifuge had been applied as a pressure generator here, generating radially increasing pressures 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 of this procedure.

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 (1971; 1972). The induction period reported by Johnson for sedimentation velocity experiments (Johnson, 1964) was

found in this study as well during the approach to equilibrium when the rotor speed selected has been too low.

The determined dependence of the swelling pressure on the polymer concentration (subsequently referred to as '*swelling pressure curve*' in the text that follows) suffered from an error. It had been assumed that all gels were swollen initially 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) has 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 (Cölfen, 1993), the general order of magnitude of the swelling pressure curves as well as their relation to each other is not changed basically.

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 *Figure 9*). 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 (three days and seven 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 (Johnson and King, 1968). In this study n was increasing with the maturing time, indicating an increased crosslinking upon maturing in the time interval up to 20 hours. This increase was more pronounced at 4° and vanishes at 18°C.

As an alternative explanation of the gelation 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 behaviour was given by Holtus (1990). Furthermore, an effect of the gelation temperature on the swelling pressure curves was observed, as could be expected due to the changes in the network crosslinking density. These results showed again that swelling pressure curves may be used as a sensitive measure of structural changes in the gel network. The reproducibility of the swelling pressure curves was found to be good.

An attempt was made to calculate the concentration independent Flory-Huggins interaction parameter χ and the crosslinking density by fitting of the swelling pressure curves to a modified Flory-Huggins equation of the type of Equation (9). This gave physically meaningless negative crosslinking densities and showed that at least the model with a concentration independent interaction parameter could not be applied.

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 (Borchard, 1991). After pointing out the general behaviour of a sedimenting gel and the nature of the swelling pressure equilibrium, irreversible thermodynamics has been applied to derive the 'generalized

Svedberg-Pedersen equation' for the swelling pressure of a binary gel:

$$\Pi_S = \omega^2 \int_{r_m^{g/s}}^{r} \frac{\rho_2}{\rho_1 \tilde{v}_1} \left(1 - \tilde{v}_2 \rho\right) r \, dr \tag{7}$$

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 gel 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 had been used already in earlier works (Holtus and Borchard, 1989; Holtus, 1990). As the theoretical considerations are given in more detail in this work, they were not mentioned before. A new but very important assumption in this study is the assumption of the swelling equilibrium at $r_m^{g/s}$. 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 (7) is not only formally similar to the Equation (1) above of Svedberg: it can actually be shown that Equation (1) is a special case of Equation (7) for highly swollen gels which is expressed in the term 'generalized Svedberg-Pedersen equation'. The application of Equation (7) 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 partial density of the polymer in the gel which is not experimentally accessible in these cases. Therefore Equation (7) has been rearranged 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 $\overline{V}_{01}$ which is fulfilled for not too concentrated gels. The resulting equation now only contained parameters which could be derived experimentally:

$$\Pi_S = \omega^2 \int_{r_m^{g/s}}^{r} \left(\rho - \frac{1}{\overline{V}_{01}}\right) r \, dr \tag{8}$$

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 of Johnson (1964).

The second part of the 'trilogy' deals with the determination of molecular parameters from the swelling pressure curves (Holtus *et al.*, 1991). 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. Substituting the difference in the chemical potentials of the solvent $\Delta\mu_1$ by $-\bar{v}_1 \Pi_S$ with $\bar{v}_1$= 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:

$$-\frac{\Pi_S \bar{v}_1}{RT} = \underbrace{\ln(1-w_2) + w_2 + \chi_{w,0} w_2^2 + \chi_{w,1} w_2^3}_{\text{Mixing Term}} + \underbrace{C_w w_2^{1/3}}_{\text{Network Term}} \qquad (9)$$

with w_2 = polymer concentration in the mass fraction scale (index 0 refers to the initial concentration), χ_w = linearly concentration dependent interaction parameter $\chi_w = \chi_{w,0} + \chi_{w,1} w_2$ 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 discussion, simply to avoid repetitions, although a strict consideration would, of course, require this. Furthermore, it must be stressed that Equation (9) is only strictly valid for nonionic gels. For polyelectrolyte gels, the mixing and network contributions must be extended by a third term which accounts for charge contributions. Nevertheless, Equation (9) was found to describe the behaviour of gelatin/water and κ-carrageenan/water well, even if no additional salt is added.

It is not just the interaction parameters of the polymer-solvent system that can be derived. For example, the network constant C_w allows us 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 and unique results.

The generalized Svedberg-Pedersen equation for a binary system (Equation (8)) as well as the modified Flory-Huggins equation (Equation (9)) have been applied to ultracentrifuge experiments with a photographic gelatin in the concentration range of 2–10% by wt. The sample has been 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 (Cölfen and Borchard, 1991), the maximum degree of swelling of the physically crosslinked networks had to be determined separately which is very tedious, time consuming, and in the case of physically crosslinked gels, sometimes even incorrect due to a dissolution of the gel.

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 *Figure 8*.

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

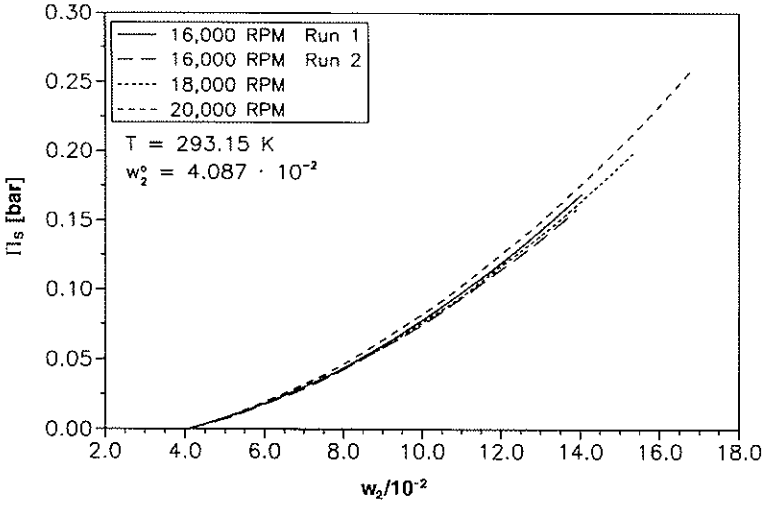


Figure 8. Swelling pressure Π_S of the system gelatin/water vs. concentration of gelatin for different rotational speeds. w_2 is the weight fraction of the polymer. Reproduced from Holtus *et al.* (1991) with kind permission of Steinkopff Verlag, Darmstadt, FRG.

