KAPPA-CARRAGEENAN

A STUDY ON ITS PHYSICO-CHEMICAL PROPERTIES, SOL-GEL TRANSITION AND INTERACTION WITH MILK PROTEINS

Dit proefschrift met stellingen van

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De Rector Magnificus van de Landbouwhogeschool,
J. P. H. VAN DER WANT

Wageningen, 9 september 1976

THOMAS HUBERTUS MARTINUS SNOEREN

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWWETENSCHAPPEN
OP GEZAG VAN DE RECTOR MAGNIFICUS,
DR. IR. J. P. H. VAN DER WANT, HOOGLERAAR IN DE
VIROLOGIE,
IN HET OPENBAAR TE VERDEDIGEN OP
WOENSDAG 10 NOVEMBER 1976, DES NAMIDDAGS TE VIER UUR,
IN DE AULA VAN DE LANDBOUWHOGESCHOOL
TE WAGENINGEN

H. VEENMAN & ZONEN B.V. - WAGENINGEN - 1976

Aan mijn ouders
Voor Toos en Maikel

Dit proefschrift verschijnt ook als Verslag van het Nederlands Instituut voor Zuivelonderzoek (NIZO) te Ede.

STELLINGEN

1

De waarde van de Flory-Fox constante, Φ, is voor polyelectrolyten aanzienlijk lager dan voor ongeladen polymeren. Het voor polyelectrolyten aannemen van een voor ongeladen polymeren gebruikelijke waarde ($\Phi = 2.5 \times 10^{21}$) kan daarom aanleiding geven tot een aanzienlijke onderschatting van moleculaire afmetingen.

CLELAND, R. L. (1968) Biopolymers 6, 1519. Hoofdstuk II van dit proefschrift.

2

Het verdient aanbeveling, gezien hun aminozuur samenstelling, de nomenclatuur voor de α_s-caseines te herzien.

3

De veronderstelling van Lawrence et al., dat in melk caseine niet de effectieve aminozuurbron zou zijn voor melkzuurstreptococcus vindt onvoldoende steun in de door hem aangehaalde gegevens.

LAWRENCE, R. C., THOMAS, T. D. and TERZAGHI, B. E. (1976) J. DAIRY Res. 43, 141.

4

Hoewel de toevoeging van struktuurvormende en onverteerbare polysacchariden aan levensmiddelen het gehalte aan dietary fibre verhoogt, zou het hypocriet zijn om een dergelijke toevoeging zonder meer aan te merken als een verrijking van ons voedsel.

5

Bij de karakterisering van aromastoffen uit komkommers heeft Forss et al. geen rekening gehouden met het optreden van nevenreakties tijdens de isolatie en identifikatie van onverzadigde carbonylverbindingen.

FORSS, D. A., DUNSTONE, E. A., RAMSHAW, E. H. and STARK, W. (1962) J. Food Sci. 27, 90.

6

In theoretische beschouwingen over complex coacervatie moet aan het model van Veis de voorkeur boven dat van Voorn gegeven worden.

VEIS, A. in „Biological Polyelectrolytes" M. DEKKER Inc. N. York (1970).
VOORN, M. (1959) Forschr. Hochpolym. Forsch. 1, 192.

7

Men dient er rekening mee te houden dat de vorming van 8 amino 1,7 nafthyridine, in de reaktie van 1,7 nafthyridine met kaliumamide in vloeibare ammoniak via een S_N (ANRORS) mechanisme zou kunnen verlopen.

PAUDTLER, W. W. and KRESS, TH. J. (1968) J. Org. Chem. 33, 1384. SIMIG GY and VAN DER PLAS, H. C. (1975). Recl. Trav. Chim. Pays-Bas 94, 125.

8

Er is in onvoldoende mate onderzocht of ethanolamine, dat veelvuldig gebruikt wordt als nakoppelingsreagens bij met bisoxiraan geaktiveerde sepharose, zich inert gedraagt t.o.v. eiwitten.

9

Het vanwege een te hoog aantal E. coli bacteriën afkeuren van oppervlaktewater als zwemwater is niet geheel juist.

10

Het zou weinig zinvol zijn om, in door melkzuurbacteriën gefermenteerde levensmiddelen, grenzen te stellen aan aantallen staphylococcus aureus.

11

De plaatsing van verkeersdrempels ter bevordering van de verkeersveiligheid is discutabel.

12

De toename van de kriminaliteit onder werkende en studerende vrouwen doet vermoeden dat alleen de sterksten de weelde van de emancipatie kunnen dragen.

13

Uit de uitlatingen van t.v. verslaggevers bij voetbalwedstrijden zou men kunnen afleiden dat aan hen een keurkorps van (scheids-)rechters verloren is gegaan.

T. H. M. SNOEREN
Wageningen, 10 november 1976

VOORWOORD

Deze dissertatie zou niet verschenen zijn als niet velen zouden hebben bijgedragen aan het resultaat. Mijn dank gaat daarom uit naar allen die direkt of indirekt bij de voltooiing van dit proefschrift betrokken zijn geweest.

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Geachte promotor Prof. Dr. W. Pilnik, ik ben u zeer erkentelijk voor de mogelijkheid die u mij geboden hebt om bij u te promoveren. Uw stimulerende belangstelling die u ondanks uw drukke bezigheden steeds hebt getoond en de grote mate van vrijheid die u mij bij dit onderzoek hebt gegeven heb ik zeer gewaardeerd.

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Alle medewerkers van de fysisch-chemische sectie van het NIZO ben ik dank verschuldigd voor hun indirekte of direkte bijdrage aan dit proefschrift. Paula Both, Corry van der Spek en Jan Brinkhuis wil ik met name noemen voor het vele experimentele werk dat zij hebben verricht.

Ir. J. Jeunink dank ik voor zijn bijdrage die hij in het kader van zijn ingenieursstudie aan dit onderwerp heeft geleverd.

Verder dank ik de heren H. J. van Brakel en J. Mondria voor het vervaardigen van de figuren, mej. M. E. Seyd en haar medewerksters voor het typewerk en de heer G. H. Stel voor zijn assistentie bij het persklaar maken van het manuscript.

Tenslotte, maar niet op de laatste plaats, Toos, wil ik jou danken voor het geduld en het begrip dat je voor mijn hobby hebt getoond.

CONTENTS

I. INTRODUCTION

1.1. POLYSACCHARIDES AS STABILIZING AGENTS

Polysaccharides are widely used as additives in the food industry as a consequence of their gel-forming and/or thickening capacities. They are commonly extracted from fruit residues, seeds or seaweeds, which yield for example pectins, starches, agar, alginates, furcellaran and carrageenan. Others, such as dextran and xanthan, are produced by microbial fermentation (Glicksman, 1969). More recently developed types of stabilizers are derived by chemical modification of natural products, e.g., low-methoxy-pectin, methylcellulose and carboxymethyl starches. Even the use of synthetic vinylpolymers has been suggested (Glicksman, 1969).

The usage of these gums to stabilize or to improve the body of such products as jams, pie fillings, sauces and mayonaise is due to their thickening or viscosity-increasing effect. Gel-formation, which is a property of a comparatively few polysaccharides is the basis of their application as suspending or gelling agents in products such as chocolate milk, puddings, desserts, aspics and mousses. The properties and applications of gums are reviewed by Whistler and Be Miller (1959) and Glicksman (1969).

In this thesis special attention will be paid to the properties of carrageenans and to the mechanism of the so-called milk reactivity on which their application in the dairy industry is based. Experience has shown that of all the known carrageenans, the so-called κ-carrageenan component is the most effective as a stabilizer of chocolate milk. It interacts in low concentrations (about 0.02%) with the casein of milk to produce complexes which have an increased viscosity.

1.2. CARRAGEENAN

Carrageenans are isolated from the cell walls of red seaweeds such as Hypnea muciformis, Chondrus crispus, Gigartina stellata and Eucheuma spinosum. These are derived from three major families of red algae whose botanical descent is shown in Fig. 1.1. Carrageenans are condensation products of D-galactose units linked together at the α 1,3- and β 1,4-positions. Smith and Cook (1953) showed that carrageenan extracted from Chondrus crispus can be separated into fractions differing in chemical composition and sulphate content, by precipitation with potassium salts. The fraction precipitated was designated as κ-carrageenan and the part remaining in solution as λ-carrageenan.

As might be expected, the physical properties of the carrageenans depend

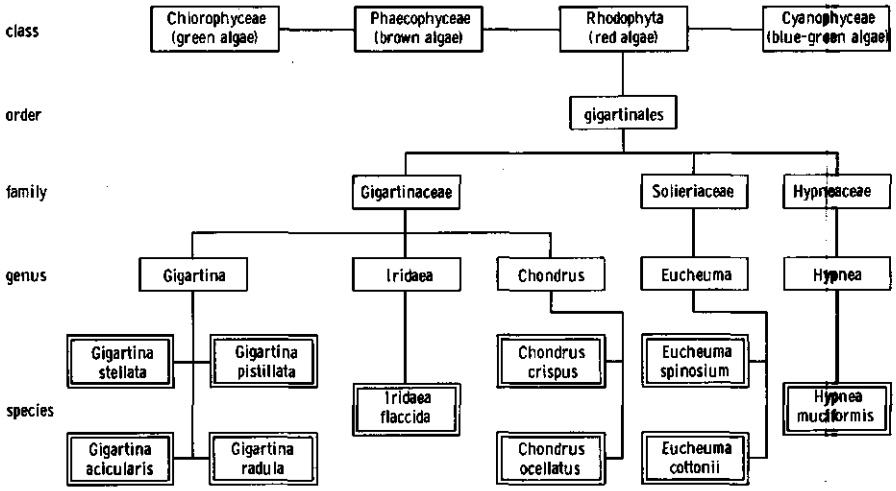

Fig. 1.1. The botanical descent of red algae. (adapted from Brochure Pierrefitte-Auby, Paris, 1972).

on their molecular weight, on their sulphate content and on the accompanying cations. The molecular weight and the sulphate content depend strongly on the season and area of harvesting and the algae species involved.

The chemical structures of the two different components isolated from Chondrus crispus by the selective potassium precipitation have been determined by O'Neill (1955). Lambda carrageenan consists of 1,4-linked galactose-2,6-disulphate and 1,3-linked galactose-2-sulphate units leading to a sulphate content of about 37%, whereas κ-carrageenan consists of 1,3-linked galactose-4-sulphate and 1,4-linked 3,6-anhydro galactose units, with a sulphate content of about 23% (cf. Fig. 1.2).

A third carrageenan component has been extracted from the red seaweed Eucheuma spinosum. The chemical structure of this so-called ι-carrageenan has been elucidated by Mueller and Rees (1967). It is structurally identical with κ-carrageenan except for the sulphate group on the c_2-position of the anhydro galactose unit and a sulphate content of about 31%. This gives ι-carrageenan a more hydrophilic character. Other carrageenans such as μ- and ν-carrageenan are considered as biological precursors of the κ- and ι-forms respectively.

Solutions of κ- and ι-carrageenan can easily gel, depending on the temperature and the cations present. Lambda-carrageenan forms only solutions of increased viscosity. This different rheological behaviour leads to different applications, as was pointed out by Glicksman (1969) and Whistler and Be

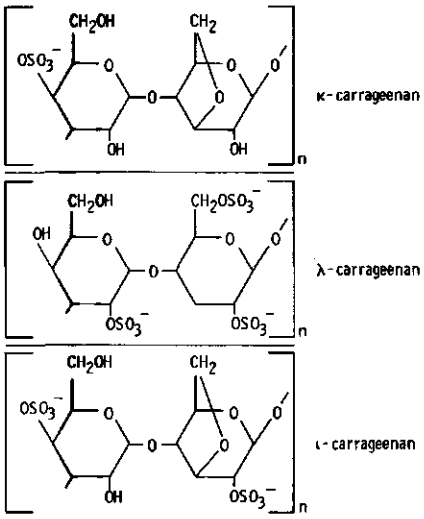

Fig. 1.2. The chemical structures of the three main components of carrageenan.

Miller (1959). Only few data are available on the molecular weight and shape of the κ-carrageenan molecule. Cook et al (1952) studied carrageenan extracted from Chondrus crispus, by ultracentrifugation and viscosity measurements, and interpreted the carrageenan molecule as an ellipsoid of revolution. This interpretation can however be questioned since the relations for an ellipsoid of revolution pertain to inpenetrable macro-molecules. Masson and Caines (1954) studied carrageenan extracted from Chondrus crispus by viscosity measurements and osmometry. They interpreted the data obtained on low molecular weight carrageenans as indicating fairly stiff rods.

Since molecular weight and shape are of primary importance for our understanding of the physico-chemical behaviour of the carrageenans, I have repeated the molecular characterization of κ-carrageenan by means of light-scattering, viscosity and ultracentrifuge experiments. It will be shown, as will be discussed in Chapter II, that the description of κ-carrageenan as a random coil is more appropriate.

As mentioned above, in the presence of different cations and depending on the temperature of the solutions, κ-carrageenan forms a gel which has been described as an interconnected network of polymer chains with the solvent and other solutes in the interstices (Rees, 1969) (cf. Fig. 1.3). Anderson et al (1969) suggested that the junctions of κ- and ι-carrageenan networks consist of double-helical crosslinks. As will be shown in Chapter III a conformational change can indeed be observed at the temperature of incipient gelation. Also the influence of the ionic strength on the gelation temperature can be interpreted as a coil-double helix transition, as will be shown in Chapter III.

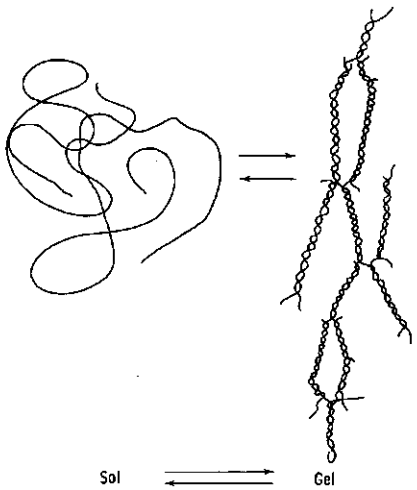

Fig. 1.3. Schematic picture of the κ-carrageenan molecule in the sol and gel state according to Rees (Rees, 1969).

Addition of κ-carrageenan to chocolate milk, in concentrations which are normally of an order of magnitude smaller than those needed for gel formation, prevents the sedimentation of cocoa particles. It has been shown that the stabilisation is based on a specific interaction between κ-carrageenan and κ-casein (Grindrod and Nickerson, 1968; Payens, 1972; and Snoeren et al, 1975). By means of experiments, to be discussed in Chapter IV, it will be demonstrated that the interaction between κ-carrageenan and κ-casein (milk reactivity) is electrostatic in nature.

In Chapter V some applications of the milk reactivity will be described. It will also be shown that the effectiveness of κ-carrageenan as a stabilizer depends strongly on its molecular weight.

1.3. CASEIN

Casein is the main protein component of cow's milk and consequently it plays an important role in the stability of dairy products. For the major part, it occurs in milk as nearly spherical colloidal particles with diameters up to about 300 nm (Schmidt et al, 1973). It has been shown that these particles, the so-called casein micelles, consist of a large number of spherical sub-units with a diameter of about 20 nm. These sub-units are called the sub-micelles (Eggmann, 1969; Schmidt and Buchheim, 1970; Schmidt et al, 1973) (cf. Fig. 1.4). Presumably the sub-micelles are cemented together by calcium bonds between the carboxylic and ester-phosphate groups of the protein moiety (Schmidt and Payens, 1976). The sub-micelles which survive exhaustive dialysis against

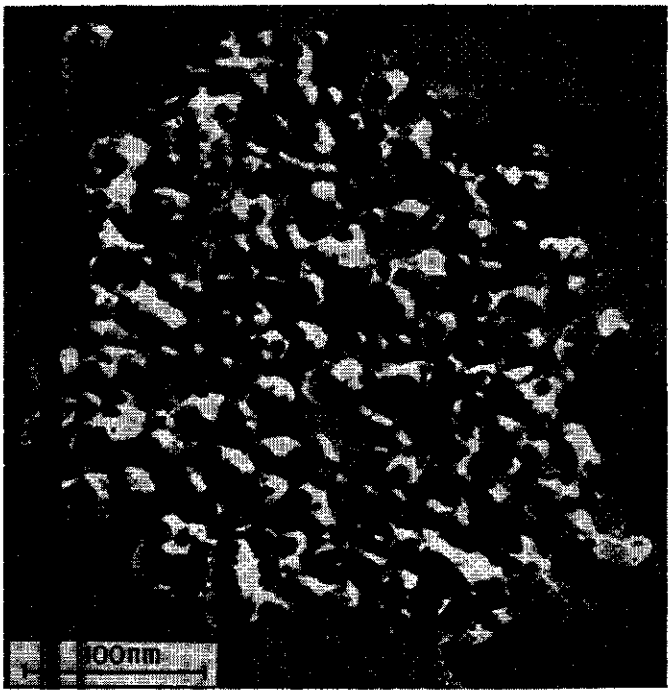

Fig. 1.4. Electronmicrograph of a casein micelle. Technique: freeze-etching.

distilled water consist of protein only. Casein is not a single protein, but it consists of three main components: α_{s1}-, β- and $\varkappa$-casein and the minor components: α_{s3}-, α_{s4}-, α_{s5}-, γ-, *R*-, *S*- and *T*-casein. The molecular weights of the three main components α_{s1}-, β- and $\varkappa$-casein are about 23600, 24000 and 19000 respectively (Mercier et al 1971, 1972, 1973). Alpha$_{s1}$- and β-casein are sensitive to calcium ions, whereas $\varkappa$-casein is not. On the contrary, the latter component is able to stabilize the other casein components against the flocculating action of calcium ions by the formation of stable micelles. This led Waugh (1958) to develop a micelle model of casein, elaborating on the earlier hypothesis of Linderström Lang (1929) concerning the role of $\varkappa$-casein as protective colloid. During the last few years many alternative models have been proposed for the casein micelle. A review has recently been given by Schmidt and Payens (1976).

The different casein components have a strong tendency to self association and complex formation, which reflects their tendency to form micelles in milk (Payens and Van Markwijk, 1963; Payens and Schmidt, 1965; Schmidt, 1969; Zittle and Walter, 1963 and Nijhuis, 1974). The self association of α_{s1}- and β-casein is mainly due to hydrophobic forces, but in case of $\varkappa$-casein aggrega-

tion by formation of covalent disulphide-bonds is also important (MacKinlay and Wake, 1971).

The stabilizing properties of κ-casein are completely destroyed by the action of the enzyme Chymosine (EC 3-4-23-4) which splits off a polypeptide of molecular weight 6 700 from κ-casein (MacKinlay and Wake, 1971). The remaining part of κ-casein, which is called para-κ-casein, is no longer able to stabilize the casein micelle against flocculation by calcium ions. Kappa-casein contains varying amounts of different carbohydrates such as galactose, galactose-amide and *N*-acetylated neuraminic acid (Wheelock and Sinkinson, 1969). The isoelectric point of κ-casein depends strongly on its neuraminic acid content. The heterogeneity of κ-casein which depends on the neuraminic acid content and on the genetic variants, can be demonstrated by starch gel electrophoresis (Schmidt et al, 1966).

As has already been mentioned above, κ-carrageenan specifically interacts with κ-casein (Grindrod and Nickerson, 1968; Payens, 1972 and Snoeren et al, 1975). It will be demonstrated that in this respect the electrical charge distribution along the polypeptide chain is of primary importance (Snoeren et al, 1975). This will be dealt with in Chapter IV.

It is worth while to pay some attention to the so called minor α_s-components viz α_{s3}- + α_{s4}- and α_{s5}-casein. Their amino acid composition and terminal ends have been reported by Ribadeau Dumas, (1970) and Hoagland et al (1971). The two proteins α_{s3} and α_{s4} seem almost identical (Ribadeau Dumas et al, 1975), and are characterized by a high phosphorus- and by a high lysine content. It was suggested by Hoagland et al (1971) that α_{s5}-casein consists of one molecule of each, α_{s3}- and α_{s4}-casein, linked through disulphide bonds. The molecular weight of α_{s3}- and/or α_{s4}-casein has been estimated by ultracentrifugation experiments (Toma and Nakai, 1973) and from the amino acid composition (Ribadeau Dumas et al, 1975) as 31 800 and 26 000 respectively.

In the Appendix of Chapter IV attention will be paid to these minor components and to their interaction with κ-carrageenan.

Other minor components such as γ, *R*, *S* and *TS* have been recognized as fragments of β-casein (Gordon et al., 1972).

II. THE PHYSICAL CHARACTERIZATION OF κ-CARRAGEENAN

2.1 INTRODUCTION

The chemical structure of κ-carrageenan has been established as a condensation product of α 1,3-linked galactose-4-sulphate and β 1,4-linked 3,6-anhydrogalactose (O'Neill, 1955). As a consequence of the α- and β-linkages the galactose rings are alternately in the 1 C and the C 1 configuration (cf. Fig. 2.1).

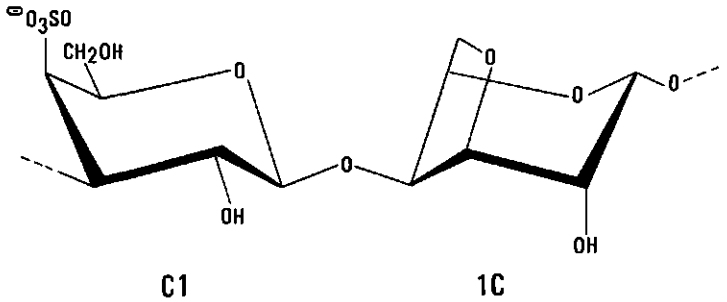

Fig. 2.1. Conformational structure of κ-carrageenan.

The hydrodynamical behaviour of the carrageenan molecule in aqueous solution as determined by ultracentrifugation and viscosity measurements has been interpreted by Cook et al. (1952) as that of an ellipsoid of revolution. Masson and Caines (1954) on the other hand interpreted – on the basis of osmometry and viscosity studies – the degraded carrageenans with number average molecular weights less than 75 000 as stiff rods. Both studies were carried out on unfractionated carrageenans.

The hydrodynamic and light-scattering measurements described in this chapter, lead to the conclusion that it is more appropriate to describe the κ-carrageenan molecule as an expanded coil. It will be shown that the factors governing the expansion can be divided into two categories. The first comprises those reflecting the chain geometry such as the size of the monomer unit, the length of the bond joining them, the valence angle and the steric hindrances to free rotation about these intermonomer unit links. These constant factors determine a basic chain dimension termed the 'unperturbed dimension'. The second category of factors determining the expansion of the κ-carrageenan coil are the so-called long-range interference effects such as the physical

volume of exclusion and the electrostatic interactions between the ionized ester sulphate groups.

For an interpretation of the physico-chemical properties of κ-carrageenan it is necessary to know the molecular parameters of κ-carrageenan such as the molecular weight, the shape of the molecule and the width of the molecular weight distribution. These parameters, therefore, were determined with fractionated κ-carrageenan samples covering an extended molecular weight range, by different physical methods such as light scattering, viscosimetry and ultracentrifugation.

2.2. MATERIALS AND METHODS

2.2.1. Materials

The κ-carrageenan samples H_0 and P100 were obtained from the commercial samples Genulacta K100 and Genulacta P100 manufactured by Københavns Pektin-fabrik, whereas sample HMR was obtained from the commercial sample Satia HMR of Pierrefitte-Auby, Paris. Sample EC was extracted from Eucheuma cottonii and was a gift from Pierrefitte-Auby. Kappa-carrageenan samples of different molecular weight were further prepared either by hydrolysis of sample H_0 (yielding the samples H_{50}, H_{20}, H_{10}, H_5 and A_{72}) and sample EC (EC_{10} and EC_5) or by ultrafiltration of sample H_0 (PM 30 and XM 100A). Hydrolysis was carried out at 100 °C in aqueous solution brought at pH 3.0 by addition of hydrochloric acid. Samples were removed after different time intervals and neutralized after cooling by addition of 0.5 M sodium hydroxide. For fractionation by ultrafiltration a commercial sample of Genulacta K100 was dissolved to a concentration of 0.2% in distilled water, that had been adjusted to pH 7 with 0.5 M sodium hydroxide. Filtration was carried out with Amicon 'diaflo' filters types PM 30 and XM 100A.

All the κ-carrageenan samples were exhaustively dialysed against distilled water which had been adjusted to pH 7 with 0.5 M sodium hydroxide, and then freeze-dried. It is worth-while to note that adjusting to pH7 before freeze-drying is necessary, since freeze-drying at lower pH values leads to hydrolysis of the product.

The commercial samples used were screened for their chemical composition by the infrared method described by Snoeren and Poll (1973) to ascertain that only κ-carrageenan was used. The estersulphate content (SO_4) of the commercial samples, determined by the method described by Hansen and Whitney (1960) was 23.1% ± 0.4%, which corresponds well with the chemical structure of κ-carrageenan presented in the introduction.

