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Massey University	

STEADY SHEAR AND OSCILLATORY RHEOLOGICAL MEASUREMENTS ON SOLUTIONS OF COMMERCIALLY AVAILABLE WHEY PROTEIN CONCENTRATES

A THESIS PRESENTED IN PARTIAL FULFILMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF TECHNOLOGY IN FOOD TECHNOLOGY

AT MASSEY UNIVERSITY

TANG QINGNONG

1990

ABSTRACT

Three commercially available whey protein concentrates (WPC), Alacen 312, Alacen 392, and Alacen 475 were studied by steady shear and oscillatory rheological methods using a Bohlin rheometer at concentrations of 5-40% and temperatures of 5-90^{o}C. The WPC solutions showed Newtonian behaviour up to a concentration of 15%, were slightly shear thinning at 20 and 25%, and exhibited time dependent or thixotropic behaviour at concentrations of 30% and above.

The apparent viscosity of Alacen 475 solutions of concentration less than 10 percent by weight could be calculated by $\eta_s = \eta_w(1+28C)$ where η_s, η_w and C are the viscosity of the solution, the viscosity of water and the fractional weight concentration. For Alacen 475 solutions of 40% concentration the structure broken down by shearing at a high shear rate of 734 s^{-1} recovered slowly when the shear rate was suddenly dropped to 147 s^{-1} or zero.

The apparent viscosity of WPC solutions was temperature dependent. It decreased at first as temperature increased until a minimum viscosity was attained and then increased rapidly with further increase in temperature. Temperature also had a marked effect on the time dependency of 20% and 30% WPC solutions - causing time dependent shear thinning at 40 and 50^{o}C and time dependent thickening at 60 and 70^{o}C.

The continuous changes in structure of WPC solutions during heat-induced gelation were followed using oscillatory rheological measurements. The effects of temperature, concentration and heating time on the formation and dynamic rheological properties of WPC gels were determined and are discussed in terms of current theories on the rheology of protein solutions and gels. A gelling model for the gelation of globular protein solutions was proposed to interpret the

development in protein gel structure during the gelling process as reflected by the continuous changes in dynamic rheological properties.

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CHAPTER 1

INTRODUCTION

Whey protein concentrate (WPC) solutions are of commercial interest because of their high nutritional value and excellent functionality in applications such as water binding, gelation, emulsification and foaming. The flow properties of WPC solutions are of practical significance in the manufacture of WPC; for example, rheological properties of dilute solutions are important in the ultrafiltration process and rheological properties of concentrated solutions are important in the evaporation and drying stages. The flow behaviour and gelling properties of WPC solutions are also very important functional properties of the product and may control the texture of commercial protein-containing foods.

Flow properties of WPC solutions are governed by molecular composition, size, shape, flexibility and degree of hydration, and by intermolecular interactions (Tung, 1978; Kinsella, 1979). These in turn are influenced by environmental conditions such as temperature, concentration, pH, ionic strength, shear rate, shear time and previous processing or treatment history (Tung, 1978). The formation and rheological properties of WPC gels may depend on composition, temperature, concentration, pH, ionic strength and the time of heating since whey protein gelation characteristics are drastically altered by these factors (Hermansson, 1979; Hillier et al., 1980; Schmidt and Morris, 1984). Information on the relationships between environmental conditions and rheological properties of WPC may be used to modify rheological behaviour of protein-containing foods so as to impart desired product textures (Tung, 1978).

A number of workers have reported steady shear and oscillatory rheological data for whey protein systems (McDonough et al., 1974; Hermansson, 1975, 1979; Pradipasena and Rha, 1977a, 1977b; Richardson and Ross-Murphy, 1981; Beveridge et al., 1984; Paulsson et al., 1986, 1989). However, more work is needed to investigate the flow properties of WPC solutions, the gelation mechanism and the rheological properties of whey protein gels.

CHAPTER 2

REVIEW OF LITERATURE

2.1 CLASSIFICATION AND STRUCTURE OF WHEY PROTEINS

The whey proteins are defined as those milk proteins remaining in the serum or whey after precipitation of the casein (Wong et al., 1988). Whey proteins are mainly composed of β-lactoglobulin, α-lactalbumin, bovine serum albumin, immunoglobulin and proteose-peptones. However, some proteose-peptone components are currently assigned to the β-casein family because they are fragments of β-casein (Andrews, 1978a, 1979b; Eigel and Keenan, 1979). There are also several minor whey proteins including lactoferrin, lactollin, glycoprotein and blood transferrin (Marshall, 1982). β-lactoglobulin is the most abundant whey protein and comprises 50% of total whey protein (Evan and Gordon, 1980). It has a monomeric molecular weight of 18,000 and exists as a dimer in milk(Schmidt and Morris, 1984), but it can form octamers under certain conditions of pH and ionic strength (McKenzie, 1971; Harper, 1984). α-lactalbumin is generally regarded as the second most important protein and makes up 20% of the whey protein (Harper, 1984). It has a molecular weight of 14,200 and tends to aggregate under acid conditions(McKenzie, 1971, Harper, 1984).