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 demixes at $\chi_w > 0.5$ if the influence of the network term in Equation (9) can be neglected. Furthermore, the χ_w-parameter of 0.497 was in very good agreement with results of other workers derived for gelatin solutions with osmosis and light scattering in the range of 0.491–0.499. 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, furthermore, 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. Furthermore, the investigated system is clearly no nonelectrolyte system, as assumed in the modified Flory-Huggins equation (Equation (9)) 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 rather well 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 (Cölfen and Borchard, 1994). 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 ten 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 (Holtus, 1990).

The third part of the paper 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 (Cölfen and Borchard, 1991). 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 gel/sol meniscus ($r_{in}^{g/s}$) is given which enables calculation of 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 as has been proved before (Johnson, 1971;1972; Holtus, 1990). Furthermore, 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 gel, it was shown that the swelling pressure curves no longer superimpose for different rotational speeds (see *Figure 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 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 (Cölfen and Borchard, 1994). The altered solvent activity caused by the superimposition of the gradient of the soluble parts was given as explanation for the observed additional deswelling of the gel at increased rotational speeds.

It was observed that the intersection of the swelling pressure curves described before (Holtus, 1990; Holtus *et al.*, 1991) could be altered by changing the rotational speed. In conclusion, it was pointed out that extreme care is necessary when 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 was confirmed in a later study (Cölfen and Borchard, 1994).

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 (Johnson, 1971; Holtus, 1990), 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 superimposition of the swelling pressure equilibrium of the gel with a sedimentation-diffusion equilibrium of the soluble parts. There is further evidence for an irreversible process such as the change of the crosslinking density of gelatin/water gels upon increase of the rotational speed (see also Johnson, 1968). 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 parts of a carrageenan sample which are generally not able to gel.

In a publication of Borchard and Cölfen (1992), the findings of the previous 'trilogy of papers' have been presented in a comprehensive way with the addition of some interesting new results which will be presented here:

It could for example be shown that a linearly concentration dependent χ_w-parameter in Equation (9) 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 previously observed highly branched structure of gelatin gels (Holtus *et al.*, 1991). A model for 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 *Figure 9*), 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. Nevertheless, 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. 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 ten 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 transforms into the glassy state, this characteristic point is the *glass transition*.

A comprehensive description of the modifications of the experimental ultracentrifuge set-up for gels (Cölfen and Borchard, 1994a,b) as well as experimental results (Holtus *et al.*, 1991) has been given by Cölfen (1993). The theory of the sedimentation of a gel derived by Borchard for binary (Borchard, 1991) and ternary gels (Borchard, 1994) was extended to an 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:

$$\left[\Delta\tilde{\mu}_i\left(c_1, c_2, c_3 \ldots . c_{N-1}\right)\right]_{T,P} = \omega^2 \int_{r=r_m^{g/s}}^{r} \left(1 - \tilde{v}_i\, \rho\right) r\, dr \quad ; i = 1,2,3,\ldots,N \tag{10}$$

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 Equation (10). But even if it would be defined for N-component systems, a better treatment of a multicomponent gel would not be possible because up to now it is not yet 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 (Cölfen and Borchard, 1995). 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 Equation (10) is of a theoretical nature in the general form. Nevertheless, it is the most general description of the sedimentation equilibrium of a gel available up to now, containing all cases treated before.

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 (1990). 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 has been reported before (see for example Holtus and Borchard, 1989), the swelling pressure curves for gelatin/water gels intersected beyond certain limits of the initial gel concentration (*Figure 9*).

Some variations of the two terms of the concentration dependent χ_w-parameter and the network constant C_w in Equation (9) 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 and not an inhomogeneity of the gel.

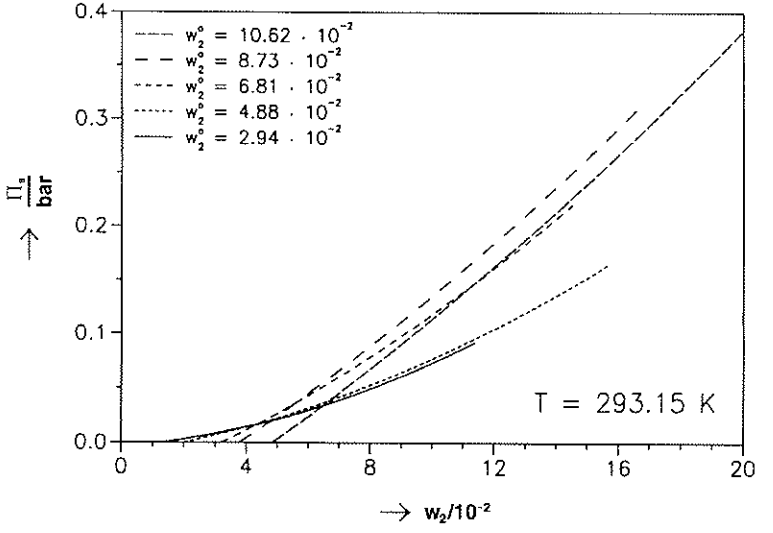


Figure 9. Swelling pressure curves for the system gelatin/water. Π_s = swelling pressure and w_2 = polymer concentration in the mass fraction scale with the index 0 for the initial gel concentration. Figure reproduced from Cölfen (1993) with kind permission of Dr Köster Verlag, Berlin, FRG.

As gelatin gels containing soluble parts cause intense experimental difficulties (Cölfen and Borchard, 1991) for the derivation of correct swelling pressure curves, a first attempt was reported to investigate the behaviour of stained soluble parts inside the gel phase during a sedimentation equilibrium experiment. Soluble parts have been extracted from gelatin/water gels as described in (Cölfen and Borchard, 1994) and stained with fluorescein-isothiocyanate. 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. Nevertheless, 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 in fact the 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. the presence of soluble parts with molecular weights above 4,000 g/mol which are normally only weakly attached to the gel network and hence readily 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 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 upheld 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 hard to understand why there is no sign of back diffusion of the soluble parts, even after a period of months from reducing the ultracentrifugal field. 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.