2.2.2. Light scattering

The excess light scattering of a polymer solution gives information about the molecular weight and the size of the polymer molecule. The relation between the scattered light and the molecular weight is given by (Tanford, 1961; Stacey, 1956):

$$Kc/R_\theta = {}^1/_{<M>_w} + 2<A_2>\, c + 0\,(c^2) \tag{2.1}$$

in which: c is the concentration of the polymer (g ml^{-1});
$<A_2>$ the average osmotic second virial coefficient (mol ml g^{-2})
$<M>_w$ the weight average molecular weight defined as

$$<M>_w = \sum_i c_i M_i / \sum_i c_i,$$

with c_i the concentration of species i in g ml^{-1}.

Further R_θ, the reduced intensity of the scattered light at angle θ and a distance r between the scattering centre and the light receptor, is given by

$$R_\theta = r^2 i_\theta / i_0 (1 + \cos^2 \theta) \tag{2.2}$$

and K is a constant given by

$$K = 2\pi^2 n_0^2 (dn/dc)^2 / N\lambda^4 \tag{2.3}$$

in which, n_0 represents the refractive index of the solvent, dn/dc the refractive index increment of the solution, λ the wavelength of the light in vacuo and N Avogadro's number.

For very dilute solutions Eqn. (2.1) reduces to

$$Kc/R_\theta = 1/<M>_w + 2<A_2>c \tag{2.1a}$$

For particles the dimensions of which exceed 0.05 λ, the optical path length of the light scattered by different points on the particle in the direction of observation may result in interference phenomena (Tanford, 1961). The phase difference is zero for the beam scattered in forward direction. As we shall see, the dimensions of the $\varkappa$-carrageenan molecule are such that interference may be expected, and the results must therefore be extrapolated to zero scattering angle to obtain the molecular weight and the second virial coefficient. This can be done by arranging the data obtained at different concentrations and angles in a so-called Zimm plot (Tanford, 1961), in which Kc/R_θ is plotted against $k'c + \sin^2 \theta/2$, where k' is an arbitrary constant, selected to obtain a well spread plot.

The weight average molecular weight of the $\varkappa$-carrageenan is then obtained by the relation,

$$1/<M>_w = \left[(Kc/R_0)_{\theta=0}\right]_{c=0} \tag{2.4}$$

The second virial coefficient is obtained from the same plot as

$$2 <A_2> / k' = \lim_{c \to 0} [d (Kc/R_\theta)_{\theta = 0}/dc] \tag{2.5}$$

From the limiting slope of the $c = 0$ curve the radius of gyration (R_g) can be derived. The radius of gyration is defined as

$$R_g^2 = \sum_i \overline{m_i r_i^2} / \sum_i m_i \tag{2.6}$$

where r_i is the distance of the element with mass m_i from the centre of mass of the molecule considered.

For polydisperse material a z-average of R_g^2 is obtained from the Zimm plot according to the relation (Tanford, 1961)

$$<R_g^2>_z = <M>_w 3\lambda^2/16\pi^2 \lim \left[d(Kc/R_\theta)_{c = 0}/dc\right] \tag{2.7}$$

$$\text{where } <R_g^2>_z = \sum_i c_i M_i R_{gi}^2 / \sum_i c_i M_i \tag{2.8}$$

The light-scattering measurements were carried out with a CENCO-TNO apparatus provided with a mercury lamp. A system of slits and lenses in combination with an interference filter produces a homogeneous, parallel and monochromatic beam of light of wavelength 546 nm. The intensity of the scattered light is determined, using the turbidity of benzene as a reference: $R_{90} = 15.6 \times 10^{-6}$ cm (Kratohvil et al., 1962). The scattering was measured between 30° and 150° at intervals of 15°.

On account of the high scattering power of dust particles the solutions to be analysed must be made completely dustfree. The κ-carrageenan solutions were therefore filtered directly into the cylindrical light scattering cell, through a filter (Gelman) with a pore size of 200 nm (Schmidt, 1969). Prior to the measurements the solution was dialysed exhaustively against the buffer (0.1 M NaCl, 0.005 M. EDTA, pH 6.7).

2.2.3. Measurements of the refractive index increment

The refractive index increment was measured with a Zeiss interferometer. The measurements were carried out with κ-carrageenan solutions which had been clarified by filtration as described in previous Section and subsequently dialysed against the buffer solution. The refractive index increment was measured relative to the dialysis buffer, so that the value obtained was at constant chemical potential of the buffer salt (Tanford, 1961).

The measurements were made at a temperature of 20°C. The κ-carrageenan concentration was determined with the phenol sulphuric acid method as described by Dubois et al. (1956).

2.2.4. The partial specific volume

The density measurements from which the value of the partial specific volume was derived were carried out with an Anton Paar Digital Densitometer D.M.A. 02 c (Kratky et al., 1969). The densities plotted as a function of the concentration yielded a straight line from which the buoyancy factor was calculated as

$$d\rho/dc = (1-\bar{v}\rho_0) \tag{2.9}$$

where ρ_0 is the density of the solvent.

2.2.5. Viscosity measurements

Viscosity measurements were carried out at 20°C ± 0.02° in Ubbelohde viscosimeters with different efflux times (87 sec. and 320 sec. for water). Prior to the measurements the dialysed solutions were filtered through a G-3 glass filter to remove dust particles. Solutions of different ionic strength were prepared by adding NaCl to the 0.005 M EDTA buffer pH 6.7. Before each experiment the viscosimeter was cleaned with concentrated nitric acid.

The relative viscosity of the $\varkappa$-carrageenan solution with respect to the dialysis buffer was calculated by:

$$\eta_r = \eta/\eta_0 = \rho(X_1 t - X_2/t)/\rho_0(X_1 t_0 - X_2/t_0) \tag{2.10}$$

where ρ and ρ_0 represent the densities of the $\varkappa$-carrageenan solution and the dialysis buffer respectively, t and t_0 are the efflux times of the solution and the buffer respectively and X_1 and X_2 are the calibration constants characteristic of the viscosimeter used.

The intrinsic viscosity, $[\eta]$, is calculated by extrapolation of $(\eta_r - 1)/c$ to infinite dilution i.e.

$$[\eta] = \lim_{c \to 0} [(\eta_r - 1)/c] \tag{2.11}$$

2.2.6. The sedimentation coefficient

Sedimentation rates were determined at a speed of 68000 r p m in a Spinco E Analytical Ultracentrifuge. The distances (r) of the maximum of the peak from a reference mark in the rotor were read from the photographic plate using a Nikon comparator model 6C.

The sedimentation coefficient (S) was calculated from the relation (Schachman, 1959).

$$S_{20,w} = (dr/dt)/\omega^2 r \cdot 2\eta_T/\eta^0_{20} \tag{2.12}$$

in which t is the time of observation (sec.);
ω the angular velocity of the rotor;

η_T the viscosity of the solution at temperature T and
η^0_{20} the viscosity of water at 20°C.

The sedimentation coefficient, $S^0_{20,w}$, of the $\varkappa$-carrageenan solution was obtained by extrapolation to zero concentration of $1/S_{20,w}$ values found at different concentrations. Measurements were made at temperatures of 20° ± 0.1°C. The $\varkappa$-carrageenan solutions were dialysed prior to experimentation against an 0.005 M EDTA buffer pH 6.7 containing 0.1 M NaCl.

2.2.7. The number average molecular weight

The number average molecular weight, $<M>_n$, can be determined by end-group analysis by different methods e.g. the hypoiodate method of Willstätter-Schudel (1918), the picric acid method of Williaman and Davidson (1924) and the copper reagent method of Nelson (1944). These methods have in common that the oxidation of the reducing end groups (aldehyde groups) occurs in alkaline medium which prevents degradation of the $\varkappa$-carrageenan chain. The relatively low sensitivity of the two former methods permits a reliable estimate only for $\varkappa$-carrageenan of number average molecular weights less than, say, 20 000. The Nelson method, which is more sensitive, can be used to molecular weights of 400 000. This method is based upon the oxidation of reducing end groups by copper (Cu^{++}), arsenomolybdate colour reagent being added as a chromogen. Before measuring the extinction at 540 nm, the $\varkappa$-carrageenan aggregates are removed by centrifugation (30 min, 1000 g).

The ratio of the weight average molecular weight and the number average molecular weight $<M>_w/<M>_n$ gives information about the polydispersity of $\varkappa$-carrageenan samples used. As we shall see for the theoretical interpretation of the solution properties of $\varkappa$-carrageenan, the polydispersity needs to be taken into account. In this study therefore the generalized molecular weight distribution function (Schulz-Zimm distribution; Morawetz, 1966) is used, in which the width of the distribution is characterized by a parameter z, which can be obtained from experimentally determined values of $<M>_w$ and $<M>_n$ ($z = <M>_n/(<M>_w - <M>_n)$).

This generalized distribution function is given by

$$N(P) = \left[y^z/\Gamma(z)\right] P^{z-1} \exp(-yP) \tag{2.13}$$

where $N(P)$ is the number of molecules of degree of polymerisation P, $\Gamma(z)$ is the gamma function of z and y is a constant.

The number, weight and z-average molecular weights are related to the parameters z and y by, (Morawetz, 1966)

$$<M>_n : <M>_w : <M>_z = (M_0/y)(z) : (M_0/y)(z+1) : (M_0/y)(z+2)$$

with M_0 the molecular mass of a monomer unit.

2.3. RESULTS

2.3.1. Light scattering

The weight average molecular weights, osmotic virial coefficients and radii of gyration determined by light scattering for various κ-carrageenan samples are collected in Table 2.1. Since the samples with the lowest molecular weight did not show any significant dissymmetry of the scattered light, their radii of gyration could not be determined (cf. Fig. 2.2). A Zimm plot for sample P100 of molecular weight 718 000 is given in Fig. 2.3.

Table 2.1. Light-scattering data, (weight average molecular weights, radii of gyration, second virial coefficient), intrinsic viscosities and number average molecular weights, of different κ-carrageenan samples.
Experimental conditions: Temp. 20°C; 0.005 M EDTA buffer, 0.1 M NaCl, pH 6.7.

Sample	$<M>_w \cdot 10^{-3}$	$<R_\theta^2>_{nm}^{1/2}$ ls	$<A_2> \cdot 10^3$ $(mol.ml.g^{-2})$	$[\eta](dl/g)$	$<M>_n \cdot 10^{-3}$
H_{50}	17.5		2.75	0.38	5.8
H_{20}	44		2.30	0.82	23.7
H_{10}	87		2.25	1.38	42.7
EC_{10}	159	51.8	2.60	2.46	80.5
H_5	248	64.1	2.50	3.23	107.5
EC_5	253	56.4	1.60	3.85	153
A_{72}	359	77.6	2.06	4.76	180
HMR	522	91.0	2.00	6.83	346
H_0	604	94.5	1.82	7.15	460
PM 30	658	98.0	1.96	7.90	
XM 100A	690	105.7	1.88	8.70	
P 100	718	102.3	1.85	8.90	
EC	836	107.8	1.30	10.40	

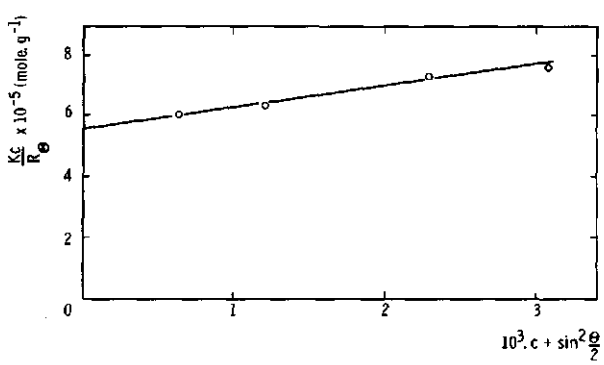

Fig. 2.2.
The concentration dependence of the light scattering of κ-carrageenan sample H_{50}. Experimental conditions: 0.005 M EDTA, 0.1 M NaCl, pH 6.7, temp. 20°C.

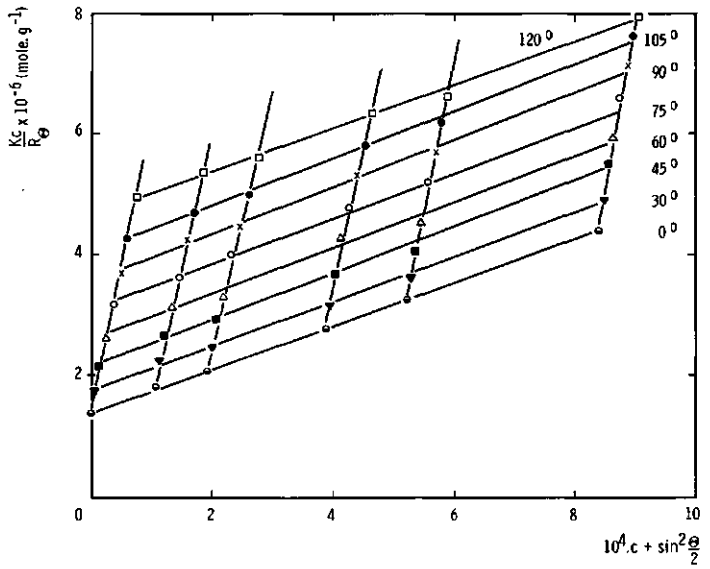

Fig. 2.3. Light scattering Zimm-plot for $\varkappa$-carrageenan sample P100. Experimental conditions identical with Fig. 2.2.

The value of the second virial coefficient decreases with increasing molecular weight. These values are comparable with the value of 1.7×10^{-3} (mol ml $\mathbf{g}^{-2}$), reported by Smidsrød (1974) for $\varkappa$-carrageenan in the presence of 0.1 M LiCl.

The radius of gyration for a macromolecule is related to the molecular weight by (Tanford, 1961)

$$R_{gi}^2 = K_{R_g} M_i^{a_{R_g}} \tag{2.14}$$

For heterogeneous macromolecules the light-scattering average molecular weight and radius of gyration can be related to each other by

$$<R_g^2>_z = \text{Constant } (q_{z\,.\,w} <M>_w)^{a_{R_g}} \tag{2.15}$$

where K_{R_g} and a_{R_g} ($a_{R_g} = 1 + \varepsilon$, see Section 2.4.3) are constants for a given polymer-solvent pair and $q_{z.w}$ is a correction factor accounting for the polydispersity of the $\varkappa$-carrageenan sample. For a Schulz-Zimm distribution $q_{z\,.w}$ is given by (Sundelöf, 1971)

$$q_{z.w} = \frac{\Gamma(a_{R_g} + z + 2)}{\Gamma(z+2)} \cdot 1/(z+1)$$

From the slope of a plot of log $<R_g^2>_z$ vs log $(q_{z.w} <M>_w)$ (cf. Fig. 2.4) the

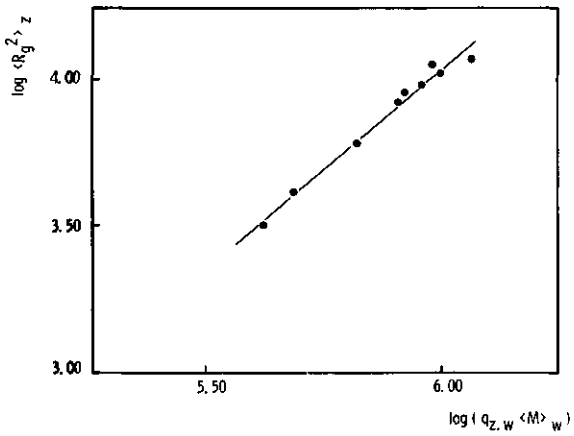

Fig. 2.4.
Plot of log $\langle R_g^2 \rangle_z$ as a function of log $(q_{z,w} \langle M \rangle_w)$ From the slope the exponent a_{R_g} has been derived as 1.20.

value of 1.20 is obtained for the exponent. Such a value is indicative of an expanded random coil (Tanford, 1961).

2.3.2. The refractive index increment

The results of the refractive index increment measurements are collected in Fig. 2.5. The refractive index increment appears to be constant and independent of κ-carrageenan concentration. A least squares analysis of the results yields a value of 0.118 (ml g^{-1}) with a standard deviation of 0.003. Jones et al. (1973) reported for non-dialysed ι-carrageenan a comparable value of 0.127. It was shown by Cleland (1968) that, as a consequence of dialysis, the refractive index increment decreases. For carboxymethylcellulose a decrease in refractive index increment of 11% was observed.

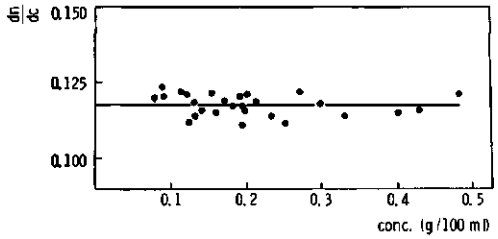

Fig. 2.5.
Refractive index increment as a function of κ-carrageenan concentration.
Experimental conditions (see Fig. 2.2).

2.3.3. Partial specific volume

The density increment of dialysed sodium salt of κ-carrageenan solutions (0.005 M EDTA, pH 6, 0.15 M NaCl) has been derived from the slope of a ρ vs concentration plot (cf. Fig. 2.6), as 0.49. Substitution in Eqn. 2.9 yields 0.51

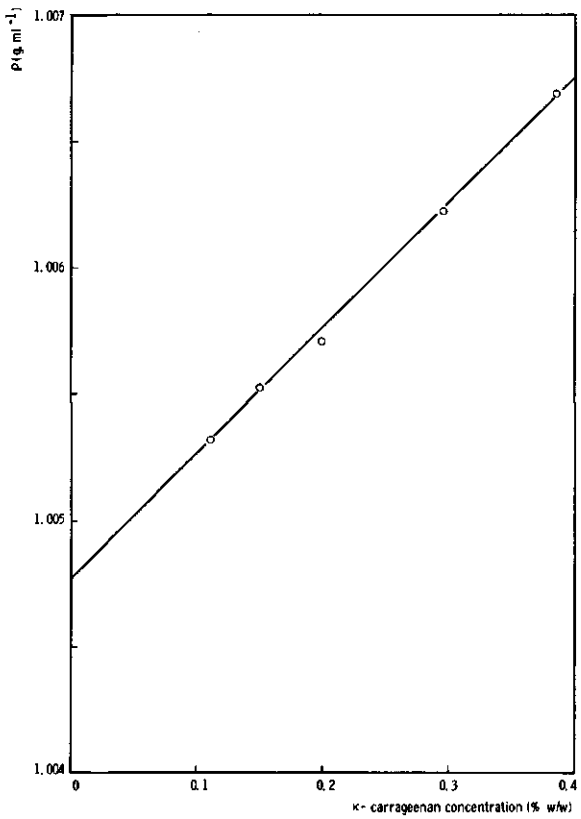

Fig. 2.6. The density of $\varkappa$-carrageenan solutions as a function of $\varkappa$-carrageenan concentration. From the slope the value of $d\rho/dc$ has been derived as 0.49. Experimental conditions 0.005 M EDTA, pH 6, 0.15 M NaCl.

ml g^{-1} for the partial specific volume. This value is in agreement with the value of 0.50 ml g^{-1} reported by Cook et al. (1952) for a carrageenan mixture.

The partial specific volume can also be calculated from the chemical composition by Traube's rule, as was done for proteins by McMeekin and Marshall (1952). The $\varkappa$-carrageenan molecule is built up from galactose units and hydrosulphate.

The partial specific volume of hydrosulphate is calculated by Eqn. 2.9, using the densities of $NaHSO_4$ solutions from the Int. Crit. Tables (Washburn, 1928) as 0.229 ml.g^{-1}. For the partial specific volume of galactose, the partial specific volume of a hexose unit has been taken as 0.613 ml.g^{-1} (Gottschalk, 1966). The partial specific volume of $\varkappa$-carrageenan thus becomes.

$$\bar{v}_{carr.} = \frac{M\ galactose.\bar{v}\ galactose + M\ hydrosulphate.\ \bar{v}\ hydrosulphate}{M_{total}} \quad (2.16)$$

The result $\bar{v}_{Na\ carrageenan} = 0.49$ ml g^{-1} corresponds well with the experimental value.

2.3.4. Viscosity measurements

The values of $(\eta_r - 1)/c$ as a function of $\varkappa$-carrageenan concentration are given in Fig. 2.7. The concentration dependence of η_r is usually expressed in terms of the relation (Tanford, 1961)

$$\frac{\eta_r - 1}{c} = [\eta] + k\,[\eta]^2 c \quad (2.17)$$

where k is the so-called Huggins constant. For $\varkappa$-carrageenan in an 0.1175 ionic strength solution the Huggins constant is 0.35, a value that is often observed for flexible polymers in good solvents (Huggins, 1942). The intrinsic viscosities of

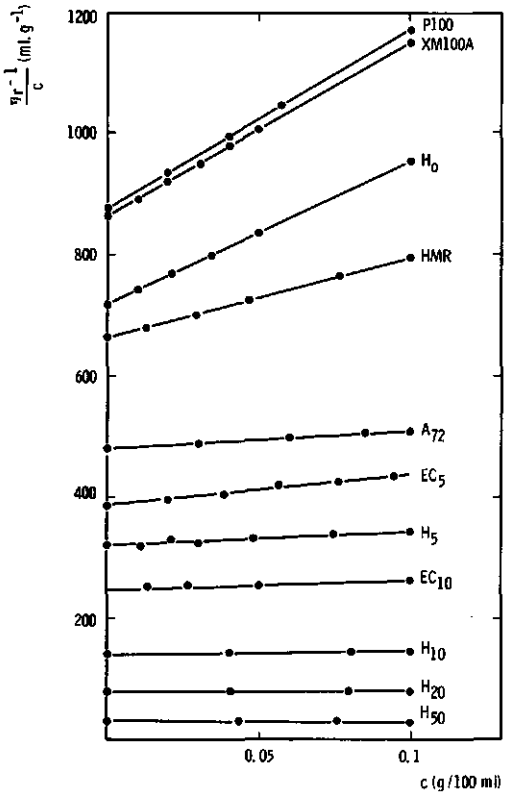

Fig. 2.7.
$(\eta_r - 1)/c$ as a function of concentration for different carrageenan samples.
Experimental conditions (see Fig. 2.2).

Table 2.2. Intrinsic viscosities and viscosity expansion factors obtained at different ionic strengths.

Sample	Ionic strength	$[\eta]$ (dl/g)	$\alpha_\eta = ([\eta]/[\eta]_\theta)^{1/3}$
H_{20}	0.0275	1.20	1.28
	0.1175	0.82	1.13
	0.2175	0.85	1.14
	∞	0.59	1.01
H_{10}	0.0275	2.02	1.36
	0.1175	1.38	1.20
	0.2175	1.40	1.20
	∞	0.96	1.06
H_5	0.0275	4.90	1.57
	0.1175	3.23	1.36
	0.2175	2.88	1.30
	∞	1.73	1.09
EC_5	0.0275	6.10	1.64
	0.1175	3.85	1.43
	0.2175	3.75	1.40
	∞	2.21	1.18
XM 100A	0.0275	14	1.84
	0.0675	10.75	1.68
	0.1175	8.70	1.58
	0.2175	7.25	1.48
	∞	3.70	1.18

κ-carrageenan fractions covering a weight-average molecular weight range of 17 500 to 836 000 are given in Table 2.1.

In Fig. 2.8 the intrinsic viscosities of different κ-carrageenan samples obtained at different ionic strengths (cf. Table 2.2) are plotted against the reciprocal of the square root of the ionic strength of the solvent. A linear relation between $[\eta]$ and $I^{-1/2}$ as proposed by Pals and Hermans (1952) was found for many polyelectrolytes (Cox, 1960; Noda et al., 1970 and Hawkins and Holtzer, 1972). Fig. 2.8 shows that such a relation also holds for κ-carrageenan. The linear, least squares extrapolation to infinite ionic strength yields the intrinsic viscosity $[\eta]_\infty$ of the κ-carrageenan at conditions where the electrostatic charge repulsion is completely suppressed through ionic screening (Noda et al., 1972).