Among the whey proteins, β-lactoglobulin and α-lactalbumin have been subjected to the most thorough study with respect to structure. These two major whey proteins exhibit a rather uniform distribution of acidic/basic and hydrophobic/hydrophilic amino acids along their polypeptide chains (Morr, 1979, 1982). β-lactoglobulin has secondary and tertiary structures described by 10% α-helix, 47% β-sheet and 43% unordered structure (Wong et al., 1988). It has two

intrachain disulphide bonds (S-S) and one thiol group (-SH) per monomer. α-lactalbumin has secondary and tertiary structures described by 26% α-helix, 14% β-sheet and 60% unordered structure (Wong et al., 1988). It contains four intrachain disulphide bonds (S-S) but no thiol groups (-SH).

Most of the whey proteins are mainly compact globular proteins ranging in molecular weight from 14,200 to 66,000 (Wong et al., 1988). These proteins fold intramolecularly, burying their thiol groups (-SH) and disulphide bonds (S-S) as well as most of their hydrophobic residues so that extensive self-association or interaction with other proteins does not occur. Their three-dimensional globular conformation resulting from secondary and tertiary structures are stabilized by intramolecular disulphide bonds, hydrogen bonds, hydrophobic interactions and ionic bonds (Haschemeyer and Haschemeyer, 1973; Cheftel et al., 1985). These intramolecular bonds can be disrupted by heat or by chemical agents leading to unfolding and hence denaturation of the protein molecules.

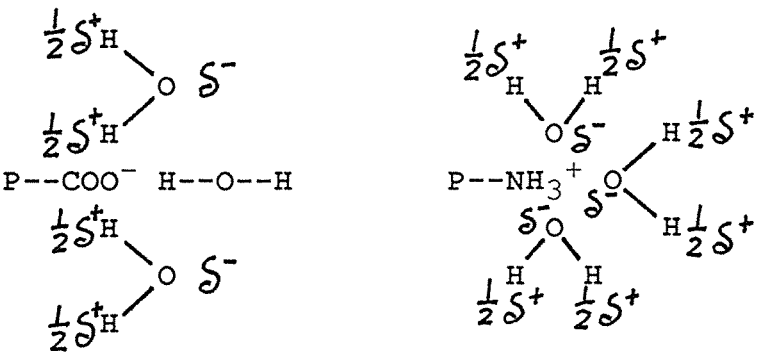
2.2 PHYSICO-CHEMICAL PROPERTIES

2.2.1 Hydration of Whey Proteins

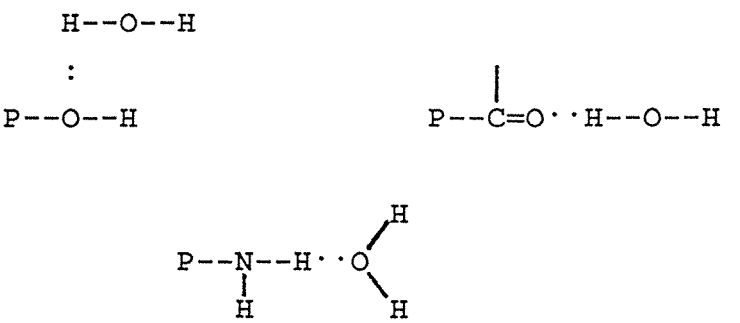
As described in Sect.2.1., the polypeptide chains of whey proteins are folded in such a way that the majority of polar hydrophilic amino acid side-chain groups are on the exterior and the hydrophobic ones buried. So these proteins are very hydrophilic.

Whey proteins interact with water through their polypeptide bonds and their amino acid side chains. Hydrophilic groups of protein molecules interact strongly with water by ion-dipole or dipole-dipole mechanisms. Hydrophobic groups of proteins interact only weakly with adjacent water, preferring a non-aqueous environment (Kinsella, 1982).

Protein-water interactions such as ionic interactions and hydrogen bonds are shown below (Cheftel et al., 1985):