An attempt was made to use the set of thermodynamic parameters derived from the swelling-pressure equilibrium curves to predict stability limits for gels (Cölfen, 1993). This was done by extrapolating the regression functions for $\chi_{w,0}$, $\chi_{w,1}$ and C_w (derived from Equation (9)) 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 also made to find out the nature of a dark zone near the cell bottom in the Schlieren pattern of gels, especially gelatin/water. A correlation was found between the mixing and the network term in Equation (9) 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 (Flory and Rehner, 1943). 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. Nevertheless, it was reported that during the sedimentation of an 18.25% by weight gelatin gel at 10°C, a dark zone in the Schlieren pattern suddenly formed within the gel phase. At this zone, a discontinuity of the polymer concentration gradient inside the gel phase was observed. This behaviour was explained by a demixing of the gel into two gels with the same crosslinking density but a different distribution of the network chains (Cölfen, 1993). This could lead to light refraction which are beyond the limits of registration by the optical system. 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 (Cölfen, 1993). Furthermore, it was demonstrated that the application of the circular sample channels in the newly designed 10-hole centrepieces yields the same results as those derived in sector shaped cells. This justified the application of the 10-hole centrepieces. Summarizing the modified ultracentrifugal technique (Cölfen and Borchard, 1994a,b) 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 acquisition 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 Equation (9), the prediction of these regions is principally 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. It was stated that the greatest disadvantage of the technique was the necessity for supplementary measurements, such as the determination of the maximum degree of swelling or the concentration dependence of the gel densities. 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 succesfully suppressed. Also, gels with a vanishing buoyancy term (e. g. $(1-\tilde{v}_2\rho)=0$) 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.

Borchard extended the theory of the sedimentation of a binary gel (Borchard, 1991) to the ternary system (Borchard, 1994). The theory treats a gel with one inert soluble component (Borchard, 1994). Basically the same approach as for the binary system (Borchard, 1991) 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 to represent 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 high 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. Experimentally, it becomes quite 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 be fully quantified experimentally.

The heterogeneous swelling equilibrium has been described with a ternary state diagram (Borchard, 1994) 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.

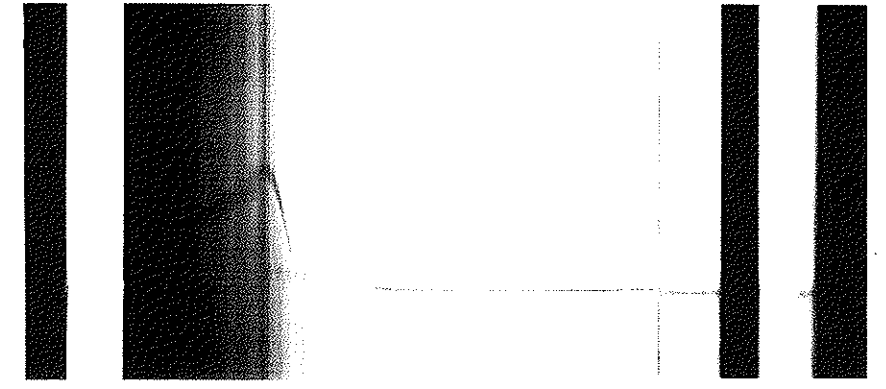
It was pointed out that the gel phase needs to move towards the cell bottom due to mass conservation. Furthermore, the addition of a soluble component to a binary gel has been discussed in terms of mass conservation. It was stressed that the total amount of soluble component in the gel can be determined by dialyzing the gel. But this implies different assumptions: The gel is insoluble under the conditions studied 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 fullfilled for physically crosslinked gelatin/water gels (Cölfen and Borchard, 1994; 1995).

The amount of the soluble component in the solution phase was suggested to be obtainable via the application of the interference optics. Here, it must be taken into account that meniscus depletion methods for the determination of the fringe shift at the meniscus solution/vapour 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 (Cölfen and Borchard, 1994). 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 (Cölfen and Borchard, 1995). It becomes quite obvious that 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.

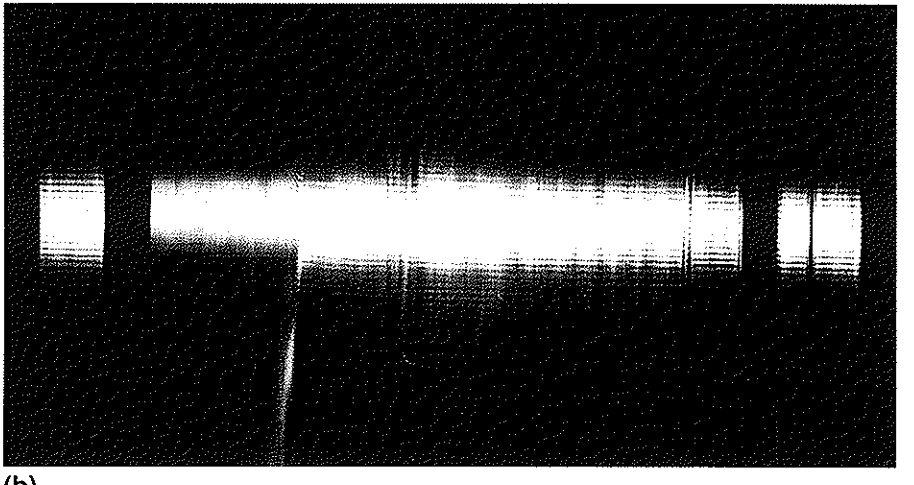
In 1994, two papers were published which were dedicated to the development of a more effective experimental set-up for the hitherto very time consuming sedimentation equilibrium experiments with gels (Cölfen and Borchard, 1994a,b). In the first part, the basic improved instrumentation applied to the Beckman Model E was described. Modifications involved a modulation system with a pulsed laser light source for Schlieren optics which has already partly been described by Holtus (Holtus and Borchard, 1989; Holtus, 1990). By means of a modified sophisticated Schlieren optical system (Cölfen and Borchard, 1994b) 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 centrepieces. 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.

The second paper dealt with several technical improvements for an efficiency increase of the sedimentation equilibrium experiments (Cölfen and Borchard, 1994b).