The relation between the intrinsic viscosity and the molecular weight is usually expressed by the Mark-Houwink relation, which for monodisperse species reads

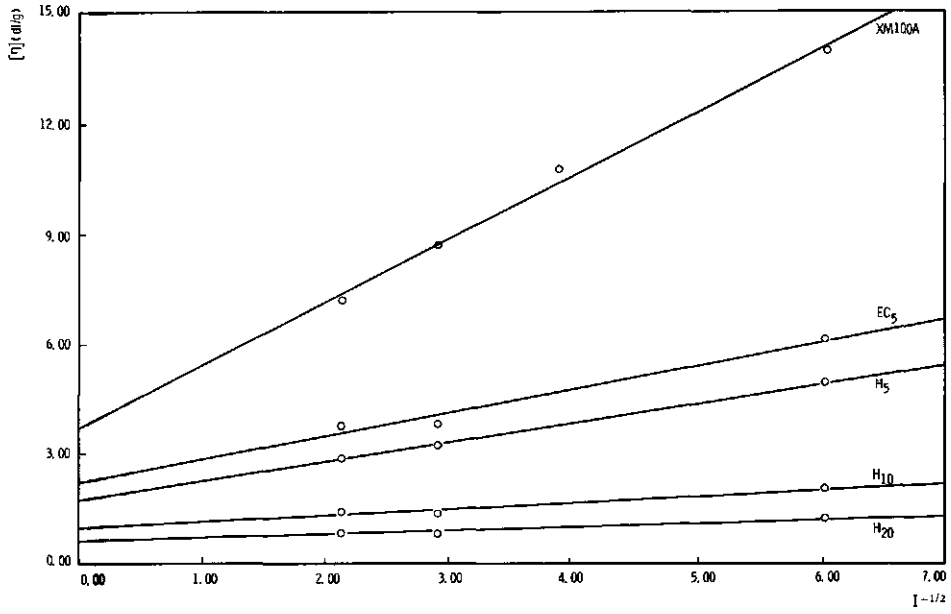

Fig. 2.8. The intrinsic viscosity as a function of the reciprocal root square of the ionic strength ($I^{-1/2}$) given for different $\varkappa$-carrageenan samples.

$$[\eta_i] = K_\eta M_i^{a_\eta} \tag{2.18}$$

In case of polydispersity this relation changes into (Appendix V):

$$[\eta] = K_\eta \, q_{v.w} < M >_w^{a_\eta} \tag{2.19}$$

where k_η and a_η are constants for a given polymer-solvent pair and $q_{v.w}$ is a correction factor for heterogeneity of the $\varkappa$-carrageenan samples. In appendix V it is shown that $q_{v.w}$ is given by

$$q_{v.w} = < M >_v^{a_\eta} / < M >_w^{a_\eta} = (\Gamma(a_\eta + z + 1)/\Gamma(z+1)).\ 1/(z+1)^{a_\eta}$$

When $a_\eta = 1$, the correction factor $q_{v.w} = 1$ and $< M >_w$ equals the viscosity average molecular weight, $< M >_v$, which is defined as

$$< M >_v = (\sum_i n_i M_i^{1+a_\eta} / \sum_i n_i M_i)^{1/a_\eta} \tag{2.20}$$

with n_i the number of molecules of molecular weight M_i.

The slope of a plot of log $([\eta]/q_{v.w})$, with $q_{v.w} = 1$, vs log $< M >_w$ gives a first approximation of a_η from which a value of $q_{v.w}$ is calculated for the next approximation (cf. Fig. 2.9). After 2 steps a constant value of a_η was obtained.

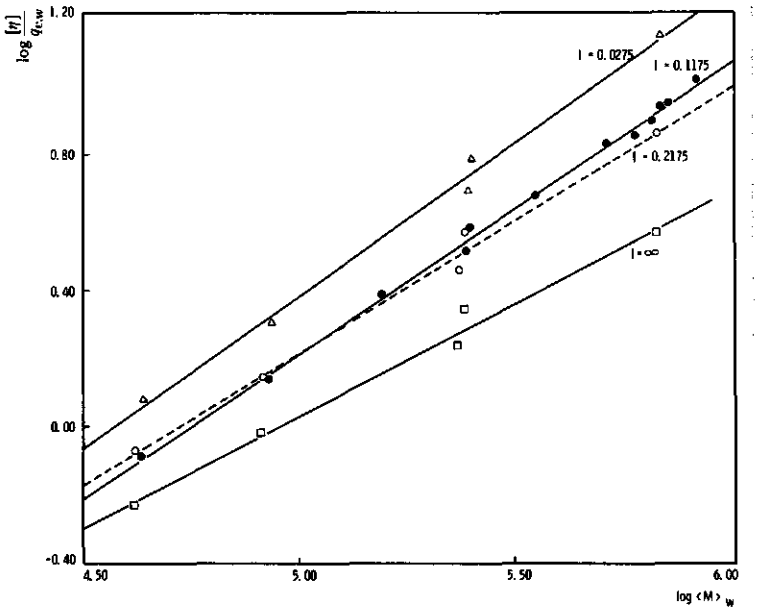

Fig. 2.9. Double logarithmic plot of $[\eta]/q_{v,w}$ vs the weight average molecular weight.

Table 2-3. Mark-Houwink parameters, K_η and a_η, estimated for κ-carrageenan solutions of different ionic strength.

Ionic strength	$K_\eta \cdot 10^5$	a_η
0.0275	7.78	0.90
0.1175	8.84	0.86
0.2175	20.9	0.78
∞	52.0	0.67

The experimental data obtained at different ionic strengths give different values for K_η and a_η which are collected in Table 2-3.

2.3.5. The sedimentation coefficient

A typical sedimentation pattern for an 0.5% κ-carrageenan solution dissolved in 0.005 M EDTA buffer pH 6.7, 0.1 M NaCl is given in Fig. 2.10. The hyper-sharpening of the peak can be explained by the strong concentration dependence of the sedimentation coefficient, an example of which is shown in Fig. 2.11. The concentration dependence of the sedimentation velocity of

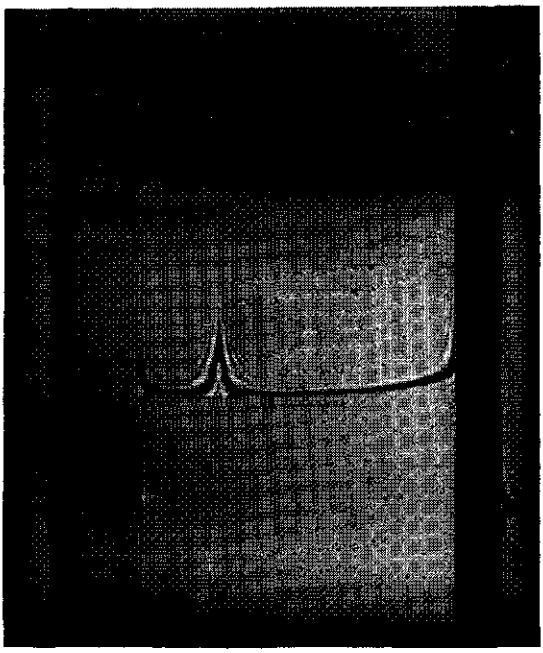

Fig. 2.10. Sedimentation pattern of $\varkappa$-carrageenan sample A_{72} in 0.005 M EDTA, pH 6.7, 0.1 M NaCl, carrageenan conc. 0.5%. Angular velocity 68000 r.p.m.

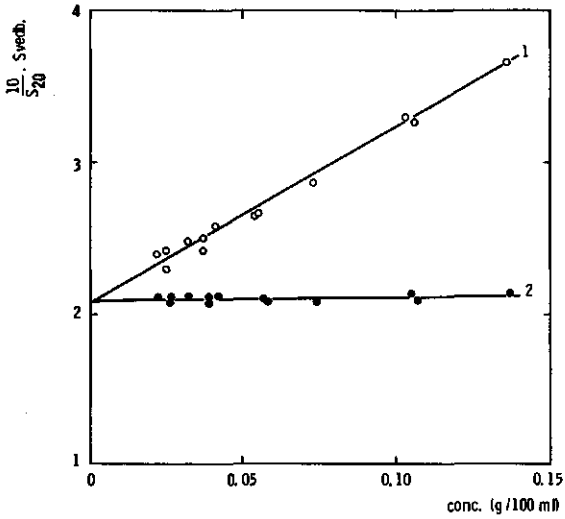

Fig. 2.11.
Sedimentation coefficient as a function of concentration for $\varkappa$-carrageenan sample A_{72}. (Exp. cond. see Fig. 2.10).
1 : $1/S_{20,w}$ against conc.
2 : $1/S_{20,w} \cdot \eta_r$ vs c.

$\varkappa$-carrageenan appears to be the result of the change in the viscosity of the solution with $\varkappa$-carrageenan concentration. From a plot of $1/(S_{20,w}\eta_r)$ vs the $\varkappa$-carrageenan concentration (cf. Fig. 2.11) it can be seen that $1/(S_{20,w}\eta_r)$ is nearly independent of the concentration. This independency of the product of relative viscosity and sedimentation coefficient was also observed for various linear polymers which behave as flexible chains (Schachman, 1959). By extrapolation to infinite dilution from the $1/S_{20,w}$ vs c plot or from the value of $1/(S_{20,w}.\eta_r)$ of the $\varkappa$-carrageenan sample A_{72} we find $S^0_{20,w} = 4.82$ Svedberg.

2.3.6. The number average molecular weight

Values for the number average molecular weight of different $\varkappa$-carrageenan samples as determined with the copper reagent method (Nelson, 1944) are collected in column 6 of Table 2.1. On account of insufficient sensitivity of this method it was not possible to determine the number average molecular weights of samples PM 30, XM 100A, P100 and EC.

For the calculation of the different factors q, which are needed as correction for polydispersity, it was assumed that the width of the molecular weight distribution of the latter samples was the same as that found for the $\varkappa$-carrageenan sample H_0. For a Schulz-Zimm distribution this means that the factor z for these samples is the same.

2.4. DISCUSSION

2.4.1. The Mark-Houwink relation

According to the statistical and hydrodynamical treatment of polymers, a value of the exponent a_η, in the Mark-Houwink relation (Eqn. 2.19) between 0.5 and 1 indicates a randomly coiled molecule, while values approaching 2 indicate a rigid rod conformation (Stuart, 1953). For an ideally flexible random coil the exponent equals 0.5, whereas higher values of the exponent are due to long-range interactions such as the exclusion of volume and electrostatic interaction. The value of $a_\eta = 0.86$ observed for $\varkappa$-carrageenan in an 0.005 M EDTA buffer pH 6.7 containing 0.1 M NaCl, therefore indicates that the shape of the $\varkappa$-carrageenan molecule under these experimental conditions can be interpreted as an expanded coiled molecule.

The value of the exponent in the Mark-Houwink relation is somewhat lower than the value reported by Smidsrød (1974) ($a_\eta = 0.98$) for low molecular weight $\varkappa$-carrageenan ($<M>_w < 170\,000$) in solutions containing 0.1 M LiCl. It was noted by Smidsrød (1974) that the plot of log $[\eta]$ against log $<M>_w$ had some curvature for molecular weights exceeding 170 000, which indicates a lower exponent for higher molecular weight samples. Smidsrød reported a value of $[\eta] = 6.6$ dl.g^{-1} for a $\varkappa$-carrageenan sample of $<M>_w = 500\,000$,

which value corresponds well with our results. Comparable values for the exponent a_η are obtained with similar polyelectrolytes such as alginates (Smidsrød and Haug, 1968) and cellulose derivatives (Brown, 1971).

Masson and Caines (1954) found a value of $a_\eta = 1.28$ for low molecular weight carrageenan in the presence of 0.1 M NaCl. This value, however, was obtained for an extract of Chondrus crispus which probably contained λ-carrageenan. The presence of λ-carrageenan with its higher charge density leads to an increase of the electrostatic repulsion as a consequence of which the value of a_η increases.

As shown in Table 2.3 the exponent in the Mark-Houwink relation decreases with increasing ionic strength, which reflects the diminished electrostatic repulsion as a consequence of the screening of the sulphate charges by the addition of salt.

For the κ-carrageenan samples used it was observed that at all ionic strengths the exponent of Eqn. 2.19 is not materially changed by replacing $<M>_v$ by $<M>_w$ i.e. $q_{v,w} \approx 1$.

2.4.2. *The unperturbed dimensions of the κ-carrageenan coil as deduced from* $[\eta]$ *and* $<M>_w$

The values of the exponents in Eqns. 2.15 and 2.19 suggest that under the experimental conditions (0.005 M EDTA, pH 6.7, 0.1 M NaCl) the κ-carrageenan molecule must be interpreted as an expanded coil. In the unperturbed state, which is defined as the conformation of the chain under conditions where long-range effects can be neglected, the size of the random coil is only governed by factors reflecting the chain geometry such as the size of the monomer unit, the length of the bond joining them, the valence angle and the steric hindrances to free rotation about the intermonomer unit links. For a random coil the dependence of $[\eta]$ on molecular mass can be used to estimate the unperturbed effective bond length l_θ, defined by Eqn. 2.23.

For monodisperse material the molecular mass dependence of $[\eta]$ can be given by the so-called Stockmayer-Fixman relation (Stockmayer and Fixman, 1963)

$$[\eta]/M^{1/2} = K_\theta + 0.51\,\Phi_\theta\,\mathrm{BM}^{1/2} \tag{2.21}$$

In Eqn. 2.21 Φ_θ is the so-called Flory-Fox viscosity constant for a θ solvent (a solvent in which the excluded volume becomes zero). Its numerical value was calculated by Flory as 2.8×10^{21} c g s units (Flory, 1953). Further B is a constant for a given polymer-solvent system. The value of K_θ is defined by (Flory, 1953)

$$[\eta]_\theta = K_\theta M^{1/2} \tag{2.22}$$

with $[\eta]_\theta$ the intrinsic viscosity of the polymer in the θ-solvent.

From the results the general S.F plot for $\varkappa$-carrageenan can now be given as:

$$[\eta]/q_{S.F} < M >_w^{1/2} = 2{\cdot}7 \times 10^{-3} + \left[0{\cdot}25 \times 10^{-5} + 0{\cdot}24 \times 10^{-5}\, I^{-1/2}\right] < M >_w^{1/2}/q_{S.F}$$

from which the relative electrostatic contribution to the total expansion can directly be derived as

$$\frac{k_{el}\, I^{-1/2}}{k_{el}\, I^{-1/2} + k_\infty} = \frac{0.24 \times 10^{-5}\, I^{-1/2}}{0.24 \times 10^{-5}\, I^{-1/2} + 0.25 \times 10^{-5}} \tag{2.27}$$

Under the experimental conditions with $I = 0.1175$ the electrostatic contribution to the total expansion is 73%.

In the above discussion of the unperturbed state of the $\varkappa$-carrageenan coil the $\varkappa$-carrageenan coil was defined as a hypothetically unrestricted chain with the same number of segments (M/M_0^*) as the real chain, but with a greater effective segment length. An alternative description is sometimes used, in which the coil has the same contour length (L) as the real chain rather than the same number of segments.

The contour length L is defined by

$$L = (M/M_0^*) l_{av} \tag{2.28}$$

In the unperturbed state, the statistical segment length, called the Kuhn statistical segment length (A_m) is defined as:

$$A_m = \overline{h_0^2}/L \tag{2.29}$$

Since $\overline{h_0^2} = (M/M_0^*) l_\theta^2$, Eqn. 2.29 may be rewritten as

$$\mathrm{A}_m = l_\theta^2 (M/M_0^*)/(M/M_0^*) l_{av} = l_\theta^2/l_{av}$$

The value of A_m for $\varkappa$-carrageenan at infinite ionic strength according to this convention is now calculated as 3.51 nm. This value is somewhat lower than values which can be derived from data reported for cellulose derivatives (Mandelkern and Flory, 1952) which are about 5 nm.

It is to be noted that for the estimation of unperturbed dimensions sometimes (Smidsrød, 1970) the polymer chain has been thought to be built up from the monomeric (monosaccharide) units instead of from the different segment bonds. It may be clear that this may lead to misleading values of l_θ and A_m, which are difficult to compare with the segment length calculated for $\varkappa$-carrageenan above.

2.4.3. The perturbed dimensions derived from viscosity data

a. The viscosity expansion factor

We have seen that under the present experimental conditions (i.e. at ionic

strength 0.1175) the $\varkappa$-carrageenan coil is expanded. The ratio of the light-scattering radius of gyration of the expanded coil to the unperturbed radius of gyration is given by the mean radius expansion factor which is defined as

$$\alpha_{R_g} = <R_g^2>_z^{1/2} / <R_{g\theta}^2>_z^{1/2} \tag{2.30}$$

in which $R_{g\theta}$ is the unperturbed radius of gyration which is related to the unperturbed end-to-end distance $(\overline{h_\theta^2})^{1/2}$ by (Tanford, 1961);

$$R_{g\theta}^2 = 1/6\ \overline{h_\theta^2} \tag{2.31}$$

The unperturbed radius of gyration can be calculated from the unperturbed end-to-end distance by Eqns. 2.23 and 2.31. To obtain a z-average value (as obtained with light scattering) of the unperturbed radius of gyration Eqn. 2.23 needs to be corrected by the polydispersity correction factor $q_{z.w}$. In the unperturbed state $q_{z.w}$ equals $<M>_z / <M>_w$, since the exponent in the relation between the radius of gyration and the molecular weight (Eqn. 2.15) equals 1 so

$$<R_{g\theta}^2>_z = 1/6\ <\overline{h_\theta^2}>_z = 1/6\ (<M>_z / M_0^*) l_\theta^2 \tag{2.32}$$

Similarly, the viscosity expansion factor α_η is defined by

$$\alpha_\eta^3 = [\eta] / [\eta]_\theta \tag{2.33}$$

The value of $[\eta]_\theta$ can be obtained from Eqn. 2.22 which for the polydisperse $\varkappa$-carrageenan samples needs to be corrected by the polydispersity correction factor $q_{v.w}$ as calculated in Appendix V.

$$[\eta]_\theta = K_\theta\ q_{v.w} <M>_w^{1/2}$$

The values of α_η for the different $\varkappa$-carrageenan samples derived from Eqn. 2.33 are collected in Table 2.4, column 3.

It has been demonstrated by Kurata and Yamakawa (1958) that the viscosity expansion factor, α_η, is related to the mean radius expansion factor α_{R_g} by

$$\alpha_\eta^3 = \alpha_{R_g}^{2.43} \tag{2.34}$$

From the viscosity expansion factors derived by Eqn. 2.33 and the unperturbed radii of gyration derived by Eqn. 2.32 the perturbed radii of gyration can be calculated for the different $\varkappa$-carrageenan samples by

$$\alpha_\eta^{1.23} = <R_g^2>_z^{1/2} / <R_{g\theta}^2>_z^{1/2} \tag{2.35}$$

The values calculated for $<R_g^2>_z^{1/2}$ in this way are collected in Table 2.4, column 4. These values are much lower (about 30%) than the experimental light scattering data which indicates that the relation $\alpha_\eta^3 = \alpha_{R_g}^{2.43}$ does not hold for $\varkappa$-carrageenan coils. Such a discrepancy between α_η^3 and $\alpha_{R_g}^{2.43}$ was also observed for poly (α-L-glutamic acid) by Hawkins and Holtzer (1972). The discrepancy between the experimental values and the values calculated by Eqn.

Table 2-4. Dimensional parameters from light-scattering and viscosity measurements for different κ-carrageenan samples.

Sample	$<R_{g\theta}^2>_z^{1/2}$ nm	$\alpha_\eta = \left[{}^{[\eta]}/_{[\eta]_\theta}\right]^{1/3}$	$<R_g^2>_z^{1/2}$ nm (Eqn. 2-35)	$<R_g^2>_z^{1/2}$ nm (Eqn. 2-38)	$<R_g^2>_z^{1/2}$ nm (light scattering)	$\Phi \cdot 10^{-21}$
EC_{10}	19.7	1.31	27.5	35.3	51.8	0.45
H_5	25.7	1.36	37.5	46.5	64.1	0.54
EC_5	24.4	1.43	38.1	45.9	56.4	0.75
A_{72}	30.2	1.45	47.7	57.8	77.6	0.58
HMR	34.1	1.53	57.6	68.4	91.0	0.60
H_0	35.6	1.51	59.4	69.1	94.5	0.54
PM 30	37.1	1.54	63.1	73.5	98.0	0.58
XM 100A	38.0	1.58	66.1	77.2	105.7	0.54
P 100	38.8	1.58	68.3	78.8	102.3	0.63
EC	41.9	1.62	75.9	87.2	107.8	0.73

2.35 may be explained by the fact that Eqn. 2.34 does not hold for polyelectrolytes, since, as a consequence of the polyelectrolyte effect, experimental values of $[\eta]$ lead to an underestimate of the molecular dimensions (Cleland, 1968).

b. The Ptitsyn and Eizner theory

The radius of gyration can also be obtained from the intrinsic viscosity as described by Ptitsyn and Eizner (1959). For flexible chains the intrinsic viscosity is related to the number average molecular weight by

$$[\eta] = \Phi(\varepsilon)\, 6^{3/2} <(R_g^2)^{3/2}>_n / <M>_n \tag{2.36}$$

where $\Phi(\varepsilon)$ is the Flory-Fox viscosity constant corrected for long-range interactions by a factor ε (for $\varepsilon = 0$ the parameter $\Phi(\varepsilon)$ equals $\Phi_\theta = 2.8 \times 10^{21}$). The parameter ε can be obtained from the exponent in the Mark-Houwink relation (Bloomfield and Zimm, 1966)

$$\varepsilon = (2a_\eta - 1)/3 \tag{2.37}$$

For κ-carrageenan in 0.005 M EDTA, pH 6.7, 0.1 M NaCl the value of the exponent a_η is 0.86, so ε equals 0.24. Ptitsyn and Eizner (1959) and Bloomfield and Zimm (1966) calculated Φ as a function of ε and tabulated $\Phi(\varepsilon)$ for different ε values. For a value of $\varepsilon = 0.24$ the value of $\Phi(\varepsilon)$ has been derived as 1.4×10^{21}.

To introduce the z-average value of the radius of gyration, Eqn. 2.36 needs to be corrected for polydispersity by a factor $q_{z,n}$, which is given by

$$q_{z,n} = (<M>_n / <M>_w)\,(<R_g^2>_z^{3/2} / <(R_g^2)^{3/2}>_n)$$

which for a Schulz-Zimm distribution becomes (see Appendix II)

$$q_{z,n} = \frac{z\Gamma(z)}{(z+1)} \left[\frac{\Gamma(z+3+\varepsilon)}{\Gamma(z+2)}\right]^{3/2} / \Gamma\,(z+3/2+3/2\varepsilon)\,)$$

Eqn. 2.36 can now be rewritten as

$$[\eta] = \Phi(\varepsilon)\, 6^{3/2} <R_g^2>_z^{3/2} / <M>_w q_{z,n} \tag{2.38}$$

The radii of gyration calculated from the viscosity by means of this equation are collected in Table 2.4, column 5. Comparison with the experimental light-scattering data (Table 2.4, column 6) shows a discrepancy for all samples investigated. This discrepancy between the light-scattering data and the radii calculated by Eqn. 2.28 may be due to an overestimation of the parameter $\Phi(\varepsilon)$ (Takahashi et al., 1967). It is shown by Takahashi et al. (1967) that the value of $\Phi(\varepsilon)$ as obtained by Ptitsyn and Eizner underestimates the value of $<R_g^2>_z^{1/2}$ for polyelectrolytes. This is in agreement with the conclusion of Cleland (1968) who suggested that the expansion of polyelectrolytes in good solvents leads to a larger decrease of $\Phi(\varepsilon)$, with respect to the ideal solvent value than in the case of non-ionic polymers. In order to investigate this point further, we calculated the values of $\Phi(\varepsilon)$ for the different $\varkappa$-carrageenan samples from the viscosity- and light-scattering data by Eqn. 2.38. This yields values for $\Phi(\varepsilon)$ varying from 0.45×10^{21} to 0.75×10^{21} (cf. Table 2.4, column 7), which values are comparable with values reported for other polyelectrolytes (Takahashi and Kagawa, 1962; Orofino and Flory, 1959; Eisenberg and Woodside, 1962; Smidsrød 1970 and 1974).

2.4.4. The interpretation of the sedimentation coefficient

For a flexible coiled molecule the radius of gyration can be calculated from the sedimentation coefficient (S^0) by the relation (Yamakawa, 1971).

$$S^0 = \frac{M(1-\bar{v}\rho)}{N \cdot 6^{1/2}\,(\eta^{3/2}/8)\,(1-\varepsilon)\,(3-\varepsilon)\,(6+5\varepsilon+\varepsilon^2)^{1/2} <R_g^2>^{1/2} \eta_0} \tag{2.39}$$

with η_0 the viscosity of the solvent and N Avogadro's number.

Relation 2.39 holds for monodisperse coils and, therefore, needs to be corrected by a factor q_s for polydispersity, given as (see Appendix III)

$$q_s = (<S^0>_w / <M>_w) <R_g^2>_z^{1/2}$$

which for a Schulz-Zimm distribution yields

$$q_s = \frac{\Gamma\left[z+(1/2)(3-\varepsilon)\right]}{[\Gamma(z+2)]^{3/2}} \cdot (\Gamma(z+3+\varepsilon))^{1/2}$$

Eqn. 2.39 can now be rewritten as

$$S^0 = \text{constant}\,(<M>_w / <R_g^2>_z^{1/2}) q_s$$

from which the radius of gyration of sample A_{72} can be derived as 80.4 nm which compares well with the value obtained for that sample with light scattering (77.6 nm).