(1) Ionic Interactions



(2) Hydrogen Bonds

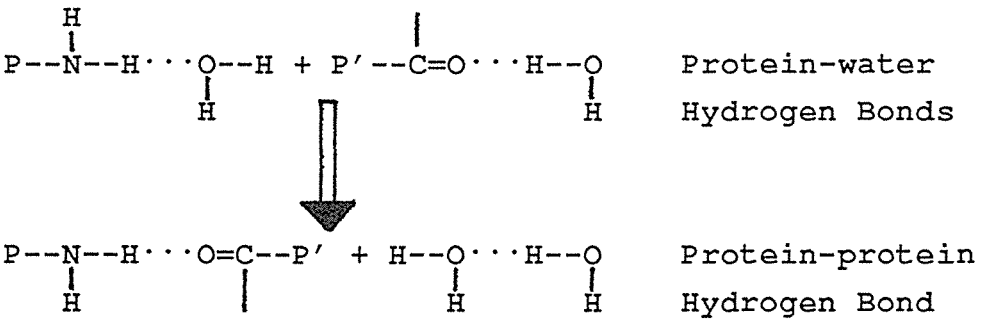
It is found that polar amino acids with ionized side chains bind the greatest amount of water, non-ionized amino acids bind an intermediate amount and the hydrophobic groups bind little or no water (Kuntz et al., 1969; Kuntz and Brassfield, 1971). The loosely held water may surround a protein molecule, being mainly clustered around the ionic side chains. The water layers around protein molecules may account for the stability of whey proteins, especially at their isoelectric points, since these water layers inhibit molecules from approaching sufficiently close to promote aggregation.

2.2.2 Intermolecular Interactions of Whey Proteins

The intermolecular interactions of whey proteins may be classified into non-covalent bonds such as hydrogen bonds, hydrophobic interactions, electrostatic interactions and ionic bonds, and covalent bonds such as disulphide bonds.

2.2.2.1 Hydrogen Bonds

Hydrogen bonds may occur among various potentially eligible groups such as hydroxyl, amino, carbonyl and amide groups. Since the natural medium of protein-protein interactions is aqueous, polar groups on the surface of a molecule will be hydrogen bonded to water. As a result, the formation of intermolecular hydrogen bonds in water first involves the rupture of hydrogen bonds between the active side groups and water, for example (Timasheff, 1964):



Hydrogen Bonding Between Protein Molecules

Generally, the contribution of hydrogen bonds to intermolecular interactions is thought to be small (Kauzmam, 1959; Tanford, 1968).

2.2.2.2 Hydrophobic Interactions

In aqueous solution, the water molecules are not free but are in a state of dynamic equilibrium, resulting from their association with each other via hydrogen bonds (Timasheff,

1964). Therefore, the molecules of liquid water form a mobile network. The network is not a rigid one and changing of neighbouring molecules occurs rapidly because of thermal motions (Tinoco et al., 1978). When a non-polar group such as the hydrocarbon side chain of an amino acid in a protein molecule is introduced into contact with water, the structure of the water is disrupted, since the water molecules cannot form hydrogen bonds with it. The net result is that water molecules around the hydrocarbon side chain become more ordered, which is associated with a large negative entropy change ($\Delta S<0$). Since there is little change in the number of hydrogen bonds, the enthalpy change (ΔH) is small. The overall free energy change for the process ($\Delta G = \Delta H - T\Delta S$) is unfavourable ($\Delta G>0$) (Haschemeyer and Haschemeyer, 1973). In order to regain a structure of minimal free energy, the water molecules tend to compress the non-polar residues into the interior of the protein molecule, leaving the polar ones on the surface. This phenomenon is called the hydrophobic effect.

When protein molecules in an aqueous solution are endowed with such a conformation that non-polar surfaces are in contact with water, in order to attain a lower free energy level the hydrophobic effects tend to compress these molecules together to form aggregates by forcing them into contact at these non-polar surfaces and thus remove the surfaces from the aqueous environment. This phenomenon is called the hydrophobic interaction (or bonding). The hydrophobic interactions are cumulative over an entire region of the protein surface. Therefore, they may result in a strong intermolecular interaction (Timasheff, 1964). As protein molecules are denatured by heat or by chemical agents, many hydrophobic amino acid side chains buried in native globular proteins are exposed to solvent. Then, intermolecular hydrophobic interactions increase markedly, which may result in the formation of whey protein gels.

2.2.2.3 Electrostatic Interactions and Ionic Bonds

Most whey proteins exhibit isoelectric points at pH values of below 6.0. For β-lactoglobulin the isoelectric point ranges from pH 4.2 to 4.5 (Kronman et al., 1964) whereas for α-lactalbumin the range is 4.4-4.6 (Pearce and Shanley, 1981). Above their isoelectric points, whey proteins tend to have an overall negative charge, while below their isoelectric points they tend to have an overall positive charge. The picture is complicated by the association and binding of various ions to the protein surface, and an electrical double layer may form which surrounds the protein molecule surface.

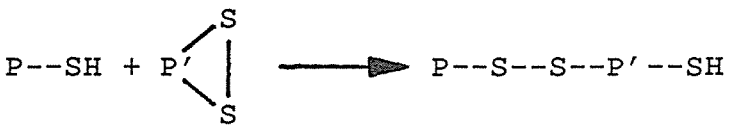
As two identical charged protein molecules above or below the isoelectric point are brought together, the electrical double layers around them will interact giving rise to a repulsion force between the molecules. This type of repulsion is found to be the driving force in the dissociation of β-lactoglobulin dimer at low pH (Timasheff, 1964).