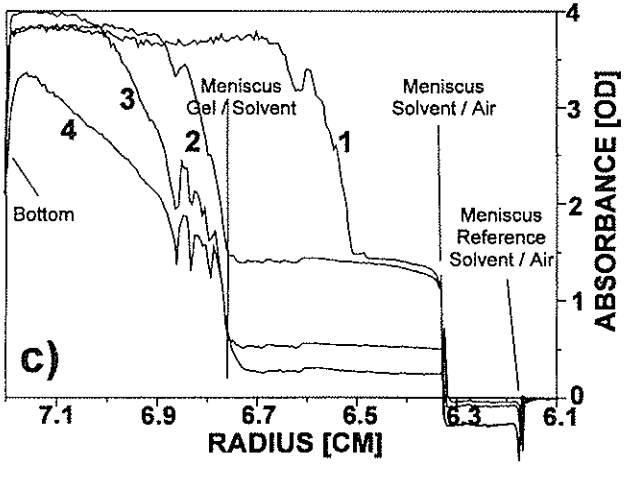
Figure 10. A sedimenting κ-carrageenan/water gel (2% by wt. at 20°C, 20,000 rpm after at least 1 day) observed with different optical detection systems. (a) Schlieren optics (the original Schlieren optics of the Beckman Model E with the mercury lamp has been used. The light intensity in the gel phase can be much increased if a laser is applied as light source), (b) Rayleigh interference optics with a He-Ne Laser as light source and water as reference solvent, (c) Beckman Optima XL-A ultra-violet 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. The time between the scans for the different wavelengths was 18 min (99 averages). Water was used as reference solvent. KEL-F (Beckman, Palo Alto, USA) centrepieces were used to avoid adhesion of the gel. All pictures do not correspond to the state of sedimentation equilibrium. Reproduced from Cölfen and Borchard (1994b) with kind permission of Academic Press, Inc., Orlando, Florida, USA.



(a)



(b)


Meniscus
Gel / Solvent
Meniscus
Solvent / Air
Meniscus
Reference
Solvent / Air
Bottom
1
2
3
4
c)
7.1
6.9
6.7
6.5
6.3
6.1
RADIUS [CM]
ABSORBANCE [OD]
0
1
2
3
4

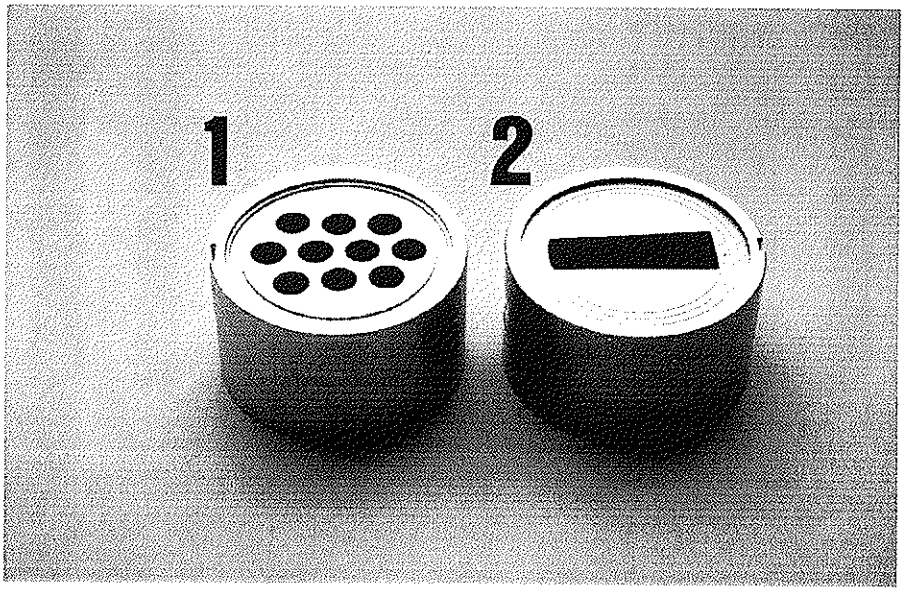


Figure 11. 10-hole short column multichannel centrepiece (1) and a conventional 4° monosector centrepiece (2).

It was pointed out that the Schlieren optical system is the detection system of choice if concentrated and turbid gels need to be investigated (*Figure 10a*). Also, it was shown that no fringes can be detected in the gel phase if the Rayleigh interference optics were used due to the intensity differences of the interfering light beams (*Figure 10b*). The high turbidity of the gels also restricted the detection with the UV-absorption optics due to the significant light scattering phenomena (*Figure 10c*).

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 multichannel centrepieces (resolution 0.3° on the spinning rotor). An important increase in the efficiency of the ultracentrifugal experiments was achieved by the introduction of 10-hole short column multichannel centrepieces with circular sample compartments (*Figure 11*). Together with the application of the 8-hole rotor, a simultaneous investigation of up to 70 samples in one equilibrium experiment was possible for the first time. If the new 10-channel centrepieces with their circular sample chambers are used, the mass balance for sector shaped sample chambers (Cölfen and Borchard, 1991) has to be modified. 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 centrepieces as well.

The centrepieces were specially designed for the Schlieren optics and allow the sedimentation equilibrium of the gel to be reached in two days instead of one week as before for the 1 cm gel columns. The restricted choice of materials for the centrepieces for experiments with gelatin/water gels was discussed. Polycarbonate was found to be

the best material for the centrepieces for this particular system whereas Polymethylmethacrylate (PMMA) was used for the manufacturing of the cell windows. PMMA was tested to be applicable up to 20,000 rpm, whereas the polycarbonate centrepieces could be used up to 40,000 rpm. It was found that even with polycarbonate centrepieces and PMMA windows, the adhesion of gelatin at the cell walls of the centrepieces 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. The benefit of this correction could be demonstrated for gelatin/water gels.

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 (Hinsken, 1995). 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, an equilibrium could be verified by reaching it from different rotational speeds (Johnson, 1971; 1972; Holtus, 1990; Cölfen and Borchard, 1991). Nevertheless, this proof of attainment of equilibrium 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) with the swelling pressure equilibrium of the gel was given as an explanation for the failure of the proof of 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 a 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 Cölfen and Borchard (1994) 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 a 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 (Cölfen and Borchard, 1995) but with the difference that the junction points are treated as crystals for κ-carrageenan

here. The crystalline regions should be detectable by X-ray techniques. Other possibilities provided 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 explanations all differ from each other and no experimental evidence has as yet been provided to validate any of these over the others. In addition, the amount of the soluble proportion (0.3–0.6 mg/ml) was stated to be of negligible magnitude as the results for the gels were found in excellent agreement with the scaling theory of de Gennes (1975). This is somewhat contradictory as, 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. What seems to be clear is that an irreversible process takes place, as observed in other studies (Johnson, 1968; Cölfen and Borchard, 1991).