I. *Molecular weight distributions*

Let us assume that the molecular weight distribution of the carrageenan samples can be described by a Schulz-Zimm distribution (Zimm, 1948)

$$N(P) = \left[y^z/\Gamma(z)\right] P^{z-1} e^{-yP} \tag{A.1}$$

in which $N(P)$ is the number of molecules with a degree of polymerization P:

$$P = M/M_0$$

M being the molecular weight of the molecule and M_0 the molecular weight of the monomer unit. Further z and y are constants characteristic of a given sample. The width of the distribution is determined by the parameter z. The gamma function of z, $(\Gamma(z))$ is defined as

$$\Gamma(z) = \int_0^\infty t^{z-1} \cdot e^{-t} \cdot dt \tag{A.2}$$

with the property

$$\Gamma(z+1) = z\Gamma(z) \tag{A.3}$$

The different molecular weight averages can now be calculated as:

$$<M>_n = \frac{M_0 \int_0^\infty \left[y^z/\Gamma(z)\right] P^z e^{-yP} dP}{\int_0^\infty \left[y^z \Gamma(z)\right] P^{z-1} e^{-yP} dP} = \frac{(M_0/y^{z+1}) \cdot \Gamma(z+1)}{(1/y^z) \cdot \Gamma(z)} =$$

$$= (M_0/y)\,(z) \tag{A.4}$$

$$<M>_w = \frac{M_0^2 \int_0^\infty \left[y^z/\Gamma(z)\right] P^{z+1} e^{-yP} dP}{M_0 \int_0^\infty \left[y^z/\Gamma(z)\right] P^z e^{-yP}\, dP} = \frac{(M_0^2/y^{z+2}) \cdot \Gamma(z+2)}{(M_0/y^{z+1}) \cdot \Gamma(z+1)} =$$

$$= \frac{M_0}{y}\ (z+1) \tag{A.5}$$

and

$$<M>_z = \frac{M_0^3 \int_0^\infty \left[y^z/\Gamma(z)\right] P^{z+2} e^{-yP} dP}{M_0^2 \int_0^\infty \left[y^z/\Gamma(z)\right] P^{z+1} e^{-yP} dP} = \frac{(M_0^3/y^{z+3}) \cdot \Gamma(z+3)}{(M_0^2/y^{z+2}) \cdot \Gamma(z+2)} =$$

$$= \frac{M_0}{y}\ (z+2) \tag{A.6}$$

The distribution (A.1) is used to investigate the relations between different molecular parameters in polydisperse systems.

II. *The relation between intrinsic viscosity and radius of gyration (Eqn. 2.38, Section 2.4.3)*

To compare light-scattering data, we are interested in a relation between the intrinsic viscosity and the z-average value of the radius of gyration. Ptitsyn and Eizner (1959) found for the relation between $[\eta]$ and the number average radius of gyration the following relation

$$[\eta] = \Phi(\varepsilon)\, 6^{3/2} <(R_g^2)^{3/2}>_n / <M>_n \tag{A.7}$$

in which $\Phi(\varepsilon)$ is the Flory-Fox viscosity constant corrected for long-range interference and electrostatic repulsions.

Let the relation between $[\eta]$, the light-scattering average $<R_g^2>_z$ and $<M>_w$ be given by:

$$[\eta] = \Phi(\varepsilon)\, 6^{3/2} <R_g^2>_z^{3/2} / (<M>_w q_{z,n}) \tag{A.8}$$

In which $q_{z,n}$ is defined as:

$$q_{z,n} = (<M>_n / <M>_w) \cdot (<R_g^2>_z^{3/2} / <(R_g^2)^{3/2}>_n)$$

For a Schulz-Zimm distribution $q_{z,n}$ then becomes

$$q_{z,n} = \frac{z}{z+1} \left[\frac{\int_0^\infty R_g^2\, M^2\, N(P)\, dP}{\int_0^\infty M^2\, N(P)\, dP} \right]^{3/2} \Bigg/ \left[\frac{\int_0^\infty (R_g^2)^{3/2}\, N(P)\, dP}{\int_0^\infty N(P)\, dP} \right]$$

Substitutin $R_g^2 = \text{const.}\, M^{a_{R_g}} = \text{const.}\, M^{1+\varepsilon}$ yields

$$q_{z,n} = \frac{z}{z+1} \left[\frac{\int_0^\infty (yP)^{(3+\varepsilon+z-1)} \cdot e^{-yP}\, d(yP)}{\int_0^\infty (yP)^{(2+z-1)} \cdot e^{-yP}\, d(yP)} \right]^{3/2} \cdot \times$$

$$\times \frac{\Gamma(z)}{\int_0^\infty (yP)^{(3/2)(1+\varepsilon)+z-1} \cdot e^{-yP}\, d(yP)} =$$

$$\frac{z\Gamma(z)}{z+1} \left[\frac{\Gamma(z+3+\varepsilon)}{\Gamma(z+2)} \right]^{3/2} \Bigg/ \Gamma\left(z + {}^3/_2\,(1+\varepsilon)\right)$$

The values of $q_{z,n}$ calculated for the different $\varkappa$-carrageenan samples are collected in Table A.1, column 3. The value of ε can be derived from Eqns. 2.15 and 2.37 by which comparable values are obtained of 0.20 and 0.24 respectively. For the calculation of the correction factors $q_{z,n}$ as given in Table A.1 a value of $\varepsilon = 0.24$ is used.

III. *The relation between the sedimentation coefficient and the radius of gyration* (Eqn. 2.39, Section 2.4.4.)

For a flexible coil of uniform molecular weight the radius of gyration is re-

lated to the sedimentation coefficient as (Yamakawa, 1971):

$$S_i^0 = \text{const. } M_i/(R_{gi}^2)^{1/2} \tag{A.9}$$

The constant is given by

$$1/\ 6^{1/2}\ (\pi^{3/2}/8)(1-\varepsilon)(3-\varepsilon)(6+5\varepsilon+\varepsilon^2)^{1/2} \cdot \eta_0 \cdot (1-\bar{v}\rho)/N$$

For heterogeneous samples the weight average sedimentation coefficient is obtained by ultracentrifugation (Schachman, 1959). Let us assume that the relation between the weight average sedimentation coefficient and the light-scattering average of the radius of gyration is given by

$$<S^0>_w = \text{const. } (<M>_w/<R_g^2>_z^{1/2})\, q_s \tag{A.9a}$$

in which q_s is defined as

$$q_s = (1/\text{const.})\,(<S^0>_w/<M>_w) \cdot <R_g^2>_z^{1/2} =$$

$$= (1/\text{const.})\ \frac{\sum_i c_i S_i}{\sum_i c_i}\left[\frac{\sum_i c_i M_i R_{gi}^2}{\sum_i c_i M_i}\right]^{1/2} \cdot \frac{\sum_i c_i}{\sum_i c_i M_i} =$$

$$= (1/\text{const.})\left[\frac{\sum_i c_i M_i}{(R_{gi}^2)^{1/2}} \Big/ (c_i M_i)^{3/2}\right] \sum_i c_i M_i (R_{gi}^2)^{1/2}$$

Substituting $R_g^2 = \text{const. } M^{1+\varepsilon}$ gives

$$q_s = \frac{\sum_i n_i M_i^{2-(1/2)(1+\varepsilon)}}{(\sum_i n_i M_i)^{3/2}}\left[n_i M_i^{1+\varepsilon+2}\right]^{1/2}$$

For a Schulz-Zimm distribution q_s can be given as

$$q_s = \frac{\int_0^\infty (yP)^{2-(1/2)(1+\varepsilon)+z-1} \cdot e^{-yP} \cdot d(yP)}{(\int_0^\infty (yP)^{2+z-1} \cdot e^{-yP} \cdot d(yP))^{3/2}}\left[\int_0^\infty (yP)^{2+1+z-1} \cdot e^{-yP} \times \right.$$

$$\left. \times\, d(yP)\right]^{1/2} = \frac{\Gamma(z+{}^1\!/_2(3-\varepsilon))}{(\Gamma(z+2))^{3/2}} \cdot (\Gamma(z+3+\varepsilon))^{1/2}$$

For sample A_{72} with $z = 1.01$ the value of q_s equals 1.26.

IV. *The relation between the radius of gyration and the weight average molecular weight* (Eqn. 2.15, Section 2.3.1.)

The radius of gyration for a macromolecule is related to the molecular weight by (Tanford, 1961)

$$R_{gi}^2 = K_{R_g} M_i^{qR_g} \tag{A.10}$$

Let for a polydisperse macromolecule the relation between the light-scattering average radius of gyration and the weight average molecular weight be given by

$$<R_g^2>_z = K_{R_g} (q_{z.w} <M>_w)^{a_{R_g}} \tag{A.11}$$

with

$$q_{z.w} = \frac{\sum_i c_i M_i R_{gi}^2}{\sum_i c_i M_i} \cdot (1/<M>_w^{a_{R_g}})$$

which for a Schulz-Zimm distribution yields:

$$q_{z.w} = \frac{\sum_i n_i M_i^{2+a_{R_g}}}{\sum_i n_i M_i^2} \cdot (1/<M>_w^{a_{R_g}}) = \frac{\Gamma(z+2+a_{R_g})}{(\Gamma(z+2))\,(z+1)^{a_{R_g}}}$$

To start the iteration procedure, assume the exponent equals 1, so the correction factor

$$q_{z.w} = \frac{<M>_z}{<M>_w} = \frac{z+2}{z+1}$$

From the slope of a plot of $\log <R_g^2>_z$ vs $\log [(z+2)/(z+1)<M>_w]$ the first approximation of the exponent a_{R_g} can be obtained, which has to be substituted in the relation for $q_{z.w}$ for the next cycle. The final values of $q_{z.w}$ are collected in Table A.1, column 4.

V. *The Mark-Houwink relation* (Eqn. 2.19, Section 2.3.4.)
For a polymer with molecular mass M_i the intrinsic viscosity is related to its molecular weight by (Tanford, 1961)

$$[\eta_i] = K_\eta M_i^{a_\eta} \tag{A.12}$$

For a polydisperse polymer the relation between the intrinsic viscosity and the viscosity average molecular weight can now be given by

$$[\eta] = K_\eta <M>_i^{a_\eta} \tag{A.13}$$

The viscosity average molecular weight is defined as (Tanford, 1961)

$$<M>_v = (\sum_i n_i M_i^{1+a_\eta} / \sum_i n_i M_i)^{1/a_\eta} \tag{A.14}$$

which for a Schulz-Zimm distribution can be written as

$$<M>_v = (M_0/y) \left\{ \frac{\Gamma(z+a_\eta+1)}{\Gamma(z+1)} \right\}^{1/a_\eta} \tag{A.15}$$

The intrinsic viscosity can be given as a function of the weight average molecular weight by

$$[\eta] = K_\eta q_{v.w} <M>_w^{a_\eta} \tag{A.16}$$

In which the correction factor $q_{v.w}$ is defined by

$$q_{v.w} = <M>_v^{a_\eta} / <M>_w^{a_\eta} = (\Gamma(a_\eta + z + 1) / \Gamma(z+1)) \cdot 1/(z+1)^{a_\eta}$$

The slope of a plot of log $([\eta]/q_{v.w})$ vs log $<M>_w$, with $q_{v.w} = 1$, gives a first approximation of a_η and a $q_{v.w}$ for the next cycle. After two steps a constant value of a_η was obtained. The values of $q_{v.w}$ calculated for the different samples and for the different values of the exponents are collected in Table A.1, column 5-9.

VI. *The Stockmayer-Fixman relation for polydisperse systems* (Eqn. 2.21. Section 2.4.2.)

For monodisperse material the Stockmayer-Fixman relation reads (Stockmayer and Fixman, 1963)

$$[\eta] = KM^{1/2} + 0.51\, \Phi\, BM \tag{A.17}$$

For polydisperse samples a correction is needed, which can be derived as follows.

The viscosity expansion factor α_η is for each species i given as (Yamakawa, 1971)

$$\alpha_{\eta i}^3 = [\eta]_i / KM_i^{1/2} = 1 + 1.55\, z^* \tag{A.18}$$

with

$$z^* = (3/2\pi)^{3/2} \cdot (B/A^3)\, M_i^{1/2},$$

$$K = \Phi_\theta\, A^3, \text{ and}$$

A and B constants

For species i therefore

$$[\eta]_i = K_\theta\, M_i^{1/2} + 0.51\, \Phi_\theta\, BM_i \tag{A.19}$$

Summation over all species yields

$$[\eta] = \frac{\sum_i c_i [\eta]_i}{\sum_i c_i} = \frac{K \sum_i c_i M_i^{1/2}}{\sum_i c_i} + 0.51\, \Phi_\theta\, B \sum_i c_i M_i / \sum_i c_i =$$

$$= K <M>_w^{1/2} q_{S.F} + 0.51\, \Phi_\theta\, B <M>_w$$

with $q_{S.F}$

$$q_{S.F} = \frac{\sum_i c_i M_i^{1/2}}{\sum_i c_i} \left[\frac{\sum_i c_i}{\sum_i c_i M_i} \right]^{1/2}$$

which for a Schulz-Zimm distribution becomes

$$q_{S.F} = \frac{\Gamma(z+3/2)}{(\Gamma(z+1))^{1/2}} \cdot \frac{1}{(\Gamma(z+2))^{1/2}} = \frac{1}{(z+1)^{1/2}} \cdot \frac{\Gamma(z+3/2)}{\Gamma(z+1)}$$

The Stockmayer-Fixman relation can now be written in terms of weight average molecular weights as

$$[\eta]/(q_{S.F}) < M >_w^{1/2} = K_\theta + 0.51\, \Phi_\theta\, B < M >_w^{1/2} q_{S.F}$$

The values of $q_{S.F}$ calculated for the different $\varkappa$-carrageenan samples are collected in Table A.1, column 10.

Table A-1. Correction factors q for polydispersity of the $\varkappa$-carrageenan samples.

Sample	z	$q_{z.n}$	$q_{z.w}$	$q_{v.w}$					$q_{S.F}$
			$(a_{R_g} = 1.20)$	$(a_\eta = 0.50)$	$(a_\eta = 0.67)$	$(a_\eta = 0.78)$	$(a_\eta = 0.86)$	$(a_\eta = 0.90)$	
H_{20}	1.17			0.944	0.952	0.963	0.978	0.981	0.94
H_{10}	0.96			0.939	0.946	0.960	0.974	0.979	0.94
EC_{10}	1.02	2.33	1.714	0.941			0.974		0.94
H_5	0.76	2.59	1.784	0.932	0.942	0.955	0.971	0.977	0.93
EC_5	1.54	2.02	1.537	0.952	0.959	0.968	0.979	0.983	0.95
A_{72}	1.01	2.33	1.685	0.940			0.974		0.94
HMR	1.96	1.85	1.456	0.958			0.981		0.96
H_0	3.23	1.57	1.391	0.971			0.986		0.97
PM 30	3.23	1.57	1.391	0.971			0.986		0.97
XM 100A	3.23	1.57	1.391	0.971	0.974	0.978	0.986	0.989	0.97
P 100	3.23	1.57	1.391	0.971			0.986		0.97
EC	3.23	1.57	1.391	0.971			0.986		0.97

III. ON THE SOL-GEL TRANSITION IN CARRAGEENAN SOLUTIONS

3.1. INTRODUCTION

It was demonstrated in Chapter II that the κ-carrageenan molecule in solutions of different ionic strength can best be described as a random coil which is more or less expanded as a result of excluded volume effects and electrostatic repulsion between the chain segments. On cooling such a κ-carrageenan solution an interaction between the κ-carrageenan molecules has been observed, leading to the formation of an infinite complex.

This phenomenon, called gel formation, is a property of κ- and ι-carrageenan, whereas the λ-component lacks this ability (Stone, 1969; Rees, 1969 and Glicksman, 1969). For κ-carrageenan it has been demonstrated that specific ion effects are involved in the gel formation (Rees, 1969; Payens and Snoeren, 1972). The latter showed that the effectivity of the cations follows a Hofmeister series.

The gel formation in solutions of κ- and ι-carrageenan is thermoreversible (Rees, 1972; Dea et al., 1972), and this fact together with the abruptness of the phenomenon suggests that gel formation might be treated as a two-state transition.

The explosiveness of gelation can be explained in two ways. First, according to Flory (1953) and Stockmayer (1944) gel formation in itself is highly cooperative due to the exponentially increasing capacity of branching polymers for further branching. Second, Rees (1969) suggested that the cross-links of a carrageenan gel consist of double helical segments, not unlike the situation encountered with the gelatin gel (Traub and Piez, 1971). The existence of ordered structures in fibres of crude carrageenan was demonstrated by Baily (1955), using X-ray analysis. More recently Anderson et al. (1969) confirmed this for purified κ- and ι-carrageenan fibres and interpreted their X-ray data as due to double helices. Also the characteristic change in the optical rotation during gelation of κ- and ι-carrageenan solutions (McKinnon et al., 1969 and Dea et al., 1972) and the extrinsic Cotton effects induced by methylene blue addition (Stone, 1972) have been interpreted as such.

This chapter deals in particular with the conformational aspect of gel formation. It is shown that for κ- and ι-carrageenan solutions there exists a linear relation between the temperature of incipient gelation and the logarithm of the salt concentration. This result is reminiscent of a similar influence of salt on the coil/double helix transitions of various polynucleotides (Dove and Davidson, 1963; Schildkraut and Lifson, 1965; Record, 1967 and Kotin, 1963).

We have therefore made an attempt to correlate the shift of the gelpoint of κ- and ι-carrageenan solutions brought about by salt with the double helix parameters reported for κ- and ι-carrageenan fibres by Anderson et al. (1969).

Parallel studies of the salt effect by penetrometry have shown that the firmness of κ-carrageenan gels increases with salt concentration. For κ-carrageenan also the influence of urea on gelpoints depression has been measured using optical rotation.

The conformational transition as observed by optical rotation becomes less explosive with decreasing molecular weight of the κ-carrageenan. This result confirms the finding of Applequist and Damle (1965) who observed a similar influence of molecular weight on the coil-double helix transition of oligoadenylic acid.

3.2 MATERIALS

The κ-carrageenan samples used were purified samples of Genulacta K 100 manufactured by Kobenhavns Pektinfabrik, Copenhagen and an extract of Eucheuma cottonii which was a generous gift from Pierrefitte-Auby, Paris. Iota- and λ-carrageenan were gifts from Pierrefitte-Auby, Paris, and Marine Colloids, Rockland, respectively. The samples were purified as described in Chapter II. Kappa-carrageenan fractions of lower molecular weight were prepared by acidic hydrolysis (cf. Chapter II).

3.3. METHODS

3.3.1. *Optical rotation*

The changes in optical rotation of carrageenan solutions were followed in a Perkin-Elmer Model 141 polarimeter at 365 nm with simultaneous recording of the temperature. A cell of 10 cm path length was used.

3.3.2 *Light-scattering*

Sol-gel transition temperatures of the κ-carrageenan solutions were determined by light scattering as described by Payens and Snoeren (1972) by recording simultaneously the light scattered at 45° and the temperature of the solution. The wave length of the light used was 480 nm.

3.3.3 *Viscosity measurements*

The setting temperatures of ι-carrageenan solutions were determined by measuring the viscosities of the solutions as a function of temperature. The viscosities were measured as efflux times using Ubbelohde viscosimeters with efflux times for water of about 300 sec.

3.3.4 *Differential scanning calorimetry*

Differential scanning calorimetry was performed in the Dupont 900 B thermal analyzer. The calibration factor converting peak area's into calories, was established as described in the manufacturer's manual, using the melting of Ga as a reference. For the enthalpy calculation the peaks corresponding to the heat release on gelation were taken. The maximum ordinate temperatures of the peaks agreed well with the setting temperatures found by light-scattering, polarimetry- and viscosity measurements. Since the enthalpy change accompanying the gelation is relatively small, the pans were completely filled to the brim with about 40 mg of carrageenan solution on the top of which the cover was put upside down. After removal of superfluous solution and weighing, the pans and the hole in the cover were sealed. They were heated to about 70°C and subsequently cooled at a rate of 5 deg./min.

3.3.5 *Penetrometry*

The gel strength was measured with a Sommer Rung KG penetrometer using a plunger of 30.2 g with a top angle of 60°. Solutions containing 0.75% κ-carrageenan were gelled with increasing amounts of potassium chloride. After setting, the gels were stored overnight at 7°C at which temperature the measurements were also carried out. Before measuring the penetration, the plunger was placed on the gel surface. The penetration was measured after different puncture times.

3.4 RESULTS

3.4.1. *Optical rotation*

Fig. 3.1 presents some typical examples of transitions observed by polarimetry with different carrageenans.

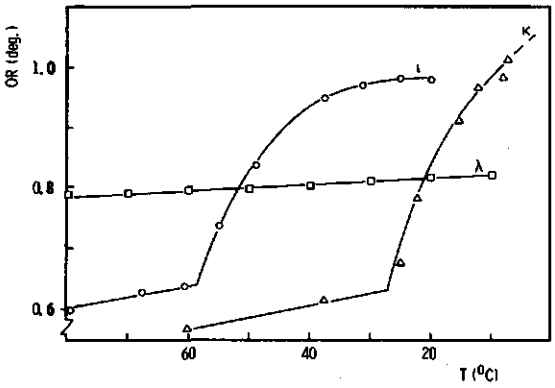

Fig. 3.1.
Temperature dependence of the optical-rotation of different carrageenan solutions in the presence of 0.3 M NaCl. Carrageenan concentration: ± 0.4%.

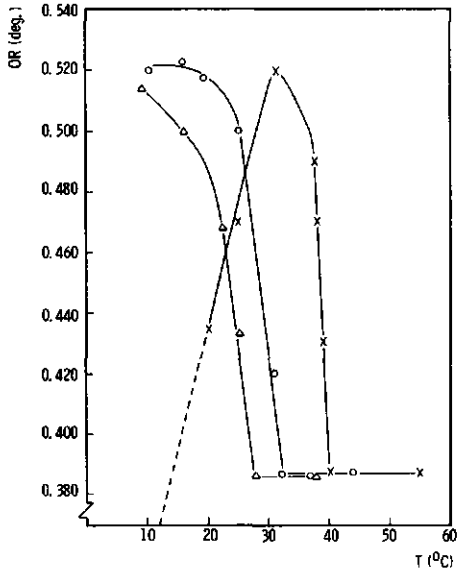

Fig. 3.2. Temperature dependence of the optical rotation of κ-carrageenan solutions in the presence of KCl.
Carrageenan concentration: 0.23%.
△ : 0.02 M KCl; ○ : 0.03 M KCl;
✱ : 0.06 M KCl.

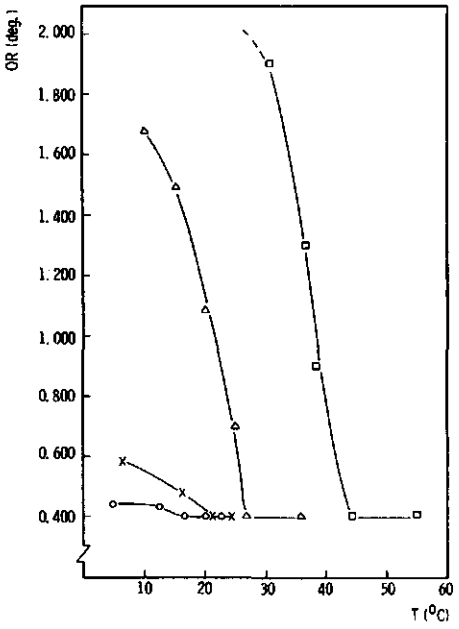

Fig. 3.3. Temperature dependence of the optical rotation of κ-carrageenan solutions in the presence of NaCl.
Carrageenan concentration: 0.25%.
○ : 0.1 M NaCl; ✱ : 0.2 M NaCl;
△ : 0.4 M NaCl; □ : 0.8 M NaCl.

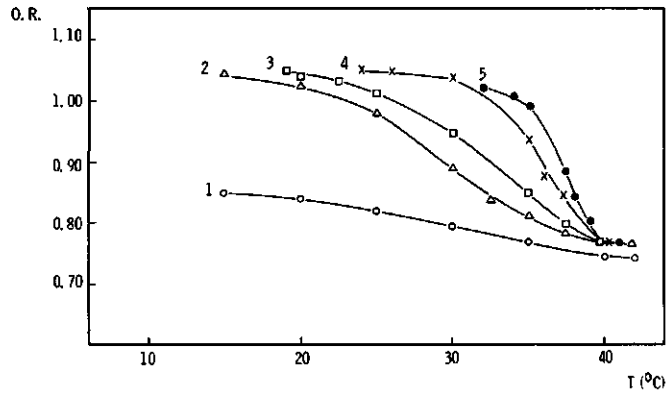

Fig. 3.4. Optical rotation of gelling κ-carrageenan solutions as a function of temperature. $1 : <M>_n = 5\,800$; $2 : <M>_n = 23\,700$; $3 : <M>_n = 42\,700$; $4 : <M>_n = 107\,500$; $5 : <M>_n = 530\,000$.
Carrageenan concentration: 0.5%; 0.05 M KCl added.