In the formation of intermolecular complexes, ionic bonding or specific attraction between two ionizable groups may be of importance, e.g. the interaction between a $--COO^-$ and $--NH_3^+$ (Timasheff, 1964). Specific ionic interaction may also occur through ion pair formation, which may result in salt bridges between two identical groups.

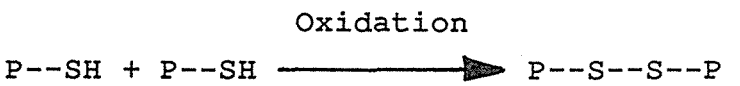
2.2.2.4 Disulphide Bonds

In the native whey proteins thiol (SH) groups and disulphide (SS) bonds are buried inside protein molecules. They are masked and unreactive due to steric hindrance (Cecil and McPhee, 1959). Therefore, disulphide bonds are not involved in protein-protein intermolecular interactions when whey proteins are in the native state. However, when whey protein

molecules are denatured by heat or by chemical agents, they unfold and hence, due to configurational changes in the protein molecule, the internal SH and SS bonds are accessible. Thus, buried SH groups are unmasked and become very active. Intermolecular disulphide bonds may then be formed by sulphydryl-disulphide (SH-SS) interchange reactions (Huggins et al. 1951) and by air or metal catalysed oxidation of SH groups as shown below (Cecil and McPhee, 1959; Wong et al., 1988). Intramolecular disulphide bonds in protein molecules may also be split when more extensive unfolding of individual protein chains is caused by heat or by chemical agents. This is then followed by involvement of exchange reactions with other protein molecules (Wong et al., 1988).



Sulphydryl-Disulphide Interchange Reaction



Air or Metal Catalysed Oxidation

2.2.3 Effect of Temperature upon Whey Protein Solutions

Proteins are sensitive to heat and their susceptibility to denaturation by heat depends on many factors, such as the nature of the protein, protein concentration, water activity, pH, ionic strength, and the kinds of ion present (Cheftel et al., 1985). Traditionally, the thermal stability order of individual whey proteins is generally considered as: immunoglobulin < serum albumin < β-lactoglobulin < α-lactalbumin (Larson and Rolleri, 1955; Lyster, 1970).

However, it also has been found in the study of the unfolding of whey proteins during heating with differential scanning calorimetry (deWit, 1983; deWit and Klarenbeek, 1984) that α-lactalbumin has the lowest denaturation temperature, although it requires the largest amount of heat per gram for unfolding. The traditional notion that α-lactalbumin is the most stable serum protein (Larson and Rolleri, 1955; Lyster, 1970; deWit, 1981) is explained by noting that α-lactalbumin is the only whey protein whose heat denaturation is reversible (deWit, 1983). It is stable against heat-induced aggregation because it renatures easily (Wong et al, 1988).

Generally speaking, the changes of whey protein molecular structure by heat can be either reversible or irreversible. For reversible changes of structure, the partially unfolded protein molecules may refold again to their original three-dimensional structure under appropriate conditions. For irreversible changes of structure, the partially unfolded molecules polymerize to form aggregates (deWit, 1981).

Reversible changes of protein molecular structure mostly occur at temperatures up to 60°C (deWit and Klarenbeek, 1984). These changes often are described as predenaturational transitions caused by a partial loss of the three-dimensional protein structure and by changes of protein hydration (Pfeil, 1981).

Irreversible changes of protein structure may occur above the denaturation temperature of a protein (deWit and Klarenbeek, 1984). These are accompanied by protein aggregation because the denatured whey protein molecules are more susceptible to intermolecular interactions such as hydrophobic interactions, disulphide bonding and ionic bonding (Morr, 1975, 1982).

By using differential scanning calorimetry, deWit and Klarenbeek (1984) conducted an investigation into the

relationship between structure, solubility, and functionality of whey proteins in three temperature ranges: 4 to 60°C, 60 to 100°C, and above 100°C. They found that in the first range (4 - 60°C) temperature caused reversible physicochemical changes such as hydrophobic association and partial unfolding, that in the second (60 - 100°C) temperature caused mainly irreversible physicochemical changes resulting in protein denaturation and that in the third (100 - 150°C) temperature caused mainly irreversible chemical changes such as Maillard reactions and cysteine breakdown.

2.3 RHEOLOGICAL PROPERTIES OF WHEY PROTEIN SOLUTIONS

2.3.1 Concentration Dependence

The viscosity of whey protein solutions has been found to rise exponentially with increase in concentration (Herbert, 1972; Mcdonough et al., 1974; Hermansson, 1975). Pradipasena and Rha (1977a) studied the viscosity of β-lactoglobulin solutions using a cone and plate viscometer and found that the apparent viscosity increased linearly with increasing concentration up to 10 percent by weight and then more rapidly as concentration increased above this value.