Certainly, further experiments are needed to clear 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 behaviour (e.g. if the original equilibrium state can be reached, even after months), the suspected self association of soluble parts (although some evidence for a reversible self-association has been given) and their behaviour during sedimentation of the gel which is accessible in principle via stained soluble parts.

However, for speeds below a critical value, reversible swelling pressure equilibria could be proved from superposition of swelling pressure – concentration curves for κ-carrageenan/water. The theoretical dependence of the concentration of the maximum swollen gel on the initial gel concentration was treated. It was 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 involved in the network constant C_w in Equation (9) than that used before and gives another argument for the semi-empirical character of the modified Flory-Huggins equation (Equation (9)) at least for the system κ-carrageenan/water and all other systems where the polymer chains at least partly helicate prior to gelation.

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 (Cölfen, 1993).

Hinsken observed so called 'double peaks' in the Schlieren patterns of the sol phase as reported before (Cölfen and Borchard, 1991) 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 in terms of the boundary shape. The question was raised why a self association of the soluble parts could be observed although they were not incorporated into the network, and 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 *Figure 9* 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 Equation (9) the crosslinking and branching degree was found to increase with the polymer concentration. Nevertheless, 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 helped to understand a similar behaviour for the system gelatin/water (Cölfen, 1993).

A quadratic concentration dependence was supposed for the network constant C_w in the small concentration range investigated: this finally led to a good agreement with the predictions of de Gennes following the C* theorem. Extrapolation to a vanishing C_w yielded a value of 0.99% by weight which was identical with the critical concentration of gel formation.

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 (Borchard and Cölfen, 1992).

The determined static shear moduli for κ-carrageenan in the double logarithmic log G* vs. log M plot showed a linear concentration dependence in analogy to the results summarized in (Borchard and Cölfen, 1992). 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 (1975). Therefore, 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 for gelling systems. This statement should be treated with extreme caution as no consideration has been included as to why all other results for gelatin/water gels (except the particular one presented) – and independent of whether static or dynamic shear moduli for gelatin/water – clearly disagreed with an exponent of 2.25 (Borchard and Cölfen, 1992). 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 furthermore 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. The curves published by other authors for higher concentrated gelatin/water gels as summarized in the paper by Borchard and Cölfen (1992) should have been taken into account as well. From this, the slope of 2.25 seems to be the exception, independent of the method of measurement used.

Furthermore, it could be established from the previous studies that the shear modulus as determined by 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 obtianed have been always higher

{indeed, for the system gelatin/water at low polymer concentrations even by factor of ten (Borchard and Cölfen, 1992) due to the viscous part of the dynamic shear modulus}. In a creep experiment, it could be stated that the shear modulus decreased by a factor of two after 24 hours experimental duration (Cölfen and Borchard, 1992). 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 (Cölfen, 1993).

Nevertheless, from the good agreement of the experimental results with the C*-theorem of de Gennes (1975) within the concentration range investigated, it was 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 (the amount of soluble parts was determined to be <0.056% by wt.) (Hinsken and Borchard, 1995) which certainly justifies this conclusion.

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 (Cölfen and Borchard, 1995). An alkaline treated gelatin of M_n = 68,000 g/mol containing soluble parts with a M_w of 2,900 g/mol was investigated. It could be shown from this study 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 it is required as proof of equilibrium (Holtus *et al.*, 1991). 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 semi-empirical Flory-Huggins theory for homogeneous networks could be applied. Nevertheless, 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 nine weeks the gel did not swell up to its original degree of swelling at a certain rotational speed, once the speed has 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 (Cölfen, 1993) cannot be upheld 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 Equation (9) 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.

Nevertheless, it could be shown that it is still 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 has been 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 (Cölfen and Borchard, 1995). 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.

As an irreversible process upon increase of the rotational speed is observed only if the gel contains soluble parts (compare for example with the observations of Holtus (1990)), the irreversibility must therefore be related to the presence of this low molecular weight component. Because of this, the results from the investigation of the concentration distribution of fluorescein-isothiocyanate stained soluble parts in gelatin/water gels at different rotational speeds (Cölfen, 1993) have been reconsidered (*Figure 12*).

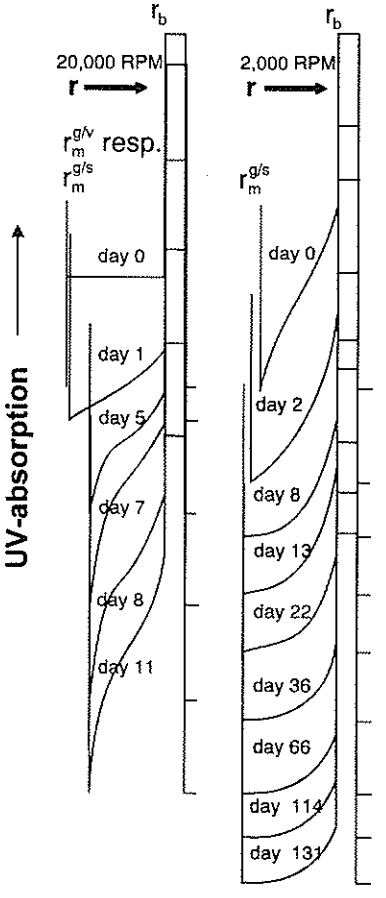


Figure 12. Ultra-violet absorption as a function of radial displacement from the centre of rotation r for a sedimentation equilibrium experiment with an 8.5% by weight gelatin/water gel 20°C. The gel contains 0.26% by weight (related to the solution) FITC-stained soluble parts (M_w = 2,900 g/mol). The 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 *Figure 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 (1993) with kind permission of Dr Köster Verlag, Berlin, FRG.

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 seven days and this agrees with previous experiments (Holtus *et al.*, 1991). From the changes in the gradient of the soluble parts (M_w = 2,900 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 furthermore similar to the gelatin in their chemical composition (Cölfen and Borchard, 1994). 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 into the gelatin network can be provided from the observation that the concentration gradient of the soluble parts still becomes steeper after the swelling pressure equilibrium of the gel has been attained. As the association is favoured 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 2,000 rpm (the minimum speed 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 (Cölfen and Borchard, 1995). The swelling pressure equilibrium of the gel is roughly reached after eight 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 = 2,900 g/mol at 2,000 rpm even when self-association occurs as the association constant was found to be small (Cölfen and Borchard, 1994).