No conformational change was observed in the λ-carrageenan solution.

In Figs. 3.2 and 3.3 some examples are given of optical rotation changes as a function of the temperature of κ-carrageenan solutions containing different amounts of KCl and NaCl respectively. One should note that in most cases the OR changes do not come to completion within the time of observation. With KCl, especially at salt concentrations exceeding 0.03 M, the initial increase of the OR is followed by a secondary process, which leads to a drastic reversal of original increases.

Similar observations have been reported by McKinnon (1969) and by Whistler (1973). With NaCl, on the other hand, the OR of the system increases steadily without reaching a plateau.

Fig. 3.4 shows typical examples of transitions observed by polarimetry with κ-carrageenan samples of different molecular weight. Obviously transitions become steeper when the molecular weight of κ-carrageenan increases.

The transition temperatures of κ-carrageenan solutions, to which different amounts of urea were added, as found by polarimetry were plotted as a function of the urea concentration in Fig. 3.5. The setting temperature decreases linearly with increasing urea concentration.

3.4.2 *Sol-gel transitions*

Fig. 3.6 presents examples of transition plots as measured by light scattering in various electrolyte solutions.

The viscosity data i.e. the efflux time of ι-carrageenan in different salt

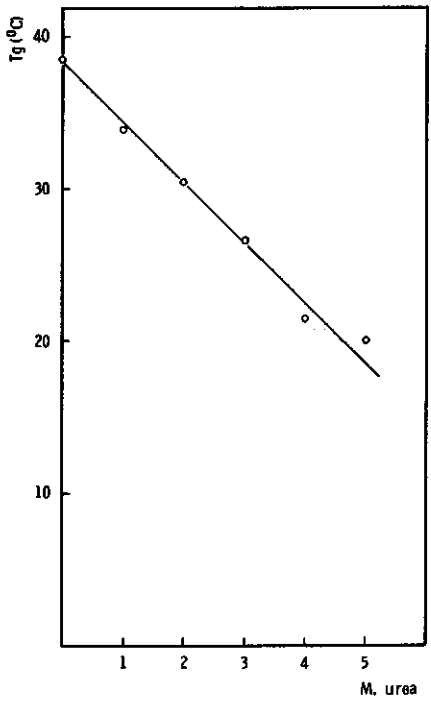

Fig. 3.5.
Sol-gel transition temperatures of κ-carrageenan as a function of urea concentration. Carrageenan concentration 0.5%; 0.05 M KCl added.

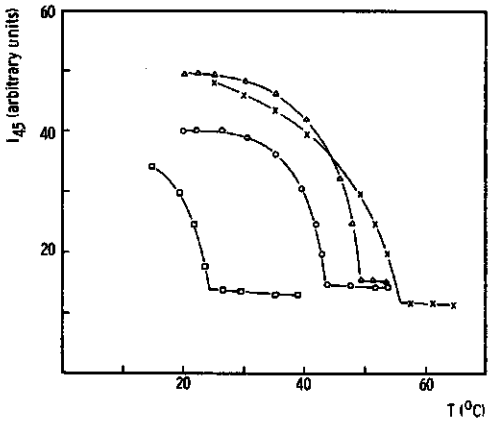

Fig. 3.6.
Light-scattering transition plots of gelling κ-carrageenan with various electrolytes added. Carrageenan concentration: 1%.
○: 0.063 M KCl; △: 0.125 M KCl;
□: 0.25 M NaCl; ✱: 1.5 M NaCl.

solutions as a function of temperature are given by Fig. 3.7. At the setting temperature a break in the curve is observed.

The shifts of the temperature of the incipient increases in light scattering and optical rotation observed for κ-carrageenan solutions 'the gelpoint', brought

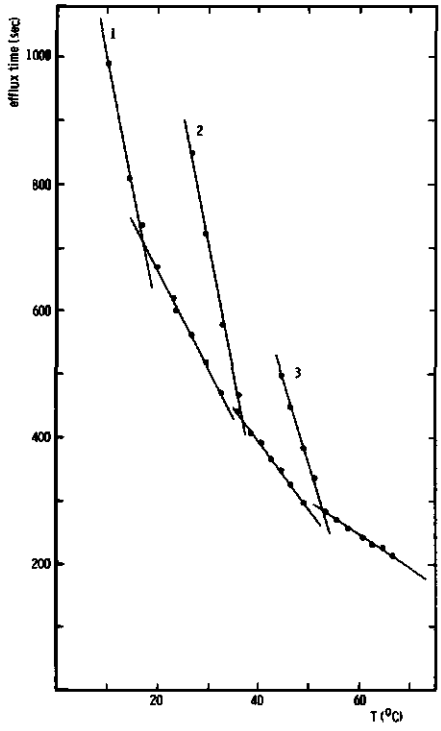

Fig. 3.7.
Temperature dependence of the efflux times of 0.1% ι-carrageenan solutions in the presence of KCl.
1 : 0.02 M KCl; 2 : 0.06 M KCl;
3 : 0.15 M KCl.

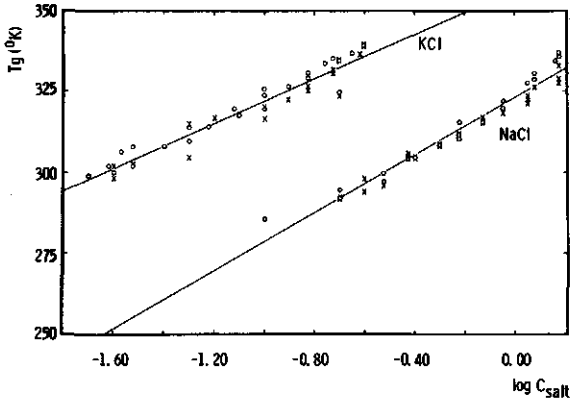

Fig. 3.8.
Dependence of the setting temperature of κ-carrageenan gels on the electrolyte concentration as measured by optical rotation and light scattering.
○: optical rotation,
± 0.25% κ-carrageenan.
× : light scattering, 1% κ-carrageenan.

about by salt addition, are collected in Fig. 3.8. With both measuring techniques the gelpoint, *Tg*, appears to be linearly dependent on the logarithm of the salt concentration. The close relation between both phenomena is demonstrated by the fact that the separate, light-scattering and polarimetry regression lines

3.4.4. *Penetrometry*

Results of the penetrometric experiment are collected in Table 3.3. It is evident that gel firmness increases with increasing salt concentration.

Table 3-3. Penetration (mm) of a conus (30.2 g) with a top angle of 60° in κ-carrageenan gels (0.75%) to which different amounts of KCl are added.

KCl concentration (M)	penetration after different puncture times (mm)			
	0.5 sec.	1 sec.	2 sec.	8 sec.
0.015	22.0	22.0	22.3	22.4
0.02	19.6	20.2	20.5	21.0
0.025	16.0	16.5	16.7	17.0
0.03	13.6	14.6	14.6	14.6
0.04	10.8	11.1	11.2	11.3
0.05	8.6	8.6	9.0	9.2
0.06	8.5	9.0	9.2	9.3
0.08	7.0	7.5	7.5	7.7
0.1	6.9	7.1	7.3	7.6
0.12	6.9	7.0	7.1	7.4
0.15	6.4	6.5	6.5	6.8
0.2	5.6	5.8	5.9	6.0
0.25	5.2	5.4	5.5	5.8

3.5. DISCUSSION

3.5.1. *The sol-gel transition of κ-carrageenan*

The increase in light scattering observed at the gelpoint of κ-carrageenan solutions is indicative of the formation of cross-links (Boedtker and Doty, 1954; Payens and Snoeren, 1972). A quantitative interpretation of this increase in terms of the number and extension of the cross-links is, however, very difficult and would require more sophisticated instrumentation than that used in this study (Keyzers et al., 1965).

The coincidence of the temperatures of the incipient changes in light scattering and optical rotation (cf. Fig. 3.8) demonstrates convincingly that the setting of the gel of κ-carrageenan is accompanied by a conformational change.

The idea that this conformational change is to be identified with a coil/double helix transition as proposed by Rees (1969, 1972) lies to hand. This becomes the more probable, because a number of authors (Dove and Davidson, 1962; Schildkraut and Lifson, 1965) have shown that for such a transition the midpoint temperature, T_m, is linearly dependent on the logarithm of the electrolyte

concentration and the difference between T_m and the gelpoint, Tg, is almost negligible on the Kelvin-scale.

The logarithmic salt effect has been explained in terms of electrical work when going from the helical to the coiled state (Schildkraut and Lifson, 1965; Record, 1967 and Kotin, 1963) and by the liberation of condensed counterions (Oosawa, 1971; Manning 1972 and Record, 1975).

3.5.2. *The conformational change of κ-carrageenan discussed in terms of the electrical work*

Let us first try to analyse the shift of the gelpoint brought about by salt in terms of difference in charge densities of the helix and the coil.

At the midpoint of the transition, T_m, the chemical potentials of carrageenan in the sol and in the gel state are equal and therefore

$$\bar{G}_s = \bar{G}_g, \tag{3.1}$$

where the subscripts s and g stand for the coil (sol) and helix (gel) state respectively. If, as customary in theories of this type, (Schildkraut and Lifson, 1965; Record, 1967; Kotin, 1963; Oosawa, 1971 and Manning, 1972) it is assumed that the free enthalpy is composed of independent electrostatic and non-electrostatic contributions, i.e.

$$\bar{G} = \bar{G}^0 + \bar{G}^{el}, \tag{3.2}$$

then it is readily shown that

$$T_m = T_m^0 + \Delta\bar{G}^{el}/\Delta\bar{S}^0 \tag{3.3}$$

where

$$T_m^0 = \Delta\bar{H}^0/\Delta\bar{S}^0 \tag{3.4}$$

and $\Delta\bar{G}^{el}$, $\Delta\bar{H}^0$ and $\Delta\bar{S}^0$ are the differences in partial molar electrostatic free enthalpy, non-electrostatic enthalpy and non-electrostatic entropy between both states respectively.

An expression for $\Delta\bar{G}^{el}$, per mole of pairing residue, which is based on discrete-charge models of the helix and coil, was given by Record (1967):

$$\Delta\bar{G}^{el} = -\frac{Ne^2Z_p^2}{D}\left[n_{s/l}\left\{\ln\frac{\varkappa l}{n_s} + \frac{2\pi\gamma}{\beta} + \sum_{m=1}^{2\pi/\beta-1}\ln(\varkappa a\cdot\sin m\beta/_2)\right\} - {}^1/_{b_c}\,(\ln\varkappa b_c + \gamma)\right] \tag{3.5}$$

with N Avogadro's number, e the elementary charge, Z_p the effective valency of the polymer's ionizable groups (cf. Schildkraut and Lifson, 1965), D the dielectric constant, and γ Euler's constant (0.5772). Further, $\varkappa$ is the reciprocal Debye Hückel length, i.e. for univalent-univalent electrolyte solution:

$$\varkappa = (8\pi e^2 NC_s/10^3 DkT)^{1/2}$$

with C_s the molar salt concentration.

The other parameters in Eqn. 3.5 refer to the geometry of the charge distributions in the helical and the coiled state. Thus n_s is the strand number, a the radius of the helix-enclosing cylinder, b_c the distance between adjacent charges on the coil, β the residue rotation in the helix and l its translational repeat distance.

Record (1967) found by straightforward differentiation of Eqn. 3.5 for the slope of the T_m vs log C_s plot

$$dT_m/d \log C_s = -1.15\, Ne^2 \left[Z_p^2/D\,(n_s/b_h - {}^1/b_c)\right] / \Delta\bar{S}^0 \qquad (3.6)$$

with $b_h = \beta l/2\pi$.

Equation 3.6 can be applied to the KCl plot of Fig. 3.8 provided the following conditions are satisfied:

1. The gelpoint, Tg, is identical with the midpoint temperature, T_m, of the coil/double helix transition. As can be seen from Fig. 3.2 the difference between Tg and T_m is indeed within a few percent.
2. At KCl concentrations beyond 0.03 M a secondary conformational process takes place as is evident from the reversal of the OR. At lower salt concentrations the contribution of this secondary process to the transition enthalpy can be neglected.
3. The dimensions of the $\varkappa$-carrageenan double helix in the gel are equal to those established by Anderson et al. (1969) in oriented fibres, i.e. $a = 0.375$ nm, $\beta = 120°$ and $l = 2.46$ nm. Further the distance between neighbouring charges on the coil is maximal, $b_c = 1.03$ nm (cf. Chapter II).
4. The coil/helix transition entropy is additively built up from non-electrostatic and electrostatic contributions

$$\Delta\bar{S} = \Delta\bar{S}^0 + \Delta\bar{S}^{el} \qquad (3.7)$$

Let us start with an estimation of the various entropy contributions. The partial molar transition entropy $\Delta\bar{S}$ is found from the heat of gelation viz.

$$\Delta\bar{S} = \Delta\bar{H}/Tg \qquad (3.8)$$

$\Delta\bar{S}^0$ can be estimated as follows. By definition

$$\Delta\bar{S}^{el} = -(\partial\Delta\bar{G}^{el}/\partial T)_p \qquad (3.9)$$

and therefore $\Delta\bar{S}^{el}$ can be obtained by differentiation of Eqn. 3.5 towards the temperature. Assuming the temperature dependence of the dielectric constant to be given by the empirical Wyman-relation (MacInnes, 1961)

$$D = 78.54\,(1 - 4 \cdot 6 \times 10^{-3}\,(T - 298) + 8 \cdot 8 \times 10^{-6}\,(T - 298)^2) \qquad (3.10)$$

Table 3-4. Partial molar entropy changes (e.u./mol. disaccharide) accompanying the gelation of different carrageenans in various salt media.

carrageen	salt	salt conc. M.	$-\Delta\bar{S}$	$+\Delta\bar{S}^{el}$	$-\Delta\bar{S}^{0}$	$Z_p^2/D \cdot 10^3$
$\varkappa$	KCl	0.025	4.40	0.34	4.74	2.90
		0.03	4.33	0.32	4.65	
		0.05	4.46	0.23	4.69	
		0.07	6.25	0.18	6.43	
		0.1	6.27	0.12	6.39	
$\varkappa$	NaCl	0.1	0.31			
		0.2	0.54			
		0.3	1.70			
		0.75	4.04			
		1.00	4.00			
ι	KCl	0.02	4.92	1.66	6.54	2.86
	NaCl	0.1	2.45	0.34	2.79	1.30
	$CaCl_2$	0.003	1.88	1.50	3.38	2.05

one then finds for $\Delta\bar{S}^{el}$,

$$\Delta\bar{S}^{el} = \frac{Ne^2Z_p^2}{2DT}\left[0 \cdot 44\,(n_s/b_h - 1/b_c) + 0 \cdot 72\,T/D\left\{n_s/l\,(\ln \varkappa l/n_s + \right.\right.$$
$$\left.\left. + 2\pi\gamma/\beta + \sum_{m=1}^{2\pi/\beta-1} \ln(\varkappa a \sin m\beta/2)) - 1/b_c\,(\ln \varkappa b_c + \gamma)\right\}\right] \qquad (3.11)$$

The values of $\Delta\bar{S}^{el}$ calculated on the basis of Eqn. (3.11) are collected in Table 3.4, together with the values of $\Delta\bar{S}$ and $\Delta\bar{S}^{0}$.

The magnitude of the electrostatic contribution will depend upon a number of factors, the most important of which is the concentration of small positive ions. The value of $\Delta\bar{S}^{el}$ decreases with increasing salt concentration as can directly be derived from Eqn. (3.11). It is seen that in the salt concentration range concerned $\Delta\bar{S}^{el}$ constitutes only a minor part of the total entropy change.

The total entropy change, $\Delta\bar{S}$, is, according to the second of the above assumptions, best estimated at 0.025 M.KCl as −4.4 e.u./mole of pairing residue. It is interesting to compare this value with the value found for the coil-double helix transitions observed with polynucleotides. Apparently the $\Delta\bar{S}^{0}$ value for the $\varkappa$-carrageenan coil/double helix transition is considerably lower than the corresponding estimates for polynucleotides, which range between −10 and −15 e.u./mole (Record, 1967 and De Voe, 1969). The difference is most probably explained by the fact that the $\varkappa$-carrageenan double helix is stabilized by only 1 hydrogen bond per disaccharide unit and, as a consequence of its thinner

structure, also offers less opportunity for stacking interactions.

Returning to the slope of T_m vs log C_s plot as given by Eqn. (3.6) we find that we are now able to compute a first approximation of the ratio Z_p^2/D from the experimental slopes, the helix parameters given by Anderson et al. (1969) and the calculated $\Delta\bar{S}^0$.

The value obtained for the ratio Z_p^2/D is then substituted in Eqn. (3.11) to recalculate $\Delta\bar{S}^{el}$. After three iteration steps the convergent values of $\Delta\bar{S}^{el}$ and $\Delta\bar{S}^0$ are obtained; actually these are the figures collected in Table 3.4. Comparison of the theoretical and experimental slopes then yields $Z_p^2/D = 2.9 \times 10^{-3}$ c.g.s. units.

Such low values for this ratio are also obtained for polynucleotides and are discussed by Record (1967) and by Schildkraut and Lifson (1965) in terms of the deviation of the Debye-Hückel approximation on which Eqn. (3.6) is based and on charge reduction as a result of counter ion binding. If it is assumed that the dielectric constant has the bulk value of $D = 80$, then the value for the sulphate charge of the $\varkappa$-carrageenan molecule derived from the ratio Z_p^2/D is reduced to approximately -0.45 e. This indeed could indicate counterion binding.

The possible occurrence of counterion binding prompts us therefore to investigate the results of Fig. 3.8 also in terms of counterion condensation.

3.5.3. *The coil-helix transition of $\varkappa$-carrageenan discussed in terms of Manning's theory*

In Manning's theory (Manning, 1972) the linear polyelectrolyte is represented as a uniform line charge. It was proposed by Manning that for the coil-helix transition of DNA both effects i.e., the electrostatic free energy change and the condensation of counterions, were important. The second effect, the counterion condensation on the polyion occurs up to the net linear charge density of the polyion is reduced below a critical value. Since the helix-coil transition is accompanied by a change in linear charge density, counterion condensation may take place when going from the coil to the helix state. As a consequence of this ion condensation the net linear charge density on the polyelectrolyte becomes sufficiently small to treat the interactions between the polyion and the mobile small ions with the Debye-Hückel approximation. Manning (1972) applied the theoretical model to explain the influence of cations on the transition temperatures of DNA and derived for the slope of the T_m vs log C_s plot

$$dT_m/d \log C_s = \frac{1.15 (\xi_c^{-1} - \xi_h^{-1}) R T_m^2}{\Delta\bar{H}} \tag{3.12}$$

with R the gas constant and ξ a dimensionless quantity which for 1–1 electrolyte solutions is defined as

$$\xi = e^2/DkTb \tag{3.13}$$

The average distance between the projections of the charges on the cylindrical axis of the locally extended polymer chain is given by b_h. It should be noted that according to this definition b_h equals half the translational repeat distances of charges on the helix in Record's Eqn. 3.5. It is seen that the slope of the T_m vs log C_s plot is largely determined by the difference in the projected charge distances, b_c and b_h, in the coil and the helix.

Manning showed that if ξ is less than unity the interactions of all small ions with the polyion may be treated in the Debye-Hückel approximation when the solution is sufficiently diluted. If, however, ξ exceeds unity, counterions will condense on the polyion. According to Eqn. 3.13, the critical projected charge density for ion condensation is found to be 0.71 nm. Therefore, for κ-carrageenan in the coiled state ($b_c = 1.03$ nm) no ion condensation occurs, whereas in the helical state ($b_h = 0.43$ nm) $\xi_h = 1.65$. According to the theory, the fraction $1 - 1/1.65 = 0.42$ of the sulphate groups on the κ-carrageenan molecule in the helical state is neutralized by condensed or bound counterions. From this result, the enthalpy obtained for the κ-carrageenan transition at 0.025 M KCl and an average value of $T_m = 315$°K the theoretical slope can be calculated using Eqn. (3.12). The value obtained in this way (72.5°) is about twice the experimental one. A similar discrepancy between theoretical and experimental slopes was found for DNA by Manning (1972). The discrepancy may be explained by the deviation from the Debye Hückel approximation on which Manning's theory is also based (Manning, 1972).

3.5.4. *The secondary process observed in κ-carrageenan solutions*

The reversal of the OR, the increase of the heat of gelation at salt concentrations beyond 0.03 M KCl and the increase of gel firmness with increasing salt concentration, all suggest that the coil/helix transition is followed by another process which is also exothermic. The nature of this secondary process is not known. Bryce et al. (1974) have recently proposed that it consists of the aggregation of double helices into the junctions of a carrageenan network. This is in agreement with recent electron microscopic work from this laboratory which

Table 3-5. The theoretical slope of the T_m vs log C_s plot and the reduced charge densities as calculated for κ- and ι-carrageenan using Eqns. 3-12 and 3-13.

carrageenan	salt	ξ_c	ξ_h	$\xi_c^{-1} - \xi_h^{-1}$	$dT_m/d \log C_s$
κ	KCl	1	1.65	0.42	72.5°
ι	KCl	1.36	3.23	0.42	62.7°
ι	NaCl	1.36	3.23	0.42	108°
ι	$CaCl_2$	1.36	3.23	0.11*	47.4°

* $(0.25/\xi_c - 0.25/\xi_h)$

bridges between the acidic groups of casein and carrageenan (Glicksman, 1969 (cf. Fig. 4.1). In the presence of calcium ions, complex formation between carrageenan and all main caseins was observed by means of electrophoresis and sedimentation experiments by Grindrod and Nickerson (1968) and Lin and Hansen (1970) respectively. This finding is in conflict with light-scattering results reported by Payens (1972) which indicated that complex formation with $\varkappa$-carrageenan took place exclusively with $\varkappa$-casein. The specific affinity of $\varkappa$-casein for $\varkappa$-carrageenan is further demonstrated in the absence of calcium ions by electrophoresis (Grindrod and Nickerson, 1968).

Interactions between proteins and polysaccharides have received considerable attention during the last few decades (Mathews, 1965; Mathews and Decker, 1968; Öbrink and Wasteson, 1971; Steven et al, 1969 and Öbrink, 1973). This is undoubtedly due to the important role of such interactions in various biochemical processes such as the organization of connective tissue (Öbrink, 1973), the action of blood anti-coagulants (Sasaki and Noguchi, 1959) and antigen-antibody interactions (Kabat, 1968).

In a number of cases complex formations between negatively charged macromolecules have been observed, and although both polymers carry a net negative charge the interaction has been recognized to be electrostatic in nature (Sasaki and Noguchi, 1959; Mathews, 1965; Öbrink and Wasteson, 1971 and Tolstogusow and Wajnermann, 1975).

In this chapter, it is also demonstrated that the interaction between $\varkappa$-carrageenan and $\varkappa$-casein, which takes place on the alkaline side of $\varkappa$-caseins isoelectric point, is of a similar nature. The observation that, of the main components of casein, only the $\varkappa$-component interacts is the more striking since the main caseins constitute a group of closely related proteins, the amino-acid compositions of which are very similar (Mercier et al, 1971, 1973 and Ribadeau-Dumas et al., 1972).

This chapter deals with the interaction between carrageenan and $\varkappa$-casein, as studied by different methods i.e., cationic dye binding, sedimentation experiments, electron microscopy and light scattering.

4.2. MATERIALS

Kappa-carrageenan (XM 100A) was prepared as described in Chapter II. Iota- and λ-carrageenan were gifts from the laboratories of Pierrefitte-Auby, Paris, and Marine Colloids, Rockland, respectively.

Alpha$_{s1}$-, β- and $\varkappa$-casein were prepared as described by Schmidt and Payens (1963); Payens and Heremans (1969) and McKenzie and Wake (1961) respectively.

All other reagents were of analytical standard grade.

4.3.1. *Cationic Dye Binding*

To an 0.0002% solution of methylene blue in an 0.005 M EDTA buffer of pH 6.7 containing 0.07 M NaCl, different amounts of $\varkappa$-carrageenan were added. The mixtures were heated for 5 min at 80°C to destroy local aggregates of carrageenan. After cooling to 20°C, the absorbance was measured in an 1 cm cuvette in a Cary 14 spectrophotometer (500 nm–700 nm).