The effect of protein concentration on the flow properties of WPC dispersions at pH 7 was investigated by Hermansson (1975). She concluded that WPC is characterized by low viscosity over a broad concentration range. The flow was found to be almost Newtonian in the range 4-12% and pseudoplastic in the range 14-16%, while at higher concentrations yield values could be measured and the flow was plastic.

Zeng and Munro (unpublished results) also conducted an investigation into the flow properties of solutions of three WPCs at concentrations of 5 to 40%. They observed that flow

was nearly Newtonian below 10%, tended to be plastic at 40% and exhibited apparent thixotropy above 30%.

2.3.2 Shear Rate Dependence

Whey protein solutions exhibit shear-thinning in some concentration ranges when subjected to a range of shear rates (Herbert, 1972; Hermansson, 1975; Pradipasena and Rha, 1977b). The effect of shear rate on the apparent viscosity of β-lactoglobulin solutions was studied by Pradipasena and Rha (1977b). They found that the apparent viscosity of 3% and 5% solutions was independent of the shear rate but at higher concentrations the apparent viscosity decreased as the shear rate increased. Herbert (1972) observed that the apparent viscosity of whey protein solutions decreased as the shear rate increased and this effect is more pronounced at higher protein concentrations.

2.3.3 Shear Time Dependence

Dispersions of whey proteins in some concentration ranges exhibit time dependent flow phenomena: shear stress or apparent viscosity may decrease or increase with time at a constant shear rate (Pradipasena and Rha, 1977b). In some cases, this change is reversible and the dispersion of protein will recover its original condition with time at rest (Hermansson, 1975). In others, the change brought about by shearing may be irreversible (Pradipasena and Rha 1977b).

Hermansson (1975) found that a dispersion of WPC at a concentration of 20% was thixotropic and the structure broken down during shearing at a high shear rate for 15 minutes was reversibly reformed after resting for 15 minutes. Zeng and Munro (unpublished results) observed that WPC solutions exhibited apparent thixotropy at concentrations over 30% at the measuring temperature of 25^{o}C: the shear stress decreased with shearing time at first

and then reached a constant value after shearing for a short time. The major difference between pseudoplastic and thixotropic systems is that in pseudoplastic fluids the structural breakdown and recovery is instantaneous, whereas in thixotropic fluids a relatively long time is required (Tung, 1978).

Pradipasena and Rha (1977b) conducted an investigation into the effect of shearing time upon the apparent viscosities of β-lactoglobulin solutions. Protein solutions (3-40%) were subjected to constant rates of shear for up to 30 minutes at 25°C. They found that for 3 and 5% solutions the apparent viscosity remained constant, showing no shear time effect over the shear rate range of 6,850-17,000 s^{-1}. For 10-30% solutions, the apparent viscosity increased with shearing time at a constant shear rate. However, the 40% β-lactoglobulin solution showed a time dependent shear thinning behaviour with its apparent viscosity decreasing with shearing time at a constant shear rate, which was distinct from the apparent rheopectic properties determined for 10-30% solutions. They assumed that the time dependent shear thinning property of the 40% solution could be caused by the breaking of aggregates since the concentration was high enough in most dispersions for the protein to exist in aggregate form. The rheopectic property of 10-30% solutions could be explained by the fact that, in shearing at high shear rates, protein molecules could be unfolded, thus changing or increasing the shape, size and effective volume of the solute in solution. They also found that changes in rheological properties were irreversible both for apparent rheopexy and for time dependent shear thinning.

2.3.4 Temperature Dependence

When dispersions of whey proteins are heated, apparent viscosities usually decrease at first (Zeng and Munro, unpublished results) and then increase rapidly above

specific temperatures (Hermansson, 1979). The effect of temperature upon apparent viscosities depends on protein concentration (Zeng and Munro, unpublished results), pH and ionic strength (Hermansson, 1979). Zeng and Munro (unpublished results) found that the increase of temperature might change the flow properties of whey protein solutions from time-independent to time-dependent, and from thixotropy to rheopexy for 40% solutions.

2.4 RHEOLOGICAL PROPERTIES AND FORMATION OF WHEY PROTEIN GELS

2.4.1 Viscoelastic Behaviour

Whey protein gels, like all other protein gels, show viscoelastic behaviour - that is, they exhibit both solid-like elastic and liquid-like viscous behaviour simultaneously. When protein gels are subjected to a stress such as a shear stress, the work of shearing deformation is not completely conserved, as in an ideal elastic solid, nor is it completely dissipated, as in an ideal liquid. Usually, if the stress is applied for a very short time, the elastic character predominates; if the stress lasts for a long time, the viscous character predominates.

Protein gels often can be deformed considerably (e.g., by 10%) and still show linear viscoelastic behaviour (Walstra and Jenness, 1984) - that is, the ratio of stress to strain is a function of time (or frequency) alone and does not depend on the stress or strain magnitude (Ferry, 1970). When a protein gel exhibits linear viscoelastic behaviour, its mechanical properties can be modelled by some suitable combination of rheological models such as the Maxwell model, the Kelvin-Voigt model and the Burgers model.