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 neighbouring polymer network chains and hence leading to the formation of a *gradient gel* (Cölfen and Borchard, 1995). If such an altered gel swells again at a lower rotational speed, this gel should not swell up uniformly in an excess of solvent after such an ultracentrifuge experiment. This feature could be verified when a sector shaped gel piece swelled up to a nearly rectangular shaped one (Cölfen and Borchard, 1995). 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 it was predicted. The observed increase of the crosslinking density leading to a gradient gel must therefore be closely related to those soluble parts present in the gelatin gels (Cölfen and Borchard, 1995).

It was pointed out that the additional crosslinking does not allow the investigation of the original gel structure anymore (Cölfen and Borchard, 1995). 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 (this is the *'Gradient method'*). Under such conditions, the crosslinking of associated/aggregated soluble parts is unlikely due to the much smaller concentration gradients inside the gel, as we consider below.

Density gradient techniques

MICROGELS

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 (Buchdahl *et al.*, 1963). 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.

Mächtle looked at the behaviour 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 (Mächtle, 1992) by analogy with the studies of Shaskoua and van Holde (1958) and Shaskoua and Beaman (1958). About 20% of the SAN was found not to be covalently bound to PBA. 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.

The gradient method

BULK GELS

In a recent paper, Hinsken *et al.* (1995) presented a new approach to evaluate sedimentation equilibrium experiments with gels. 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 of the occurrence of a dark zone near the cell bottom (Svedberg and Pedersen, 1940; Johnson, 1964; Metcalfe, 1965; Johnson and King, 1968; Holtus, 1990; Cölfen, 1993), low speeds are used so that no sedimentation of the macroscopic gel phase occurs (see case a) in *Figure 1*). The local slopes of the swelling pressure curves are calculated using a differentiated form of the generalized Svedberg-Pedersen equation (Equations 7 and 8) for a binary system (Borchard, 1991).

As the concentration range between the meniscus gel/vapour 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 little 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 are like. A further large advantage of the gradient method is that irreversible changes in the network which were observed for gelatin/water (Cölfen and Borchard, 1995) 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 (Cölfen and Borchard, 1995). Therefore, it has been suggested that the gradient method can be used as a test for statistical theories which describe the gelation process.

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 whether they have been reached from either higher or lower rotational speeds. The Schlieren patterns presented were evaluated quantitatively by integration of the Schlieren curve. As the gels were investigated in a monosector cell, no baseline is present which is normally required for the proper integration procedure. 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 polymer concentration gradients evaluated 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 superpose which has to be expected for superposing swelling pressure–concentration curves indicating an equilibrium. Instead, curves for an equilibrium at 8,000 and 6,000 rpm were found to be similar. These results indicate present inaccuracies of the gradient method.

As no further experiments, particularly those using doublesector cells (baseline), were performed in this study, it it is impossible to assess what difficulties or limitations arise when the gradient method is applied even with good quality Schlieren patterns with baselines. The promising advantages expected for its application thus remains to be confirmed. The potential source of error, 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 optical system is the only realistic choice for a concentration determination in the gel phase as both the Rayleigh interference and the UV-absorption optics fail (Cölfen and Borchard, 1994b): see also *Figure 10.*

To derive a complete swelling pressure–concentration curve for a gel with a certain network structure, it has been suggested that it could be constructed 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 of physically crosslinked gels is not altered upon drying has not to date been 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 theory.

Conclusions

The results from more than 60 years of ultracentrifugal investigations of gels permit the following conclusions to be drawn:

- Thermodynamic, elastic and structural parameters of bulk gels can be characterized in an effective and elegant manner by sedimentation equilibrium since the ultracentrifugal field causes a continuous radial dependent deswelling of the gel. Usually, these experiments require several weeks, but short column multichannel centrepieces have been suggested, which reduce this time to a few days and allow the possibility of investigating up to 70 samples simultaneously in one experiment if an 8-hole rotor is used.
- Sedimentation velocity experiments can rapidly characterize the same parameters as mentioned above for microgels as well as the sedimentation coefficient of bulk gels which can possibly be related to structural parameters
- Sedimentation velocity experiments allow the quantitative determination of soluble components in bulk and microgels
- Density gradient centrifugation of microgels is a very sensitive method for the investigation of small structural changes or the effects of grafting reactions etc.
- With bulk gels, one may encounter extensive experimental difficulties due to adhesion of the gel and the high turbidity and polymer concentration which restricts optical detection
- It would be of considerable benefit if the on-line Schlieren system (Clewlow *et al.*, 1997) available for older ultracentrifuges could be adapted to the commercially available Beckman Optima XL-I as quickly as possible: the Schlieren optical system is the only optical system for the ultracentrifuge which will allow the application of all of the technology described in this review.

OUTLOOK FOR THE FUTURE

Due to the rapidly increasing technical possibilities, especially in the computer and electronics sector, it is expected that the efficiency of the application of ultracentrifuge technologies to the characterisation of biopolymer gels can be further increased on a large scale with the incorporation of on-line recording techniques. This will certainly also inspire new applications.

Some trends can already be observed. It has become clear for example 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 have not yet been exactly theoretically treated. The general thermodynamics of anisotropic deformation has however been treated by Borchard (1975) but this has not yet been applied to the ultracentrifugation of gels. Further problems arise from the failure of the Schlieren optical system in the region of the cell base and, most important, additional crosslinking of the gel by soluble components. All these problems can probably 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 using the gradient method and investigating the swelling pressure equilibrium in a single gel phase where no sol phase has yet been introduced (Hinsken *et al.*, 1995). 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 properies 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. Here, one simply uses the ultracentrifuge to create a desired concentration gradient and to detect if a suspected instability occurs at the predicted polymer concentration or not.

As many technologically important gels are polyelectrolytes, it is expected that the theoretical treatment of the sedimentation behaviour of polyelectrolyte gels will be brought forward, resulting in a proper characterization of such systems by analytical ultracentrifugation.