To mixtures of 0.0002% methylene blue and 0,0038% $\varkappa$-carrageenan various amounts of different caseins were added. After heating for 5 min at 80°C they were cooled to 20°C and their absorbances at 665 nm were measured as described above.

4.3.2. *Sedimentation experiments*

Stock solutions of carrageenan and protein in 0.005 M EDTA buffer, pH 6.7, were diluted to final concentrations of about 0.02% and 0.2% respectively. In the sedimentation experiments with mixtures of $\varkappa$-carrageenan and α_{s1}- and β-casein the pH was 5.9 and the carrageenan and protein concentrations were 0.03% and 0.3% respectively. The ionic strength of the solutions was adjusted by the addition of NaCl. To study the influence of pH on the interaction between $\varkappa$-carrageenan and $\varkappa$-casein, an experiment was carried out in 0.2 M NaCl with various pH-values. After mixing, the solutions were heated for 5 min at 80°C and subsequently cooled to 20°C, after which they were centrifuged at 90 000 g for 60 min in a Spinco Model L-2 centrifuge equipped with an SW50 rotor. The residual amounts of non-sedimented protein and carrageenan in the supernatant were determined as a function of ionic strength. Parallel experiments were carried out with the pure protein and carrageenan solutions.

Supernatant $\varkappa$-casein was determined at pH 12 (to ensure the availability of all chromophores) from the absorbance at 291 nm measured with an Unicam SP 500 spectrophotometer, using the value 12.6 cm^2/g for the specific absorptivity (Vreeman, to be published). Supernatant α_{s1}- and β-caseins were determined by measuring the absorbance at 278 nm using absorptivities of 10 cm^2/g (Thompson and Kiddy, 1964) and 4.6 cm^2/g (Garnier et al, 1964) respectively. Corrections for scattered light were made by subtracting the absorbance value measured at 320 nm.

Carrageenan was determined with the phenol sulphuric acid method as described by Dubois et al. (1956).

4.3.3. *Electron microscopy*

Solutions of carrageenan and protein were prepared in 0.07 M NaCl or NH_4 acetate, adjusted to pH 6.6 by the addition of ammonium hydroxide. The

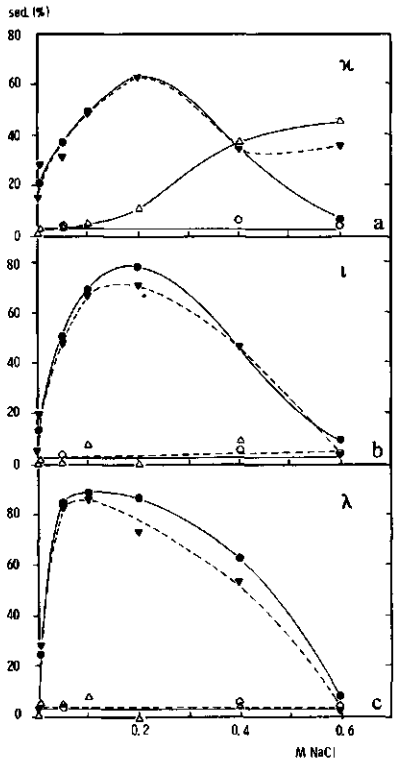

Fig. 4.4. Influence of ionic strength on the relatieve amounts of κ-casein and carrageenan sedimented in mixtures and separate solutions at 90000 g. Experimental conditions 0.005 M EDTA, pH 6.7.
○ and ● 0.2% κ-casein in separate and in mixed solutions respectively.
△ and ▲ 0.02% κ-, ι- or λ-carrageenan in separate and in mixed solutions respectively.

complex formation (Grindrod and Nickerson, 1968; Payens, 1972 and Snoeren et al, 1975). On the other hand under identical conditions the sedimentation behaviour of the separate components is found to be completely different at ionic strengths below 0.5. In particular the maxima at about $I = 0.2$ are not observed with the separate component solutions. Similar results were obtained with ι- and λ-carrageenan (Figs. 4.4b and 4.4c).

Similar experiments with κ-carrageenan, in which the κ-casein was replaced by α_{s1}- or β-casein, confirmed the earlier finding of Payens (1972) that only κ-casein interacts specifically with κ-carrageenan. As is shown by Figs. 4.5a and 4.5b, the sedimentation behaviour of α_{s1}- and β-casein and κ-carrageenan is essentially the same in the mixed and separate solutions.

In Fig. 4.6 the relative amounts of κ-casein and κ-carrageenan sedimented, are given as a function of pH. The complex formation decreases with increasing pH, indicating that the interaction decreases when the net negative charge on the protein increases.

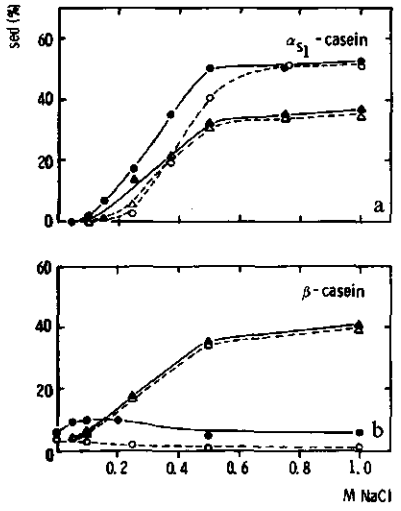

Fig. 4.5. Influence of ionic strength on the relative amounts of $\varkappa$-carrageenan and protein sedimented in mixtures and separate solutions at 90 000 g. Experimental conditons 0.005 M EDTA, pH 5.9.
○ and ● 0.3% α_{s1}- and β-casein in separate and in mixed solutions respectively.
△ and ▲ 0.03% $\varkappa$-carrageenan in separate and mixed solutions respectively.

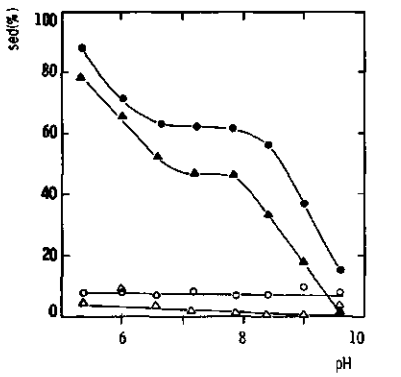

Fig. 4.6. The influence of pH on the complex formation between $\varkappa$-carrageenan and $\varkappa$-casein. Experimental conditions 0.005 M EDTA, 0.2 M NaCl.
○ and ● 0.2% $\varkappa$-casein in separate and in mixed solutions respectively.
△ and ▲ 0.02% $\varkappa$-carrageenan in separate and in mixed solutions respectively.

4.4.3. *Electron microscopy*

In the electron micrographs $\varkappa$-casein consists of independent spherical particles with a diameter of 20 nm (Figs 4.7a and 4.7b). In the presence of $\varkappa$-carrageenan it is seen that the protein particles become aligned in threadlike structures (Figs 4.8a and 4.8b). Since the thickness of the threads in Fig. 4.8a is of the order of 20 nm they clearly do not represent the $\varkappa$-carrageenan chains proper, the thickness of which is of a smaller order of magnitude (Snoeren et al, 1976). In Fig. 4.8b in addition to the complexes some free $\varkappa$-carrageenan chains with a diameter of about 2 nm are visible. Comparable interaction products are also observed with mixtures of $\varkappa$-casein and the other carrageenan components (Snoeren et al, 1976).

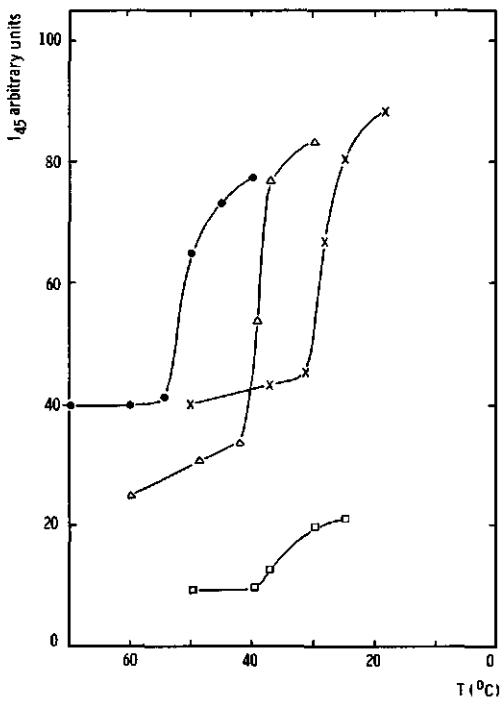

Fig. 4.9.
The intensity of the light scattered at 45° (in arbitrary units) of mixtures of κ-casein (0.3%) and κ-carrageenan (0.03%) as a function of temperature.
●: 0.167 M KCl; △: 0.05 M KCl; □: 0.5 M NaCl; × : 0.30 M NaCl.

4.4.4. *Light scattering*

Fig. 4.9 represents typical light scattering plots of κ-carrageenan- κ-casein mixtures at different ionic strengths as a function of temperature. (Such transition plots were not observed for the separate κ-carrageenan solutions in such low concentrations.) From these plots it can be concluded that a complex formation takes place which is of an abrupt nature comparable to the sol-gel transition observed with more concentrated solutions of pure κ-carrageenan (cf. Chapter III). A similar transition has been observed for ι-carrageenan-κ-casein mixtures (Fig. 4.10). In Fig. 4.11 the light scattering plots of λ-carrageenan-κ-casein mixtures in different salt solutions are given. The transition is lacking in these plots, indicating that gel formation does not take place with κ-casein-λ-carrageenan mixtures.

When the shifts of the transition temperatures of κ-casein solutions to which κ- or ι-carrageenan has been added are plotted as a function of the logarithmic ionic strength, straight lines are obtained (Figs 4.12 and 4.13). This linear relationship is reminiscent of a similar salt effect observed in the separate carrageenan solutions (cf. Chapter III).

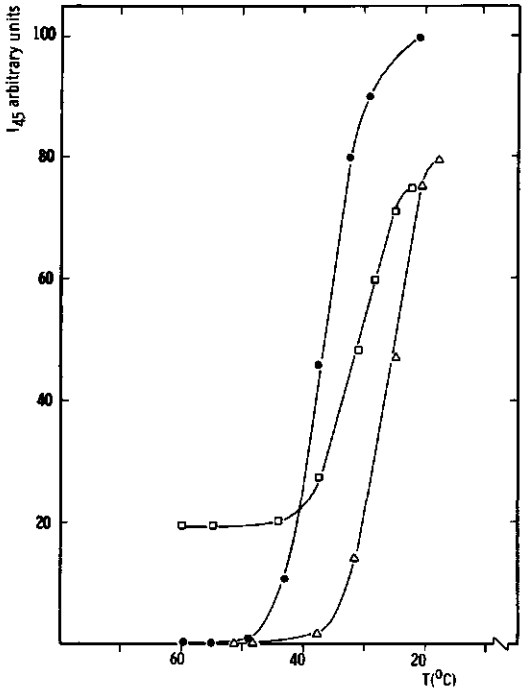

Fig. 4.10.
The intensity of the light scattered at 45° (in arbitrary units) of κ-casein (0.25%) and ι-carrageenan (0.037%) mixtures as a function of temperature.
●: 0.12 M KCl; □: 0.125 M NaCl; △: 0.0625 M KCl.

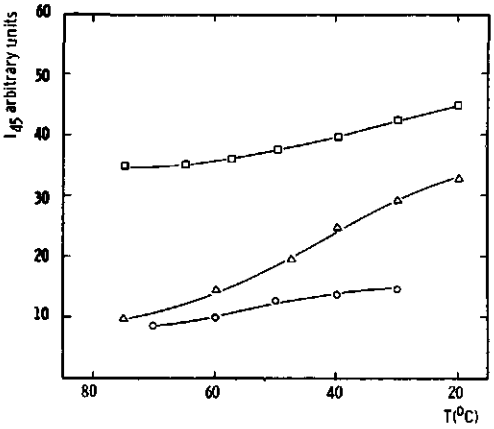

Fig. 4.11.
The intensity of the light scattered at 45° (in arbitrary units) of κ-casein (0.3%) and λ-carrageenan (0.03%) mixtures as a function of temperature.
○: 0.0625 M KCl; □: 0.15 M NaCl; △: 0.25 M NaCl.

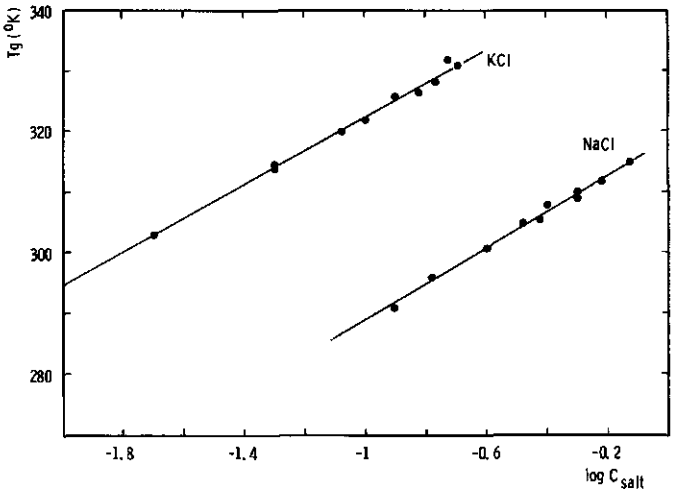

Fig. 4.12.
Gel point temperature vs the logarithmic salt concentration for $\varkappa$-casein (0.3%) and $\varkappa$-carrageenan (0.03%) mixtures.

The regression of the setting point temperature, Tg, on the logarithmic salt concentration is given by

$$Tg = T^0 + A \log C_{salt}$$

It is seen from the results collected in Table 4.1 that the T^0 values obtained for the carrageenan-$\varkappa$-casein mixtures and those obtained for the corresponding pure carrageenan solutions differ by only 1%, whereas the slopes of the Tg vs log C_{salt} plots for the mixtures correspond well with the slopes of these plots for the pure carrageenan. The setting points of the $\varkappa$-carrageenan-$\varkappa$-casein mixtures in the presence of NaCl are, especially at low salt concentrations ($C_{salt} < 0.3$), higher than the setting points of the corresponding $\varkappa$-carrageenan solutions. These higher values lead to lower values of the slope of the Tg vs log C_{salt} plot which are 44.59° and 31.20° for the pure $\varkappa$-carrageenan solution

Table 4-1. The constants T^0 and A and the correlation coefficient R^2 as obtained from the relation $T_m = T^0 + A \log C_{salt}$ for carrageenan ($\pm$ 0.03%) $\varkappa$-casein ($\pm$ 0.3%) mixtures. The data between the brackets correspond with the values obtained with pure carrageenan solutions.

type of carrageenan	salt	T^0	A	R^2
$\varkappa$	KCl	352.3 (356.3)	30.56 (34.40)	0.950
	NaCl	320.1 (323.2)	31.20 (44.59)	0.987
ι	KCl	370.7 (366.3)	47.70 (43.66)	0.933
	NaCl	360.5 (361.8)	48.69 (46.22)	0.936

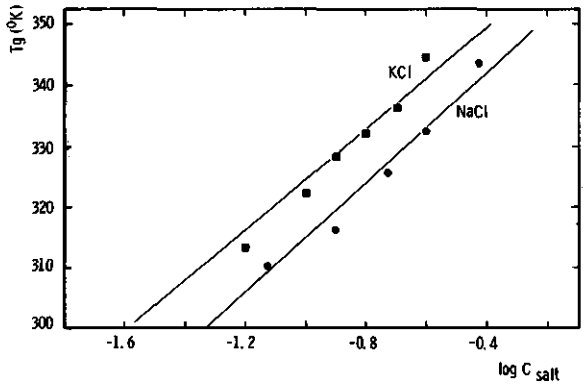

Fig. 4.13.
Gel point temperature vs the logarithmic salt concentration for κ-casein (0.25%) and ι-carrageenan (0.037%) mixtures.

and for the κ-carrageenan-κ-casein mixture respectively. At higher NaCl concentrations the setting point temperatures are almost the same as those observed with pure κ-carrageenan, which is demonstrated by the values of T^0 which differ by only about 1%.

4.5. DISCUSSION

4.5.1 *The interaction between carrageenan and κ-casein*

Fig. 4.3 demonstrates that under comparable experimental conditions, viz $I = 0.07$ and pH 6.7, κ-casein is the only main casein component which is able to displace the cationic dye methylene blue from the κ-carrageenan molecule. This suggests that only κ-casein interacts with κ-carrageenan and that the interaction is electrostatic in nature, since the interaction between κ-carrageenan and methylene blue has been recognized to be electrostatic (Schoenberg and Moore, 1964).

More direct evidence for such a specific interaction can be obtained from sedimentation experiments at varying ionic strength. Indeed, as is shown in Figs 4.4 and 4.5, mixing of κ-carrageenan with various casein components leads only in the case of κ-casein to aggregates which, at an ionic strength below 0.5, differ in sedimentation behaviour from the separate components. Fig. 4.4a further demonstrates that the interaction between κ-casein and κ-carrageenan depends strongly on the ionic strength and reaches its maximum at an ionic strength of $\pm$ 0.2. The occurrence of the maximum can be understood as follows. At the left of the maximum, i.e. at decreasing ionic strength, the thickness of the electrostatic double layer apparently increases to such an extent that the repulsion due to negative charges on both polymers increases, which leads to a decreased complex formation. At the right of the maximum

i.e. at ionic strengths exceeding 0.2, salt addition leads to a more effective screening of the charges on the polymers, which results in the suppression of the electrostatic interaction. Such an effect is reminiscent of the suppression of the complex coacervation of gum arabic and gelatin (Overbeek and Voorn, 1957). In the latter case, however, the net charges of both polymers have opposite signs, whereas at pH 6.7 $\varkappa$-casein and $\varkappa$-carrageenan are both negatively charged, since the iso-electric point of $\varkappa$-casein is situated at pH 4.4 (Swaisgood and Brunner, 1962; Snoeren et al, 1975).

This paradoxical electrostatic interaction can, however, be understood by an inspection of the amino acid sequence of the $\varkappa$-casein molecule (cf. Fig. 4.14) which shows that an extensive, positively charged region exists between the residues 20 and 115 of $\varkappa$-casein. Such an accumulation of positive charges is lacking in α_{s1}- and β-casein, where positive and negative amino acid side chains are evenly distributed along the polypeptide chain.

More specifically the electrostatic interaction can be accounted for by a screened Debye-Hückel potential (Tanford, 1961),

$$V = e^2/D \sum_i \sum_j \exp(-\varkappa r_{ij})/r_{ij}, \tag{4.1}$$

where the summation extends over all pairs of interacting charges on both polymers a distance r_{ij} apart. In this equation $\varkappa$ is the Debye-Hückel parameter defined as

$$\varkappa = (10^3\, DkT/8\pi e^2 Nc)^{-1/2}$$

From Eqn. 4.1 it can readily be computed that the electrostatic interaction virtually vanishes beyond intercharge distances, say, twice the reciprocal

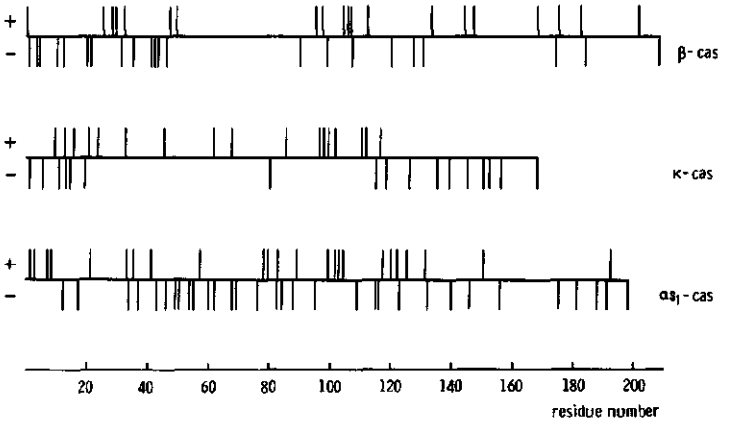

Fig. 4.14. Schematized charge distribution along the casein molecules as adapted from the sequence given by Mercier et al. (1971, 1972 and 1973).

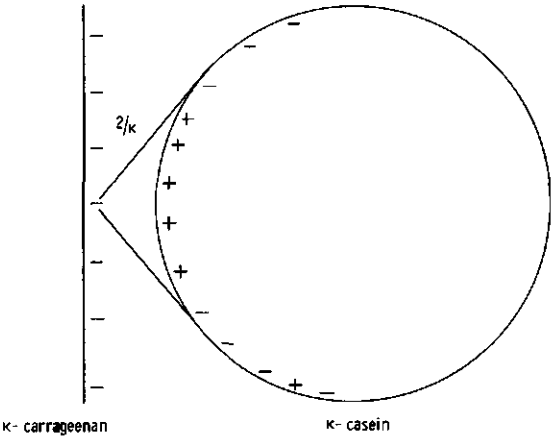

Fig. 4.15.
Schematized picture of the electrostatic interaction between κ-carrageenan and κ-casein.

Debye-Hückel length. The ionic strength of the maximum can now be interpreted as yielding that screening distance beyond which neighbouring negative charges on the κ-casein molecule start to interfere with the electrostatic attraction. This idea enables us to make an estimate of the size of the interaction region. Substituting the salt concentration at the maximum of Fig. 4.4 in the relation for $\varkappa$ leads to $1/\varkappa = 0.6$ nm. The upper estimate of the range of interaction is therefore about 1.2 nm (cf. Fig. 4.15). As shown by the schematic picture of Fig. 4.15, this should also give an estimate of the order of magnitude of the size of the interaction site itself. Obviously the positive site in κ-casein, which is involved in the electrostatic attraction, does not extend over the whole positive region of the κ-casein molecule but has the rather limited size of, say, 1–1,2 nm.

It also seems probable that this positive charge cluster is located on the surface of the κ-casein molecule, since it is difficult to imagine that the expanded coil of κ-carrageenan could penetrate into the κ-casein core. The 40% reduction in the interaction which is observed between pH 5 and pH 7 (cf. Fig. 4.6) suggests that in addition to other positive charged residue side chains, histidine also plays an important role in the interaction. In this respect it is interesting that all histidines (residue numbers 98, 100 and 102) present in κ-casein are located near the chymosine labile Phe-Met bond between residues 105 and 106, which has also been located on the κ-casein molecular surface (Thomas, 1973). It is therefore tempting to locate the active site in κ-casein involved in the electrostatic interaction with κ-carrageenan somewhere between the residues 97 and 112, where 1 Arg, 3 His and 2 Lys are accumulated.

Unfortunately under the experimental conditions κ-casein forms polymers, the secondary and tertiary structures of which are not well known (McKinlay

and Wake, 1971). This impedes a quantitative estimation of the electrostatic interaction by Eqn. (4.1).

4.5.2. *The sol-gel transition in mixtures of carrageenan and κ-casein*
All results discussed so far indicate that the electrostatic interaction with κ-casein takes place with all types of carrageenan, suggesting that the milk reactivity should be a common property to all carrageenans. This, however, is in conflict with practical experience from which it is known that λ-carrageenan is not able to act as a stabilizer in dairy products.

The abrupt change in light scattering observed at the setting temperature of mixtures of κ-casein and κ- or ι-carrageenan (cf. Figs. 4.9 and 4.10) is typical for a sol-gel transition and is comparable with the transition observed with more concentrated solutions of κ- and ι-carrageenan. In mixtures of κ-casein and λ-carrageenan the light-scattering jump is not observed (cf. Fig. 4.11), indicating that in λ-carrageenan-κ-caseine mixtures no sol-gel transition takes place, which is in line with practical experience. Apparently network formation in carrageenan-κ-casein mixtures is restricted to those types of carrageenan that are also able to form a gel in the absence of the protein.

The sol-gel transition of mixtures of κ- and ι-carrageenan with κ-casein may now be discussed by the following model. Carrageenan chains are adsorbed on the surface of the κ-casein molecule, which is induced by electrostatic interactions between both polymers. It is generally accepted that only a part of segments of the adsorbing polymer is really in contact with the surface (trains), the other part remaining in the solution in the form of free loops or tails (Jenkel and Rumbach, 1951; Silberberg, 1962 and Hoeve, 1971). The free loops and tails will behave like free carrageenan molecules, which at the setting temperature of the gel associate to form a network. The mechanism of this association was discussed in Chapter III, where it was shown that the gel points of pure carrageenan are linearly dependent on the logarithm of the salt concentration. As shown by Figs. 4.12 and 4.13 and in Table 4.1, in mixtures of carrageenan and κ-casein the same dependence of the gel point on the salt concentration is found, whereas the gel points themselves are also comparable. This confirms indeed that in such mixtures free carrageenan loops and tails are responsible for the formation of the network.