In order to minimize alteration of internal structure, rheological evaluation of protein gel formation or protein

gel properties must be made using very low stress or strain. In oscillatory rheological measurements small deformations and short time spans are used so that the structure of a protein gel is not altered and so that the requirements of linear viscoelasticity theory are satisfied (Tung, 1978). In oscillation experiments a strain which is made to vary with time in a sinusoidal way is imposed on a sample. A sinusoidally varying stress will result. For an ideal elastic solid, the stress will be in phase with the strain, while for an ideal viscous fluid, the stress will be 90^{o} out of phase. When a viscoelastic material such as a protein gel sample is subjected to sinusoidally oscillating strain, the stress is neither exactly in phase nor 90^{o} out of phase but is intermediate in response with some of the energy input stored and recovered in each cycle and some dissipated as heat (Tung, 1978).

If a sinusoidally varying strain $\gamma(t)$ given by

$$\gamma(t) = \gamma_o \sin(\omega t) \qquad (1)$$

where γ_o = the maximum strain
ω = the angular frequency
t = time

is applied, the following sinusoidally varying stress $\tau(t)$ is obtained:

$$\begin{aligned}\tau(t) &= \tau_o \sin(\omega t + \delta) \\ &= \tau_o \{\sin(\omega t)\cos\delta + \cos(\omega t)\sin\delta\}\end{aligned} \qquad (2)$$

where τ_o = the maximum stress
δ = the phase angle (loss angle) between the strain and the stress

Within the linear region τ_o is by definition proportional to γ_o. Equation (2) can be written as:

$$\tau(t) = \gamma_o \{\tau_o/\gamma_o \cos\delta \sin(\omega t) + \tau_o/\gamma_o \sin\delta \cos(\omega t)\} \quad (3)$$

The elastic part of the stress, which is the part of the stress in phase with the strain, corresponds to the storage modulus G', which is defined as:

$$G'(\omega) = (\tau_o / \gamma_o) \cos\delta \quad (4)$$

The viscous part of the stress, which is the part of the stress out of phase with the strain, corresponds to the loss modulus G", which is defined as:

$$G''(\omega) = (\tau_o / \gamma_o) \sin\delta \quad (5)$$

Then, equation (3) becomes:

$$\tau(t) = \gamma_o \{G'(\omega)\sin(\omega t) + G''(\omega)\cos(\omega t)\} \quad (6)$$

The loss tangent(tanδ), the dynamic viscosity (η') and the complex modulus (G^*) are expressed as :

$$\tan\delta = G''/G' \quad (7)$$

$$\eta' = G''/\omega \quad (8)$$

$$|G^*| = \tau_o/\gamma_o \quad (9)$$

$$G^* = G' + j\,G'' \quad (j^2 = -1) \quad (10)$$

$$|G^*| = (G'^2 + G''^2)^{1/2} \quad (11)$$

The storage modulus (G') is a measure of energy stored during each test cycle whereas the loss modulus (G") measures the energy dissipated. The phase angle (δ) indicates the extent of viscous or, conversely, elastic character of a protein gel at a particular test frequency (ω). For a

perfectly elastic gel, G" and δ would both be zero while for an ideal fluid, G′ and δ would be zero and 90° respectively. The relationship between G′, G", G^* and δ is shown vectorially in Fig. 2.1.

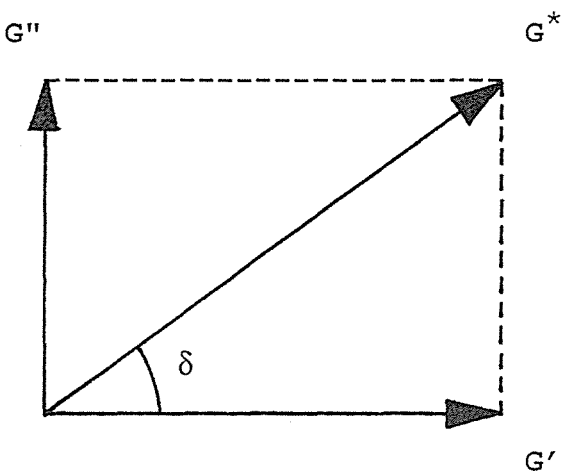


Fig.2.1 Vector diagram showing the relationship between G′, G", G^* and δ.

2.4.2 Formation and Properties of Whey Protein Gels

Formation of a protein gel structure during a gelling process can be studied using nondestructive oscillatory rheological temperature and time scans. This oscillatory rheological technique has been used by a number of workers to monitor protein gel formation (Beveridge et al., 1984; Beveridge and Timber, 1985; Bohlin et al., 1984; Goldsmith and Toledo, 1985; Paulsson et al., 1986, 1989), but few studies of heat-induced gelation of WPC using this technique have been published.