The determination of the sedimentation coefficient s via the movement of the centre of mass raises hopes that it will be possible to relate s to the structure of the gel (via a parameter like the empirical parameter n of Johnson's group (see Johnson and Metcalfe, 1967). 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 (Shaskoua and van Holde, 1958; Shaskoua and Beaman, 1958; Lange, 1986; Mächtle *et al.*, 1995). As this characterization method avoids the main disadvantages of the investigation of a bulk gel phase – namely anisotropic deformation and adhesion – the calculation of thermodynamic parameters from the swelling behaviour might have future potential for gel characterization, although the ultracentrifugal investigation of microgels has, up to now, been more or less applied to studies of the efficiency of a grafting reaction (Shaskoua and van Holde, 1958; Shaskoua and Beaman, 1958; Mächtle, 1992). As long as parameters like the Flory-Huggins interaction parameter or the molar mass of the crosslinked chains are desired, a sedimentation velocity experiment with microgels should be the method of choice. For this, a significant potential of applications is evident 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 Hinsken *et al.* (1995) 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 developed for the Schlieren optical system (Clewlow *et al.*, 1997). With such on-line systems, exact kinetic studies of the gel sedimentation are for the first time possible and the accuracy of the determination of s via the movement of the centre of mass should be much better than possible at the moment.

An application of a real time optical system can also be seen in monitoring the kinetics of swelling of extremely low amounts 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 1,000 rpm to allow swelling of the particles.

Another interesting application is the investigation of the diffusion of small protein molecules (which are detectable via the uv-absorption optics) in polysaccharide gels: the gel itself will be invisible for uv-absorption optics so 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 and may yield important information, especially for the food industry if one thinks of flavour 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. One could then think of more complicated situations where the radial concentration of the gel is extensively varied by application of a high centrifugal field so that in one experiment the influence of the gel concentration on the protein diffusion can be monitored.

Another application for ultracentrifuge techniques would be the characterization of hybrid gels with inorganic components, a topic which appears to be gaining in industrial importance in recent years. For example, if an inorganic compound is synthesized in a gel phase, it is of interest how well the inorganic particle sticks to the functionalized polymer. In most cases, the inorganic matter has a higher density than the gel so that sedimentation of the inorganic particles should be expected if they are not (or weakly) complexed by the polymer. If the inorganic particles absorb in the UV/VIS, their concentration can independently be determined by the absorption optics, whereas Schlieren optics would deliver the complete concentration gradient. Also, possible changes in the gel structure by the inorganic component can be characterized using the discussed techniques.

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

- Simultaneous structural, thermodynamic and elastic characterization of a large number of gels (up to 70 at the 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
- Characterization of polyelectrolyte gels
- Kinetic swelling studies as well as kinetic studies of the gel sedimentation applying real time detection systems
- Diffusion studies of proteins in polysaccharide gels
- Characterization of organic/inorganic hybrid gels
- Stability investigations of gel systems *via* the accessible set of thermodynamic parameters and possible experimental verification by sedimentation velocity

Because of the importance of gel technologies in the food and increasingly the

pharmaceutical and health-care industries, the potential of ultracentrifugation methodologies as reliable and complementary gel characterisation technologies to traditional rheological approaches should be taken very seriously.

Acknowledgements

All authors and editors are acknowledged for their kind permission to reproduce the figures used in this review. I would especially like to thank Dr P. Johnson (Cambridge, UK) for making available the PhD theses of his former students to me.

References

BABSKIJ, V. G. AND S'EDIN, A.A. (1977). Investigation of gel forming structures by means of Analytical Ultracentrifugation. *Khim. Biol. Nauki.* (Dopov AN Ukr. Ser. B) **10**, 926–929.

BLOOMFIELD, V.A. (1976). Ultracentrifugal compression of gels. *Biopolymers* **15**, 1243–1249.

BORCHARD, W. (1975). Zur Thermodynamik von elastischen Mischphasen. *Habilitation*, Clausthal, 108.

BORCHARD, W. (1975a). Über das Quellungsverhalten von Polystyrol verschiedener Netzwerkdichte in Cyclohexan. *Progress in Colloid & Polymer Science* **57**, 39–47.

BORCHARD, W. (1991). Swelling pressure equilibrium of swollen crosslinked systems in an external field. I: Theory. *Progress in Colloid & Polymer Science* **86**, 84–91.

BORCHARD, W. AND CÖLFEN, H. (1992). Characterization of thermoreversible gels by means of sedimentation equilibria. *Macromoecular Chemistry, Macromolecular Symposium* **61**, 143–164.

BORCHARD, W. (1994). The sedimentation diffusion equilibrium of a ternary gel. *Progress in Colloid & Polymer Science* **94**, 82–89.

BORCHARD, W. AND HINSKEN, H. (1997). The sedimentation velocity of a gelled polymer. *Progress in Colloid & Polymer Science* **107**, 172–179.

BUCHDAHL, R., ENDE, H. A. AND PEEBLES, L.H. (1963). Detection of structural differences in polymers by density gradient ultracentrifugation II: Detection of Microgel. *Journal of Polymer Science Part C* **No. 1**, 143–152.

CLEWLOW, A.C., ERRINGTON, N. AND ROWE, A.J. (1997). Analysis of data captured by an on-line image capture system from an analytical ultracentrifuge using Schlieren optics. *European Biophysical Journal* **25**, 311–317.

CÖLFEN, H. (1993). Bestimmung thermodynamischer und elastischer Eigenschaften von Gelen mit Hilfe von Sedimentationsgleichgewichten in einer Analytischen Ultrazentrifuge am Beispiel des Systems Gelatine/Wasser. *PhD thesis*, Duisburg; 1. Auflage Verlag Köster, Berlin 1994.

CÖLFEN, H. (1995). Analytical ultracentrifugation of gels. *Colloid & Polymer Science* **273**. 1101–1137.

CÖLFEN, H. AND BORCHARD, W. (1991). Swelling pressure equilibrium of swollen crosslinked systems in an external field. III: Unsolved problems concerning the systems gelatin/water and κ-carrageenan/water. *Progress in Colloid & Polymer Science* **86**, 102–110.

CÖLFEN, H. AND BORCHARD, W. (1994). Soluble parts in gelatin/water gels. *Acta Polymerica* **45**, 325–329.

CÖLFEN, H. AND BORCHARD, W. (1994a). A modified experimental set-up for sedimentation equilibrium experiments with gels. Part 1: The instrumentation. *Progress in Colloid & Polymer Science* **94**, 90–101.

CÖLFEN, H. AND BORCHARD, W. (1994b). A modified experimental set-up for sedimentation equilibrium experiments with gels. Part 2: Technical developments. *Analytical Biochemistry* **219**, 321–334.

CÖLFEN, H. AND BORCHARD, W. (1994c). Ultrasensitive Schlieren optical system. In *Biochemical Diagnostic Instrumentation.* Eds. R.F. Bonner, G.E. Cohn, T.M. Laue, A.V. Priezzhev. Proceedings, SPIE **2136**, 307–314.