4.5.3. *The milk reactivity of κ- and ι-carrageenan*
In milk the κ-casein is incorporated in the casein micelle (Schmidt and Payens, 1976). It may be expected, however, that a part of the κ-casein is located on the surface of the micelle, where it is again available for interaction with carrageenan. The milk reactivity, i.e. the interaction between κ- or ι-carrageenan and the casein in milk resulting in the formation of a network, may therefore be explained in the same way as the results obtained with the model experi-

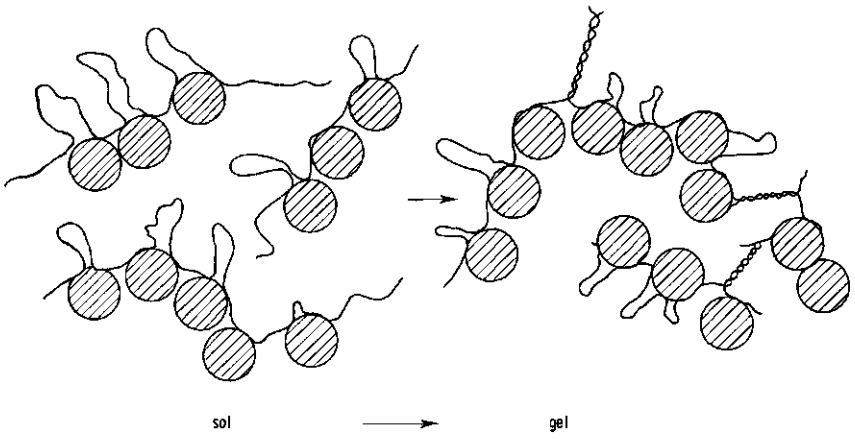

Fig. 4.16. The sol-gel transition as proposed for $\varkappa$- and ι-carrageenan in milk.

ments. According to this picture, in milk to which $\varkappa$- or ι-carrageenan is added, electrostatic complex formation takes place leading to the adsorption of carrageenan on the micelle surface. On cooling below the setting point, the formation of a network is initiated by the formation of double helices between the free carrageenan tails and loops (cf. Fig. 4.16).

It has been postulated that calcium ions are responsible for the milk reactivity (Glicksman, 1969; Lin and Hansen, 1970 and Payens, 1972) and that calcium bridges are formed between the acidic groups of casein and carrageenan. Since, however, λ-carrageenan shows no milk reactivity it is clear that the formation of calcium bridges between the carrageenan chains and casein cannot be of primary importance. This conclusion is further substantiated by the fact that no light scattering transition typical of a sol/gel transition is observed with $\varkappa$-carrageenan and α_{s1}- or β-casein in a solution simulating the ionic conditions prevailing in milk (Payens, 1972).

THE PREPARATION OF $\varkappa$-CASEIN BY ELECTROSTATIC AFFINITY CHROMATOGRAPHY USING $\varkappa$-CARRAGEENAN AS COLUMN MATERIAL

1. *Introduction*

It has been demonstrated in Chapter IV that, of the main caseins present in milk, only $\varkappa$-casein interacts with carrageenans. In milk $\varkappa$-casein is incorporated in the casein micelle. Many methods have been described for the preparation of $\varkappa$-casein from whole casein (McKenzie and Wake, 1961; Zittle and Custer, 1963; Toma and Nakai, 1974 and Nijhuis and Klostermeyer, 1975). The procedures described by McKenzie and Wake and by Zittle and Custer are either very laborious or require strong reagents, such as alcohol and sulphuric acid. The preparation of $\varkappa$-casein by Agarose Gel chromatography as described by Toma and Nakai (1974) is based on the property of $\varkappa$-casein to form polymers by s–s interaction, which ability is lacking with the other main components of casein. Nijhuis and Klostermeyer used affinity chromatography for the preparation of $\varkappa$-casein. The method used by these authors, is based on the covalent binding of cysteine- and cystine-containing proteins to Activated Thiol Sepharose. It is, however, impossible to prepare pure $\varkappa$-casein by this technique, since the minor α_s-caseins, α_{s3}-casein and α_{s4}-casein, also contain cysteine or cystine residues (Hoagland et al., 1971 and Ribadeau Dumas 1975), and are, therefore, isolated together with $\varkappa$-casein.

In this section a new method of preparing $\varkappa$-casein is described, which is based on the interaction between $\varkappa$-carrageenan and $\varkappa$-casein and on the disruption of this complex by salt addition (cf. Chapter IV, section 4.3.2). At room temperature $\varkappa$-carrageenan is insoluble in potassium chloride solutions (cf. Chapter III). The dispersion of $\varkappa$-carrageenan at room temperature in an 0.075 M KCl solution therefore yields swollen gel-like particles which can be used as a matrix for affinity chromatography.

2. *Materials and methods*

Materials

The $\varkappa$-carrageenan used in this experiment was an extract of Eucheuma cottonii and was a gift of Pierrefitte Auby, Paris.

Kappa-, β- and α_{s1}-casein used for reference in starch gel electrophoresis, were prepared as described by McKenzie and Wake (1961), Payens and Heremans (1969) and Schmidt and Payens (1963) respectively.

The elution buffers used were:

Buffer I; 0.005 M EDTA, pH 6, 0.075 M KCl and 3 M urea

Buffer II; 0.005 M EDTA, pH 8, 0.1 M KCl, 0.9 M NaCl and 3 M urea
Buffer III; 0.005 M EDTA, pH 6, 0.15 M KCl and 3 M urea.

Chromatography

To 5 g $\varkappa$-carrageenan which has been dispersed homogeneously in 20 ml ethanol, 200 ml of an 0.005 M EDTA buffer pH 6 containing 0.075 M KCl and 3 M urea (Buffer I) were added. After swelling for 72 hours at room temperature, the gel particles were collected by centrifugation. The sedimented material was washed three times with Buffer I and thereafter transferred to a column of dimensions 2 × 15 cm.

Acid-precipitated whole casein (300 mg) obtained from pooled skim milk (Schmidt, 1969) was dissolved in Buffer I to a final concentration of about 8% and applied to the column. Elution was started with Buffer I. After the release of the first peak, this buffer was replaced by Buffer II (0.005 M EDTA, pH 8 containing 0.1 M KCl, 0.9 M NaCl and 3 M urea).

To study the influence of ionic strength on the elution pattern, Buffer I was replaced by Buffer III (0.005 M EDTA pH 6, containing 0.15 M KCl and 3 M urea).

The absorbance at 280 nm of the effluents (downward flow) was determined continuously with a Uvicord, type 8301 A photometric detector (LKB). The fractions collected were exhaustively dialysed against distilled water which was adjusted to pH 7 by the addition of 0.5 M NH_4OH prior to lyophilization.

Characterization of the caseins was performed by starch gel electrophoresis at pH 8.6 as described by Schmidt (1964). The gel contained 12% starch and was made with a buffer consisting of 0.076 M tris, and 7 M urea. An addition of 0.022 M mercaptoethanol was made to the gel. Amino acid analysis was performed on the small protein sample released from the $\varkappa$-carrageenan column by Buffer III (Peak 2 Fig. A.3). Samples of (about 1 mg) protein were hydrolyzed in 0.5 ml of constant-boiling HCl in evacuated tubes at 110 °C for 24, 48 and 72 hours respectively. The hydrolysates were evaporated in vacuo at 40 °C and analysed with a Jeol JLC 5 AH amino acid analyzer.

3. *Results*

Fig. A-1 shows the elution pattern of acid precipitated whole casein. Buffer I (0.005 M EDTA, pH 6, 0.075 M KCl, 3 M urea) releases only one peak, which by starch gel electrophoresis was identified as a mixture of α_{s1}- and β-casein. Buffer II (0.005 M EDTA pH 8, 0.1 M KCl + 0.9 M NaCl, 3 M urea), releases a second peak which consists of $\varkappa$-casein and the minor α_s-components i.e. α_{s3}- + α_{s4}-casein (cf. Fig. A.2 sample 8).

From 300 mg whole casein 227 mg of the α_{s1}-, β-casein mixture and 59 mg of the mixture of $\varkappa$-, α_{s3}- and α_{s4}-casein was obtained.

When the column loaded with whole casein is eluted by Buffer III (0.005 M

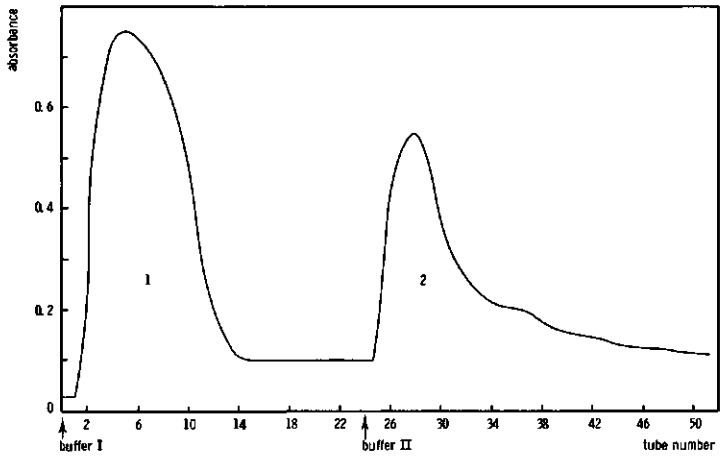

Fig. A.1. Electrostatic affinity chromatography of whole casein on a $\varkappa$-carrageenan column. Experimental conditions: Sample weight 300 mg; column size: 2 × 15 cm. Elution buffers:
Buffer I: 0.005 M EDTA, pH 6, 0.075 M KCl and 3 M urea
Buffer II: 0.005 M EDTA, pH 8, 0.1 M KCl, 0.9 M NaCl and 3 M urea.

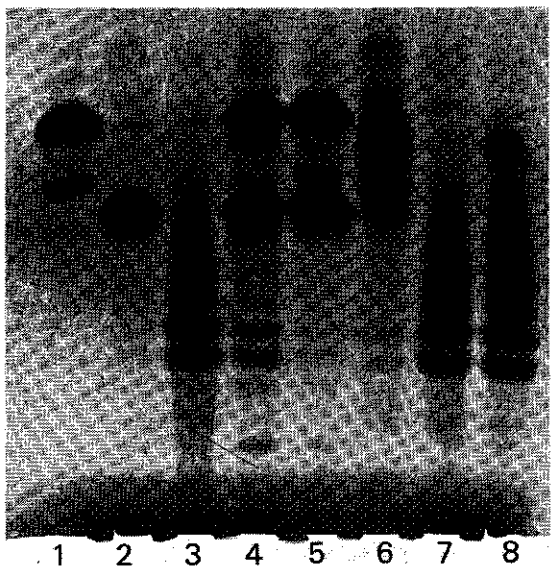

Fig. A.2. Starch gel electrophoresis of the fractions isolated by the electrostatic affinity chromatography.
1 = α_{s1}-casein (reference); 2 = β-casein (reference);
3 = $\varkappa$-casein (reference); 4 = whole casein as applied to the column
5 = α_{s1}- + β-casein released from the $\varkappa$-carrageenan column by Buffer III
6 = α_{s3}- + α_{s4}-casein released from the $\varkappa$-carrageenan column by Buffer III (Peak 2 Fig. A.3)
7 = $\varkappa$-casein released from the $\varkappa$-carrageenan column by Buffer II (peak 3 Fig. A.3)
8 = α_{s}- + α_{s4}- + $\varkappa$-casein released from the $\varkappa$-carrageenan column by Buffer II (peak 2 Fig. A.1)

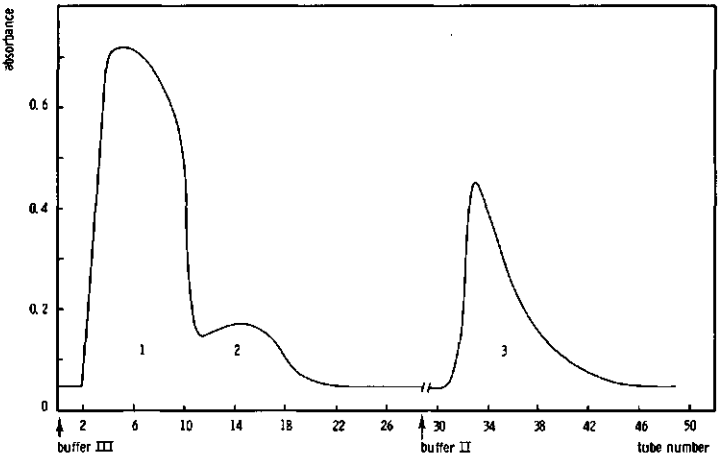

Fig. A.3. Electrostatic affinity chromatography of whole casein on a $\varkappa$-carrageenan column. Experimental conditions as Fig. A.1; Elution buffers:
Buffer III: 0.005 M EDTA, pH 6, 0.15 M KCl and 3 M urea
Buffer II: 0.005 M EDTA, pH 8, 0.1 M KCl, 0.9 M NaCl and 3 M urea.

EDTA, pH 6, 0.15 M KCl and 3 M urea) after the first peak (α_{s1}- and β-casein) a second small peak is released (Fig. A.3) which is due to α_{s3}- and α_{s4}-casein (cf. Fig. A.2 sample 6). A third peak representing pure $\varkappa$-casein is released by Buffer II (0.005 M EDTA pH 8, 0.1 M KCl, 0.9 M NaCl, 3 M urea) (cf. Fig. A.2 sample 7). The amino acid composition of the small sample released by Buffer III (Fig. A.2 sample 6) was also determined. The relative amounts of amino acids (μmol) per ml hydrolysate found after different times of hydrolysis are shown in Table 4 A.1. For the calculation of the mol ratio of the amino acid residues the asparagine content was taken as 20 mol per mol of protein. This content was found by Ribadeau Dumas et al. (1975) for α_{s3}- and α_{s4}-casein. The mol ratio estimated in this way is given in Table 4A.1 column 6. From the results, which are similar to those of Ribadeau Dumas (1970), we may conclude that this sample consists of α_{s3}- and α_{s4}-casein.

4. *Discussion*

The results of the chromatographic experiments once more indicate that under the experimental conditions the $\varkappa$-casein interacts specifically with $\varkappa$-carrageenan. It is further shown that in addition to $\varkappa$-casein, the minor α_s-components α_{s3}- and α_{s4}-casein also interact with $\varkappa$-carrageenan. The relative mobilities of these components in starch gel electrophoresis are 1.04 and 1.00 respectively. These minor components are accompanied by two undefined

Table 4A.1. Amino acid composition of the casein sample A3-2 (μmol amino acid/ml hydrolysate)

	24 h	48 h	72 h	average value	Mol ratio
ASP	0.0864	0.0881	0.0881	0.0875	20
THR[1])	0.0665	0.0657	0.0623	0.0685	15–16
SER[1])	0.0669	0.0629	0.0569	0.0710	16
GLU	0.1954	0.2000	0.2008	0.1987	45–46
PRO	0.0651	0.0656	0.0637	0.0648	15
GLY	0.0165	0.0173	0.0162	0.0167	4
ALA	0.0433	0.0439	0.0439	0.0437	10
CYSS[3])					2
VAL[2])	0.0598	0.0659	0.0668	0.0668	15
MET	0.0156	0.0154	0.0157	0.0156	3–4
ILE[2])	0.0472	0.0504	0.0520	0.0520	12
LEU	0.0672	0.0687	0.0684	0.0681	15–16
TYR	0.0521	0.0542	0.0525	0.0529	12
PHE	0.0287	0.0286	0.0274	0.0282	6–7
TRP	not determined	nd	nd	nd	
LYS	0.1094	0.1108	0.1103	0.1102	25
HIS	0.0195	0.0192	0.0195	0.0194	4–5
ARG	0.0304	0.0301	0.0283	0.0296	7

[1]) THR en SER – obtained by extrapolation
[2]) VAL en ILE – values obtained after 72 h of hydrolysing
[3]) CYSS – determined with the method as described by Moore (1963)

components, which in starch gel electrophoresis have relative mobilities of 0.88 and 0.82 respectively compared to the mobility of α_{s4}-casein ($\alpha_{s4} = 1.00$). These components are present in very low concentrations and are also present in the $\varkappa$-casein prepared by the method of McKenzie and Wake (1961). It has been demonstrated by Vreeman (Vreeman, H. J. to be published) that these components are not split by the milk clotting enzyme chymosin, indicating that they are not due to $\varkappa$-casein.

From the yields in Experiment I it is clear that $\varkappa$-, α_{s3}- and α_{s4}-casein together constitute about 20% of whole casein. The $\varkappa$-casein of whole casein has been estimated by Ribadeau Dumas (1968), by determining C-terminal amino acids split from whole casein by the enzyme carboxypeptidase A_1, as 11.4%. Apparently the α_{s3}- and α_{s4}-casein content is of the same order of magnitude. Manning et al. (1971), however, reported a $\varkappa$-casein content in whole casein of 22.5%. This value was determined from sulphydryl groups present in whole casein, assuming that only $\varkappa$-casein contained these groups. Recent investigations of Hoagland et al. (1971) and Ribadeau Dumas et al. (1975) have demonstrated that α_{s3}- and α_{s4}-casein also contain cystine (2 residues per mol). The value of

22.5% as estimated by Manning is therefore due to the total content of $\varkappa$-, α_{s3}- and α_{s4}-casein, which agrees satisfactorily with our estimate of 20%.

The release of α_{s3}- and α_{s4}-casein from $\varkappa$-carrageenan by Buffer III suggests that also the interaction between $\varkappa$-carrageenan and α_{s3}- and α_{s4}-casein is electrostatic in nature. It is tempting to assume that in α_{s3}- and α_{s4}-casein a positive cluster is also present, this supposition may be strengthened by the fact that the lysine content in α_{s3}- and α_{s4}-casein is high (25 residues/mol) as compared with β- (11 res/mol) and α_{s1}-casein (14 res/mol).

It is demonstrated in this Appendix that by electrostatic affinity chromatography, using $\varkappa$-carrageenan as column material, electrophoretically pure $\varkappa$-casein and relatively pure α_{s3}- and α_{s4}-casein can be prepared.

V. THE MOLECULAR WEIGHT OF κ-CARRAGEENAN AS A PARAMETER OF SEDIMENTATION AND CREAMING PHENOMENA OF SOME FLUID MILK PRODUCTS

5.1 INTRODUCTION

In dairy products, such as chocolate milk and evaporated milk, sedimentation and or creaming of certain constituents may occur.

In chocolate milk the cocoa particles, which are suspended in the milk plasma, sediment as a consequence of their density, being higher than that of the surrounding plasma. To retard this defect, thickening agents are added. Results of technological experiments with various types of stabilizers indicated that in this respect κ-carrageenan is much more effective than other types of stabilizers (Glicksman, 1969). The effect even of a small amount of κ-carrageenan (about 0.025%) is due to its unique interaction with the casein micelles (Glicksman, 1969; Andersen, 1962 and Lin and Hansen, 1970). It has been demonstrated that of the main caseins of milk i.e. α_{s1}-, β- and κ-casein, the κ-component is involved in this interaction (cf. Chapter IV; Grindrod and Nickerson, 1969 and Payens, 1972). In the Appendix to the previous chapter it is further demonstrated that the minor α_s-caseins, i.e. α_{s3}- and α_{s4}-casein are also involved in this interaction.

Creaming in sterilized canned evaporated milk on prolonged storage occurs since the density of the suspended 'fat-protein complexes' is lower than that of the surrounding medium. According to Stoke's law, important parameters in (gravity) creaming are: the diameter of the fat globules, the difference in density between milk plasma and fat globules and the viscosity of the suspending medium. To affect these parameters, attention has been paid to technological modifications during manufacture. Thus, it has been shown that homogenization retards creaming in evaporated milk (Webb and Holm, 1939). Hunziker (1949) suggested that during homogenization of concentrated milk casein micelles are adsorbed on to the freshly formed surface of the milk fat globules. The existence of these so-called 'fat-protein complexes' has been confirmed by electron microscopy (Buchheim and Knoop, 1970); Eggman, 1969 and Henstra and Schmidt, 1970). Thus homogenization affects both diameter and density of the fat globules.

The viscosity of canned sterilized evaporated milk changes during manufacture and storage. As a result of homogenization and sterilization, thickening occurs. On storage, the viscosity of the evaporated milk decreases to a 'basic storage viscosity' (Mulder and Walstra, 1974 and Deysher et al., 1944). Depending on process conditions, prolonged storage may result in an increase

of the viscosity, sometimes leading to age thickening (Heintzberger et al., 1972).

The use of stabilizers in evaporated milk in order to retard fat separation (creaming), has not been widely applied. The use of carrageenan as a stabilizer in evaporated milk has been reported by Wilcox (1958) and Sabharwal (1972).

It may be assumed that addition of κ-carrageenan to chocolate or to evaporated milk leads to the formation of a thixothropic network as a consequence of the interaction between κ-carrageenan and casein (Andersen, 1962; Payens 1972).

Indirect evidence for such a network is obtained from the retarded sedimentation of cocoa particles in chocolate milk (Andersen, 1962), and from the improved storage properties – as far as fat separation concerns – of evaporated milk (Wilcox, 1958 and Sabharwal, 1972). The evidence of a sol-gel transition as obtained by light-scattering (Payens, 1972; Chapter IV) is also indicative for the formation of such a network. Additional proof for network-like structures has been afforded by electron microscopic investigations, as discussed in Chapter IV. From these data it was concluded that the network formation could be ascribed to coil/double helix transitions of free tails or loops of κ-carrageenan molecules proper which are adsorbed on the casein particles. The possibility of network formation, therefore, may depend on the lengths of the loops and tails. Since Stromberg et al. (1965) demonstrated that the loop length of adsorbed polymers increases with molecular weight, it therefore seems realistic to suggest that the possibility of network formation of κ-carrageenan containing milk systems increases with the molecular weight of the κ-carrageenan.

The effect of the molecular weight of κ-carrageenan on the sedimentation of cocoa particles in chocolate milk and on fat separation in evaporated milk was studied in a series of experiments in which κ-carrageenans of various molecular weights were added to these products.

5.2. MATERIALS AND METHODS

5.2.1. *Materials*

The κ-carrageenan samples were prepared from Genulacta K 100 (a commercial sample, manufactured by Københavns Pektinfabrik). Sample EC was a gift of Pierrefite Auby. Carrageenan samples of different molecular weight were made either by hydrolysis or by ultrafiltration as described in Chapter II. Weight- and number-average molecular weights were determined by light scattering and the amount of reducing end-groups, respectively (cf. Chapter II). These molecular weights are given in Table 5.1.

Table 5-3. Viscosity (m Pa.s) of chocolate milk to which various amounts of high molecular weight κ-carrageenan are added.

sample	concentration %	viscosity (m Pa.s)
H_5	0.02	16
	0.025	18
	0.03	25
A_{72}	0.02	17
	0.025	18
	0.03	25
XM 300	0.02	19
	0.025	24
	0.03	31
EC	0.02	26
	0.025[1])	32
	0.03[1])	35

[1]) In this chocolate milk overstabilizing occurs leading to syneresis.

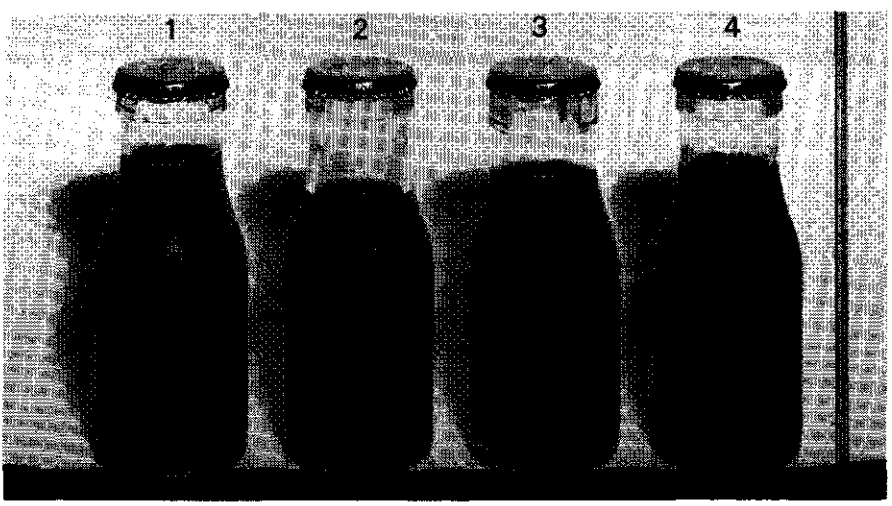

Fig. 5.2. Chocolate milks to which various amounts of the κ-carrageenan sample XM 300 ($<M>_w$ = 660 000) were added, after storage for 10 days at 20°C.
1: Blank; 2: 0.02%; 3: 0.025%; 4: 0.03%.