Paulsson et al. (1989) studied heat-induced gelation of β-lactoglobulin by a dynamic rheological method and found that the onset of gelation of β-lactoglobulin was not clearly influenced by either the pH or the protein concentration.

The complex shear modulus (G^*) of the protein gels depended on protein concentration and pH at 40°C. The value of the loss modulus (G") was small compared to the storage modulus (G'). It was also observed (Paulsson et al., 1986) that bovine serum albumin had good, β-lactoglobulin intermediate and α-lactalbumin poor thermal gelation properties. Beveridge et al.(1984) studied protein gel formation of WPC as a function of time and temperature. They found that after the gel point the increasingly elastic character of WPC gels was clearly shown by the storage modulus (G') increasing with time at constant temperature, and the rate and extent of such increases were temperature dependent.

CHAPTER 3

OBJECTIVES

The objectives of this study were to investigate the rheological properties of three WPC solutions together with the effects of concentration, shear rate, shear time and temperature on these properties. The breakdown of the protein solution structure by shear, and the recovery of this structure, were also to be investigated.

protein gel structure development during heat-induced gelation was to be studied along with an evaluation of the effect of concentration and temperature on the formation rate of gel structure. The effects of temperature and protein concentration on protein gel properties were also to be examined. A main objective of the study was to gain a more complete understanding of the mechanism of protein gelation to enable easier control of protein gel properties by appropriate manipulation of environmental conditions such as protein concentration, temperature, pH and ionic strength.

Temperature dependence, concentration dependence and time dependence of the liquid-gel transition for WPC solutions also were to be investigated by three kinds of experiments: constant temperature, constant concentration and temperature-scanning experiments.

CHAPTER 4

EXPERIMENTAL

4.1 PREPARATION OF WPC SOLUTIONS

Samples of Alacen 312, Alacen 392 and Alacen 475, all commercially available WPC powders, were obtained from the New Zealand Dairy Research Institute (NZDRI). Alacen 312, Alacen 392 and Alacen 475 are lactic casein, cheese and rennet casein WPC powders. The compositions of the three WPC powders are shown in Table 1.

Table 1. COMPOSITIONS OF WPC POWDERS (%)*

Components	Products		
	Alacen 312	Alacen 392	Alacen 475
Protein (TN x 6.38)	76.5	76.5	75.9
Moisture	3.9	3.9	4.4
Fat	4.5	6.5	6.7
Ash	3.3	3.3	3.0
Lactose	11.8	9.8	10.0

* Source: New Zealand Dairy Research Institute.

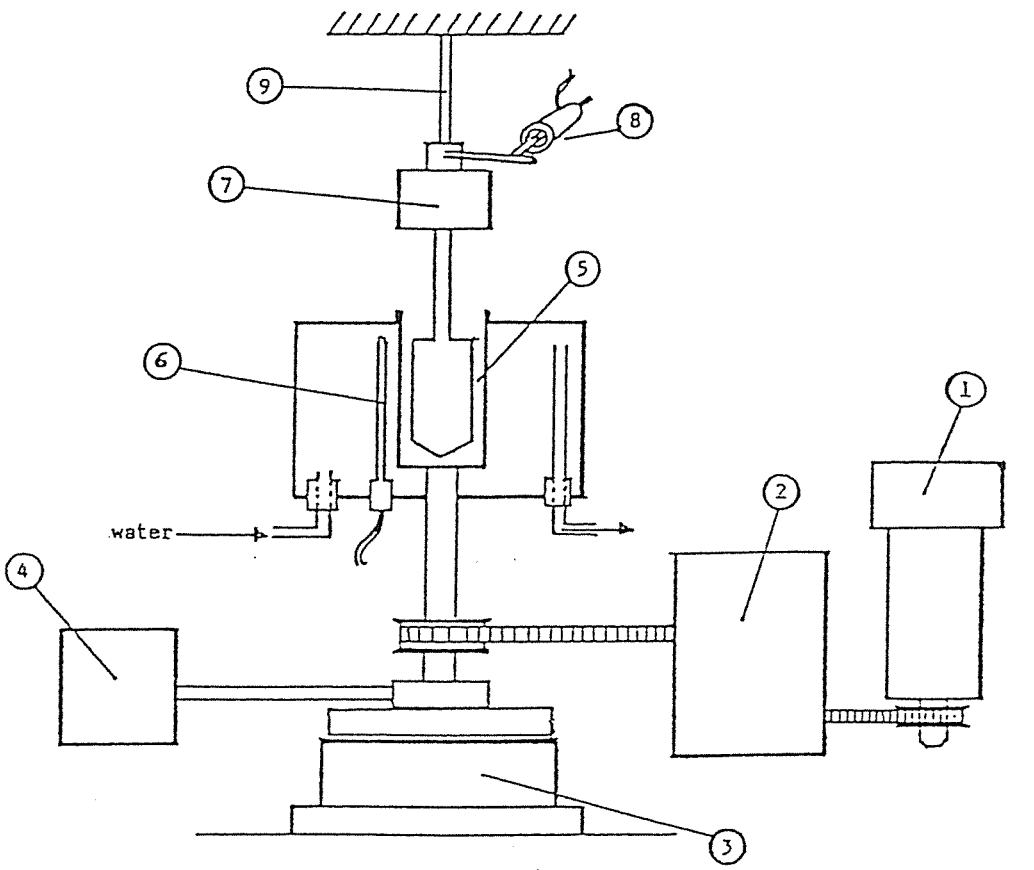
WPC solutions of 5-40% (w/w) were prepared by reconstituting WPC powder with distilled water in a stomacher. Dispersed air in WPC solutions was removed by leaving solutions in a vacuum chamber at an absolute pressure of less than 1KPa and room temperature until the visible foam had disappeared (Tiu and Boger, 1974). The natural pH values of the solutions varied with concentration and usually ranged from pH 6.4 to pH 6.8. Prepared samples were kept in a refrigerator overnight before rheological measurements.