CÖLFEN, H. AND BORCHARD, W. (1994d). Determination of the partial specific volumes of thermoreversible gelatin/water and κ-carrageenan/water gels. *Macromolecular Chemistry and Physics* **195**, 1165–1175.

CÖLFEN, H. AND BORCHARD, W. (1995). Influence of soluble parts in gelatin/water gels on their network structure in an ultracentrifugal field. *Macromolecular Chemistry and Physics* **196**, 3469–3485.

CÖLFEN, H. AND HARDING, S.E. (1994). Unpublished observations.

DE GENNES, P.G. (1975). *Scaling concepts in Polymer Physics*. London: Cornell University Press.

FLORY, P.J. AND REHNER, J.R. (1943). Statistical mechanics of cross-linked polymer networks II: Swelling. *Journal of Chemical Physics* **11**, 521–526.

FLORY, P.J. AND GARRETT, R.R. (1958). Phase transitions in collagen and gelatin systems. *Journal of the American Chemical Society* **80**, 4836–4845.

HINSKEN, H. AND BORCHARD, W. (1995). Continuous swelling pressure equilibria of the system κ-carrageenan/water. *Colloid & Polymer Science* **273**, 913–925.

HINSKEN, H., SELIC, E. AND BORCHARD W. (1995). Formation of reversible concentration gradients during the centrifugation of gels. *Progress in Colloid & Polymer Science* **99**, 154–161.

HINSKEN, H. (1998). Eine neue Methode zur Bestimmung Kinetischer und thermodynamischer Großen thermoreversibler Gele mit Hilfe der analytischen Ultrazentrifuge: Die Gradientenmethode am Beispiel des Systems κ-Carrageenan/Wasser. *PhD thesis*, Duisburg.

HOLTUS, G. AND BORCHARD, W. (1989). Swelling pressure equilibrium of physical networks in the field of an analytical ultracentrifuge. *Colloid & Polymer Science* **267**, 1133–1138.

HOLTUS, G. (1990). Untersuchung der Quellungsdruckgleichgewichte von wäßrigen Gelatine-Gelen in einer Analytischen Ultrazentrifuge. *PhD thesis*, Duisburg.

HOLTUS, G., CÖLFEN, H. AND BORCHARD, W. (1991). Swelling pressure equilibrium of swollen crosslinked systems in an external field: II: The determination of molecular parameters of gelatin/water gels from the swelling pressure–concentration curves. *Progress in Colloid & Polymer Science* **86**, 92–101.

JOHNSON, P. AND METCALFE, J.C. (1963). Sedimentation studies of gelatin gels. *Journal of Photographic Science* **11**, 214–224.

JOHNSON, P. (1964). A sedimentation study on gel systems. *Proceedings of the Royal Society of London* **A278**, 527–542.

JOHNSON, P. AND METCALFE, J.C. (1967). Physico-chemical studies on gelatin gels from soluble and insoluble collagens. *European Polymer Journal* **3**, 423–447.

JOHNSON, P. AND KING, R.W. (1968). Sedimentation studies on gelatin gels. *Journal of Photographic Science* **16**, 82–88.

JOHNSON, P. (1968). Physicochemical studies on strongly interacting systems. In *Solution properties of natural polymers*. Special publication No. **23**, pp 243–262. London: Royal Society of Chemistry.

JOHNSON, P. (1971). Velocity and equilibrium aspects of the sedimentation of agar gels. *Journal of Photographic Science* **19**, 49–54.

JOHNSON, P. (1972). Velocity and equilibrium aspects of the sedimentation of agar gels. In *Photographic Gelatin*. Ed. R.J. Cox, pp 13–27. London, New York: Academic Press

JOHNSON, P. AND RAINSFORD, K.D. (1972). The physical properties of mucus: Preliminary observations on the sedimentation behaviour of porcine gastric mucus. *Biochimica et Biophysica Acta* **286**, 72–78.

KING, R.W. (1967). Physical and Chemical Studies on Gelatin Gels and Sols. *PhD thesis*, Cambridge.

LANGE, H. (1986). Determination of the degree of swelling and crosslinking of extremely small polymer gel quantities by Analytical Ultracentrifugation. *Colloid & Polymer Science* **264**, 488–493.

MÄCHTLE, W. (1992). Analysis of Polymer Dispersions with an Eight-Cell-AUC-Multiplexer: High Resolution Particle Size Distribution and Density Gradient Techniques. In *Analytical Ultracentrifugation in Biochemistry and Polymer Science*. Eds. S.E. Harding, A.J. Rowe and J.C. Horton, pp 147–175. Cambridge: Royal Society of Chemistry.

MÄCHTLE, W., LEY, G. AND STREIB, J. (1995). Studies of microgel formation in aqueous and organic solvents by light scattering and analytical ultracentrifuge. *Progress in Colloid & Polymer Science* **99**, 144–153.

MCBAIN, J.W. AND STUEWER, R.F. (1936). Anwendungen des einfachen luftgetriebenen Zentrifugenkreisels auf kolloidchemische Probleme. *Kolloid-Zeitschrift* **74** Heft 1; 10–16.

METCALFE, J.C. (1965). A physico-chemical study of gelatin gels. *PhD thesis*, Cambridge.

MÜLLER, H.G., SCHMIDT, A. AND KRANZ, D. (1991). Determination of the degree of swelling and crosslinking of latex particles by Analytical Ultracentrifugation. *Progress in Colloid & Polymer Science* **86**, 70–75.

SHASKOUA, V.E. AND VAN HOLDE, K.E. (1958). Graft Copolymers: Synthesis and Characterization. *Journal of Polymer Science* **28**, 395–411.

SHASKOUA, V.E. AND BEAMAN, R.G. (1958). Microgel: An Idealized Polymer Molecule. *Journal of Polymer Science* **33**, 101–117.

STEENSGAARD, J., HUMPHRIES, S. AND SPRAGG, P. (1992). Measurement of sedimentation coefficients. In *Preparative Centrifugation, A practical Approach*. Ed. D. Rickwood, p 193. Oxford: Oxford University Press.

SVEDBERG, T. AND PEDERSEN, K.O. (1940). *The Ultracentrifuge*. pp 29–33. Oxford: Oxford University Press, or Svedberg, T. and Pedersen, K. O. (1940). *Die Ultrazentrifuge*. pp 26–29. Dresden: Steinkopff Verlag.