($<M>_w$ <100 000) does not retard the sedimentation of the cocoa particles. The high molecular weight species ($<M>_w$ >100 000) proved to be very effective, although in the concentration used (0.020%) complete stabilization could not be achieved. It is further demonstrated by the results of Table 5.2 that the viscosity of the chocolate milks increases with the molecular weight of the κ-carrageenan added. Table 5.3 summarizes the data on the relation between the viscosity and the amount of added high molecular weight κ-carrageenan. As shown, the viscosity increases with κ-carrageenan concentration. Serum separation was observed in chocolate milks containing 0.025% and 0.03% κ-carrageenan of sample EC. The beneficial effect of an increase of the κ-carrageenan concentration is clearly demonstrated in Fig. 5.2.

5.3.2. *Evaporated milk*

The influence of the molecular weight of κ-carrageenan on viscosity and fat separation as observed for evaporated milk, at κ-carrageenan concentrations of 0.005% and 0.01% is given in Fig. 5.3 and 5.4 respectively. Addition of low molecular weight κ-carrageenan ($<M>_w$ <100 000) only improved storage stability at a concentration of 0.01% during the first months of storage, which may be due to an increased viscosity.

The higher molecular weight samples were more effective in this respect

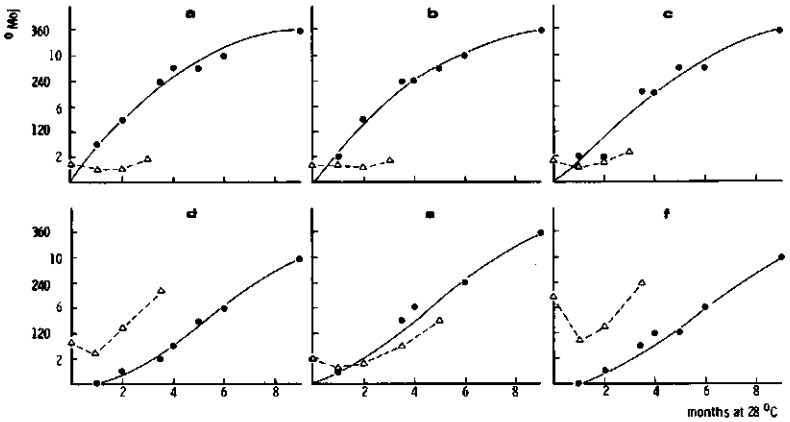

Fig. 5.3. Rate of viscosity change and fat separation during storage of samples of evaporated milk containing 0.005% κ-carrageenan of different molecular weight.
a: Blank; b: $<M>_w$ 44000; c: $<M>_w$ 87000; d: $<M>_w$ 248000; e: $<M>_w$ 359000; f: $<M>_w$ 660000.
·———· Fat separation (0–12; 0 = absence of creaming; 12 = thick, sticky cream layer).
△- - - △ Viscosity (0–360° Mojonnier, arbitrary units).

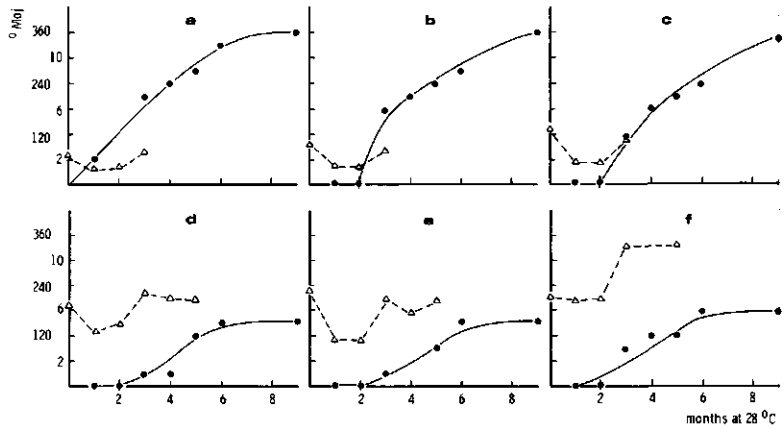

Fig. 5.4. Rate of viscosity change and fat separation during storage of samples of evaporated milk containing 0.01% m/m κ-carrageenan of various molecular weights. For further explanation, see Fig. 5.3.

(cf. Figs. 5.3 and 5.4). In the samples to which 0.005% or 0.01% κ-carrageenan XM 300 ($<M>_w = 660\,000$) was added, some serum separation was observed. The fat content of the top, middle and bottom layers of samples of evaporated milk stored for 2, 4, 6 and 9 months at 20°C, and to which 0.005% κ-carrageenan of different molecular weight were added is presented in Table 5.4. The results of these objective determinations agree with those of the subjective test (cf. Fig. 5.3).

The effect of the concentration of the κ-carrageenan sample XM 300 on the viscosity and the fat separation behaviour of evaporated milk is given in Fig. 5.5. It is seen that fat separation decreases with increasing concentration of

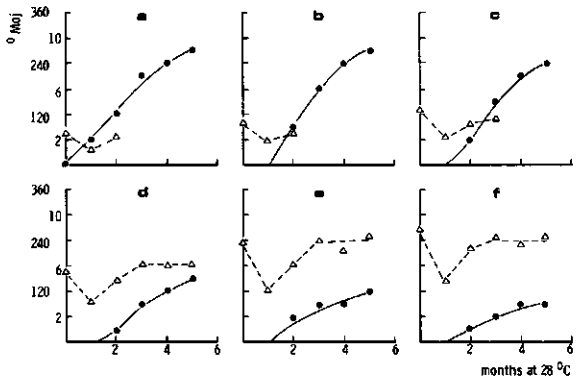

Fig. 5.5.
The effect of the concentration of a κ-carrageenan of high molecular weight ($<M>_w$ 660 000) on the rate of viscosity change and fat separation of samples of evaporated milk (9/22) during storage.
a: Blank; b: 0.001% carrageenan; c: 0.002%; d: 0.003%; e: 0.004%; f: 0.005%. For further explanation, see Fig. 5.3.

Table 5-4. Fat content in top, middle and bottom layers[1]) of samples of evaporated milk (9/22) containing 0.005% of κ-carrageenan of different molecular weight. Storage time 2, 4, 6 and 9 months respectively.

storage time	κ-carrageenan sample fat %	blank	H_{20}	H_{10}	H_5	A_{72}	XM 300
2	top	12.11	12.46	11.22	9.66	9.77	9.54
	middle	7.83	7.62	7.66	8.91	8.94	8.74
	bottom	7.22	7.15	7.16	7.72	7.57	8.06
	fat, recovery	99.3	98.7	100	99.6	100.8	102
4	top	13.45	13.57	12.98	10.30	10.54	9.97
	middle	6.79	6.92	6.97	8.88	9.06	8.64
	bottom	6.42	6.45	6.43	6.96	6.64	7.47
	fat, recovery	100.6	100.4	100.2	100.5	100.8	101.3
6	top	14.87	14.88	14.18	11.55	9.82	10.64
	middle	6.37	6.36	6.44	9.21	8.66	8.63
	bottom	5.51	5.69	5.99	5.87	6.03	6.97
	fat, recovery	101.4	101.6	101.2	93.2	100.9	101
9	top	16.67	14.79	15.21	11.35	12.44	11.21
	middle	5.40	5.47	5.47	8.90	8.99	8.57
	bottom	5.24	5.15	5.34	5.05	4.54	6.50
	fat, recovery	100.5	97.5	96.5	96.9	98.4	98.42

[1]) Top, middle and bottom layer about 40, 60 and 68 g respectively (total content: 168 g).

Table 5-5. Fat content(% m/m) in top, middle and bottom layers[1]) of samples of evaporated milk (9/22) containing various amounts of κ-carrageenan (XM300, $<M>_w$ = 660 000).
Storage time: 9½ months at 28°C.

layer	κ-carrageenan (% m/m)					
	0	0.001	0.002	0.003	0.004	0.005
top	18.29	17.98	15.82	14.13	12.02	12.06
middle	6.35	6.85	7.37	9.31	9.76	9.08
bottom	5.75	6.06	6.20	5.76	5.93	6.77
fat, recovery	98.3	97.3	98.3	97.3	96.7	98.0

[1]) top, middle and bottom layers about 40, 60 and 68 g respectively (total content 168 g).

κ-carrageenan. The fat content of the top, middle and bottom layers in samples of evaporated milk stored for 9½ months at 28°C and to which κ-carrageenan XM 300 had been added in various concentrations, is given in Table 5.5.

5.4. DISCUSSION

5.4.1. *The influence of the molecular weight of κ-carrageenan on stabilizing properties*

The results of the above experiments demonstrate that the stabilizing effect of κ-carrageenan in both types of products strongly depends on the molecular weight as well as on the concentration of the stabilizer added. From Chapter IV it is known that κ-carrageenan interacts with the casein, which interaction has been recognized to be electrostatic in nature. It is shown in Chapter IV that κ-, α_{s3}- and α_{s4}-casein are involved in this complex formation. This interaction leads to the adsorption of κ-carrageenan segments on the casein surface, whereas the remaining part is present as free loops or tails which will behave as free κ-carrageenan chains. If the interaction products are cooled to a temperature below the setting point of κ-carrageenan, the free loops and tails interact by double helix formation, which results in a three dimensional network. In such a network a κ-carrageenan matrix is therefore present, to which casein particles or 'fat-casein complexes' adhere. Such a network retards sedimentation of the cocoa particles and the rising of the fat globules.

It was shown by Stromberg et al. (1965) that the mean square distance of polymer segments from the adsorbing surface increases with the first power of the molecular weight of the polymer. This would indicate that in our experiments the length of the loops and tails increases with the molecular weight of the κ-carrageenan used, and therefore also the possibility of three dimensional network formation.

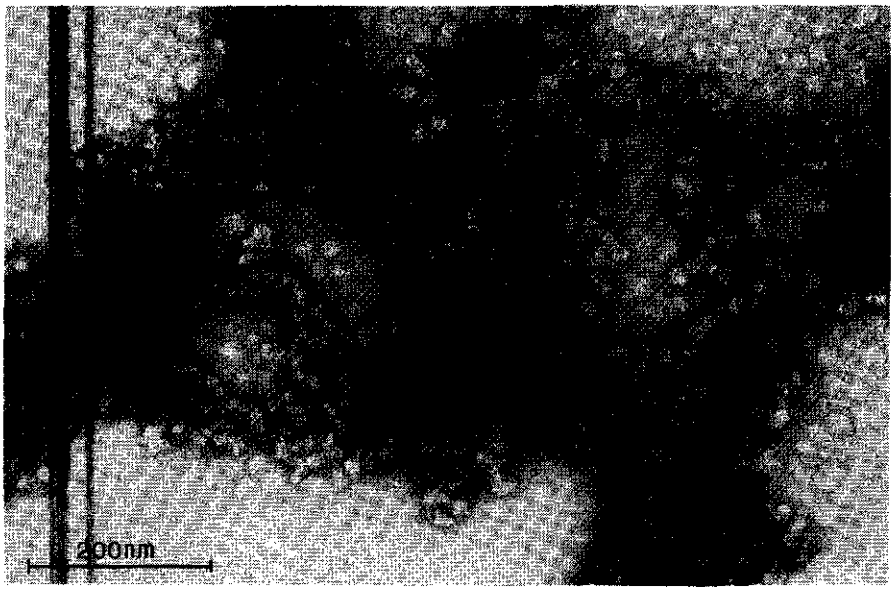

Fig. 5.6. Electron micrograph of the complex formed by κ-casein and low molecular weight κ-carrageenan ($< M >_w$ 44 000).
Experimental conditions: 0.15% κ-casein and 0.015% κ-carrageenan.
Technique used: negative staining with uranyl acetate.

Flory (1953) demonstrated that network formation only occurs if the functionality (i.e. the possible interaction sites on a polymer) exceeds 2. For our systems it may be obvious that the functionality of the casein-κ-carrageenan complexes formed by the electrostatic interaction depends on the lengths of the loops and tails. Since these lengths depend on the molecular weight of the κ-carrageenan used, the functionality of the complexes may be correlated to the molecular weight of the κ-carrageenan.

Let us assume that a κ-carrageenan sample with an arbitrary molecular weight of M_c is needed to produce a κ-carrageenan-casein aggregate of functionality 3, necessary for network formation. Then it is clear that κ-carrageenan with lower molecular weights ($< M >_w < M_c$) only can induce the formation of aggregates without network formation. Although these aggregates may produce an increase in product viscosity, they are not able to stabilize products such as chocolate milk and evaporated milk adequately. The electron micrograph of κ-casein to which low molecular weight κ-carrageenan is added ($< M >_w = 44000$) demonstrates the formation of such aggregates (cf. Fig. 5.6). This hypothesis is further supported by the fact that in κ-casein-κ-carra-

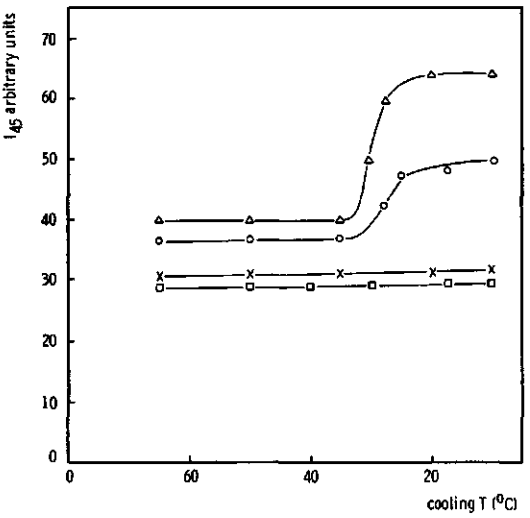

Fig. 5.7.
Light scattering plots of κ-casein (0.3%)-κ-carrageenan (0.03%) mixtures dissolved in milk ultrafiltrate.
△: $<M>_w$ κ-carrageenan = 660 000; ○: $<M>_w$ = 248 000; ✳: $<M>_w$ = 87 000; □: $<M>_w$ = 44 000.

geenan mixtures in simulated milk ultrafiltrate (Jennes and Koops, 1962) at the gel-point of κ-carrageenan no increase in light scattering was observed if low molecular weight κ-carrageenan ($<M>_w$ <100 000) had been used (cf. Fig. 5.7).

On the other hand, when κ-carrageenan samples of molecular weight $> M_c$ are used, an infinite network may be formed, which is accompanied by an increase in structural viscosity. Such a network considerably retards fat separation in evaporated milk and the sedimentation of cocoa particles in chocolate milk. The electron micrograph of κ-casein to which high molecular weight κ-carrageenan ($<M>_w$ = 660 000) has been added indeed demonstrates that a network is formed (cf. Fig. 5.8). Moreover the abrupt increase in light-scattering intensity at the setting point temperature of pure κ-carrageenan, as observed with κ-casein-κ-carrageenan mixtures ($<M>_w$ >100 000), is an indication that network formation takes place (cf. Fig. 5.7).

It is observed that the steepness of the transition increases with molecular weight (Fig. 5.7). This provides evidence that the lengths of the κ-carrageenan loops or tails involved in the coil-double helix transition increase with molecular weight (cf. Chapter III, section 3.5.8.), which is in agreement with the observation of Stromberg et al. (1967) that the loop length of adsorbed polymers increases with increasing molecular weight.

The results of the experiments indicate that the critical molecular weight of κ-carrageenan (M_c) needed for gel formation in milk systems is about 100 000.

Fig. 5.8. Electron micrograph of the network formed by $\varkappa$-casein and high molecular weight $\varkappa$-carrageenan ($<M>_w = 660\,000$).
Experimental conditions as given in Fig. 5.6.

5.4.2. *General conclusion*

The results dealt with in Chapters IV and V lead to the conclusion that milk reactivity is due to two processes:

1. electrostatic interaction between carrageenan and casein, and subsequently;
2. network formation by the interconnection of free carrageenan loops or tails via the coil-double helix transition discussed in Chapter III.

The electrostatic interaction in which $\varkappa$-, α_{s3}- and α_{s4}-casein are involved leads to the formation of carrageenan-casein complexes, and takes place with all types of carrageenan. Network formation, however, is restricted to those carrageenans which are also able to gel without casein, e.g. $\varkappa$- and ι-carrageenan. Further, the loops or tails have to be sufficiently large to ensure gel formation.

From the knowledge obtained concerning the milk reactivity we may infer that milk reactivity – in the sense as described in the preceding chapter – for polymers can be expected if the following conditions are fulfilled:

a. the presence of negative charges, necessary for the electrostatic interaction;
b. gel-forming ability in milk salt solutions in absence of protein, and
c. a sufficiently large molecular weight to ensure gel formation.

SAMENVATTING

Het doel van het onderhavig onderzoek was de karakterisering van κ-carrageen in oplossing en het vinden van een verklaring voor de melk reaktiviteit, d.w.z. de eigenschap van carrageen om reeds bij zeer lage concentraties bepaalde zuivelprodukten te stabiliseren. In dit onderzoek is verder aandacht besteed aan de sol-gel overgang van carrageen en aan de invloed van het molecuulgewicht op het stabiliserende effect van κ-carrageen in enkele zuivelprodukten.

In hoofdstuk I is een korte beschrijving van de verschillende carrageentypen en van de caseïnecomponenten in melk gegeven.

In hoofdstuk II worden κ-carrageen-monsters met gewichtsgemiddelde molecuulgewichten van 17500–836000 bestudeerd met verschillende technieken zoals lichtverstrooiing, viscosimetrie en ultracentrifugering. Uit de waarden van de exponenten in de Mark-Houwink vergelijking ($[\eta] = K_\eta \cdot q_{v.w} < M >_w^{a_\eta}$) en in de relatie tussen $< R_g >_{l.s}$ en het molecuulgewicht ($< R_g^2 >_z = K_{R_g} (q_{z.w} < M >_w)^{a_{R_g}}$), met respectievelijk waarden $a_\eta = 0.86$ en $a_{R_g} = 1.20$, blijkt dat het κ-carrageen molecuul in waterige oplossing ($I = 0.1175$) het best beschreven kan worden als een geëxpandeerd kluwen. De expansie wordt veroorzaakt door het uitgesloten volume en door elektrostatische repulsies. De elektrostatische interactie van de sulfaatgroepen van het κ-carrageen neemt af met toenemende ionsterkte als gevolg van de ladingsafscherming door ionen.

Onder de omstandigheden waarbij lange-afstands interacties te verwaarlozen zijn ($I \to \infty$ en $M \to 0$) kan het κ-carrageen molecuul beschreven worden als een kluwen dat opgebouwd is uit statistische segmenten met een lengte van 0.83 nm. De Flory-Fox viscositeits constante Φ berekend uit de viscositeits- en lichtverstrooiingsresultaten verkregen bij een ionale sterkte van 0.1175 is aanzienlijk lager (0.45×10^{21}–0.75×10^{21}) dan in het geval van ongeladen polymeren in goede oplosmiddelen ($\Phi = 2.5 \times 10^{21}$).

Het is verder gebleken dat de meeste moleculaire grootheden beïnvloed worden door de heterogeniteit van de κ-carrageen monsters. In de meeste relaties tussen de moleculaire grootheden zijn daarom correctiefactoren gebaseerd op een Schulz-Zimm molecuul gewichtsverdeling toegepast.

In hoofdstuk III wordt de wanorde-orde overgang die optreedt bij het gelpunt in κ-carrageen oplossingen bestudeerd met behulp van optische rotatiemetingen en lichtverstrooiing. Het blijkt dat de overgangstemperaturen gemeten met beide technieken samenvallen. Dit wijst er op dat de sol-gel overgang gepaard gaat met een conformatie-verandering. Dergelijke conformatieovergangen worden ook gevonden in oplossingen van ι-carrageenan maar

niet in die van λ-carrageenan. De overgangstemperaturen blijken lineair afhankelijk te zijn van de logarithme van de zoutconcentratie, hetgeen wordt toegeschreven aan een kluwen-dubbel helix overgang van de carrageen componenten bij het gelpunt.

De overgangstemperatuur (T_m) en de steilheid van de overgang nemen af naarmate het aantalgemiddelde molecuulgewicht ($<M>_n$) van κ-carrageen afneemt. Dit verschijnsel is verklaard met behulp van de kluwen-dubbel helix overgang. Het blijkt dat voor κ-carrageen de dubbel-helix initiatie-parameter vrij hoog is (0.315 l/mol) wat de sterke afhankelijkheid van de overgang van het molecuulgewicht verklaart.

De enthalpie van de sol-gel overgang is gemeten met behulp van differential scanning calorimetrie. De enthalpie verandering neemt voor κ-carrageen toe met de ionsterkte hetgeen wordt verklaard met de aanname van een aggregatieproces, volgend op de kluwen-dubbel helix overgang. Voor ι-carrageen oplossingen worden vergelijkbare enthalpieën gemeten.

In hoofdstuk IV wordt aangetoond dat van de drie hoofdcomponenten van caseïne (α_{s1}-, β- en κ-caseïne) alleen κ-caseïne reageert met carrageen. Deze specificiteit van κ-caseïne moet toegeschreven worden aan de aanwezigheid van een ophoping van positieve elektrische ladingen in het κ-caseïne molecuul, waardoor elektrostatische interactie met de negatief geladen sulfaatgroepen van het carrageen mogelijk is. In α_{s1}- en β-caseïne is een dergelijke positieve cluster afwezig en kan geen wisselwerking met carrageen optreden. Deze elektrostatische interactie welke maximaal is bij een ionsterkte van 0.2 treedt op met alle carrageen typen. Carrageen segmenten worden geadsorbeerd aan het κ-caseïne deeltje terwijl een deel van het carrageenmolecuul in oplossing blijft als z.g.n. vrije lussen en staarten. De gelpunten, die in oplossingen van κ- of ι-carrageen en κ-caseïne worden waargenomen zijn vergelijkbaar met die in overeenkomstige oplossingen van het carrageen alleen. Dit wijst er op dat de sol-gel overgang in carrageen-κ-caseïne mengsels uitsluitend een gevolg is van interactie tussen de geadsorbeerde carrageen-lussen en -staarten onderling en dat κ-caseïne als zodanig hierbij geen rol speelt. In overeenstemming hiermee is het feit dat λ-carrageen, wat geen gelen vormt, geen melkreaktiviteit vertoont.

In de Appendix van hoofdstuk IV is aangetoond dat behalve κ-caseïne ook de zgn. minor α_s-caseïne componenten, α_{s3}- en α_{s4}-caseïne, reageren met κ-carrageen. Ook deze interactie heeft een elektrostatisch karakter. Met behulp van affiniteits chromatografie werd een mengsel van κ-, α_{s3}- en α_{s4}-caseïne geïsoleerd. Deze componenten maken tezamen 20% uit van het totale caseïnegehalte.

In hoofdstuk V is aangetoond dat het molecuulgewicht van κ-carrageen een zeer belangrijke factor is voor de melkreaktiviteit en dus voor zijn vermogen om zuivelprodukten te stabiliseren. Kappa-carrageen monsters met een mole-

$\Delta\bar{S}$	difference in total entropy (eqn. 3–4)
T_g	gelpoints temperature
$\bar{v}$	partial specific volume (eqn. 2–9)
y	constant (eqn. 2–13)
z	characteristic for the breadth of the molecular weight distribution (eqn. 2–13)
z_p	effective valency (eqn. 3–5)
α_{R_g}	mean radius expansion factor (eqn. 2–30)
a_η	viscosity expansion factor (eqn. 2–33)
β	residue rotation (eqn. 3–5)
β'	equilibrium constant (eqn. 3–15)
Γ	gamma function (eqn. 2–13)
γ	Euler's constant (eqn. 3–5)
ε	correction for long range interactions (eqn. 2–36)
η_r	relative viscosity (eqn. 2–10)
$[\eta]$	intrinsic viscosity (eqn. 2–11)
θ	theta conditions
$\varkappa$	Debye Huckel parameter (eqn. 3–5)
λ	wave length of the light
ξ	reduced charge density (eqn. 3–12)
ρ	density (eqn. 2–9)
Φ	Flory-Fox viscosity constant (eqn. 2–21)
ω	angular velocity

CURRICULUM VITAE

De vooropleiding tot de studie aan de Landbouwhogeschool kreeg ik op de R.K. lagere school te Dongensche Vaart (N.Br.), de lagere landbouwschool te Raamsdonksveer, de avond-U.L.O. te Breda en de John F. Kennedy H.B.S. te Dongen.

Van 1966 tot 1972 studeerde ik aan de Landbouwhogeschool te Wageningen in de richting levensmiddelentechnologie. Het ingenieursdiploma werd in april 1972 behaald met als vakken levensmiddelenchemie (hoofdvak, verzwaard), organische chemie en industriële bedrijfskunde.

Van juli 1970 tot juli 1972 was ik verbonden als part-time leraar scheikunde aan de HAVO Maris-Stella te Dongen.

In juni 1972 volgde mijn aanstelling als wetenschappelijk medewerker bij het Nederlands Instituut voor Zuivelonderzoek (N.I.Z.O.) te Ede.