4.2 BOHLIN VOR RHEOMETER

The Bohlin VOR Rheometer System (Bohlin Rheologi AB, Lund, Sweden) was used in both viscometry (steady shear) and oscillation modes. The principle of operation of the Bohlin Rheometer System is that a controlled shear rate or strain is applied to the sample and the resulting shear stress is monitored. Figure 4.1 shows the principal parts of the rheometer (Bohlin VOR Software User Manual, Bohlin Rheologi AB, Sweden, 1987).

A steady rotational speed in the viscometry mode is obtained by using the DC motor drive (1) with the clutch (3) disengaged. The software controls the speed by an output of a set value motor speed, and the gear boxes (2) are used for giving reductions of speed. Small amplitude, position controlled, angular deflection is used in the oscillation mode and this motion is obtained through the position servo actuator (4) with the clutch (3) engaged.

The sample cell (5) consists of a rotating cup or plate and a virtually fixed bob, plate or cone, with the sample contained between the fixed and the rotating part. The torque on the fixed part is transmitted to a torsion bar through a shaft supported by an air bearing (7). The torque measurement involves both the torsion bar (9) and the LVDT (8). The choice of torque bar determines the sensitivity, while the small angular deflection as measured by the LVDT gives the desired torque signal to the software. The temperature is measured by the sensor (6) in the circulating temperature controlled water. A temperature range of 5^{o}C to 90^{o}C is available along with the facility for a programmed temperature sweep with a heating rate of up to 6^{o}C/min.



1 - DC motor with tacho servo

2 - Gear boxes

3 - Clutch

4 - Position servo actuator

5 - Sample cell

6 - Temperature sensor

7 - Air bearing

8 - LVDT torque measurement

9 - Torsion bar

Fig.4.1-The principal parts of the Bohlin Rheometer.

4.3 STEADY SHEAR AND OSCILLATORY RHEOLOGICAL MEASUREMENTS

The concentric cylinder measuring system C25, consisting of a 25 mm diameter fixed bob and a 27.5 mm diameter rotating cup, was used in all experiments. All measurements were made with 10-12 ml of solution. For all oscillatory tests and for steady shear tests at high temperatures, high concentrations or long times the sample was covered with a thin layer of liquid paraffin to prevent evaporation of water. In oscillatory tests the frequency and the shear strain amplitude, unless otherwise noted, were set to 1 Hz and 0.0309 (angular amplitude = 0.00309 radian) respectively. The results of oscillatory measurements were continuously shown on the PC monitor of the rheometer in terms of phase angle, dynamic viscosity, and storage and loss moduli. Shear rate sweeps to illustrate shear rate dependency or time dependency of viscosity were determined by first increasing and then decreasing the rate of shear (Pradipasena and Rha, 1977b). Time sweeps at constant shear rates were also conducted to investigate time dependency of viscosity.

CHAPTER 5

STEADY SHEAR RHEOLOGICAL PROPERTIES OF WPC SOLUTIONS

5.1 CONCENTRATION DEPENDENCE

The effect of protein concentration on the apparent viscosity is shown in Fig.5.1. The apparent viscosity of WPC solutions increased with increase in concentration. The effect of concentration may perhaps be explained by assuming three separate regions: a dilute region, a semi-concentrated region and a concentrated region. In the dilute region where the concentration was up to 10%, the protein solution obeyed Einstein's equation and a linear relationship existed between viscosity and weight percent concentration. In this region, the relationship between concentration and viscosity of Alacen 475 solutions could be expressed as:

$$\eta_S = \eta_W(1 + 28C)$$

where η_S = viscosity of solution.
η_W = viscosity of water (0.9 mPas at 25°C).
C = fractional weight concentration.

In the semi-concentrated region in which the concentration was higher than 10% and lower than 30%, a deviation from Einstein's equation was apparent from the non-linear dependence of viscosity on the protein concentration. In the concentrated region, which corresponded to levels above 30%, the apparent viscosity of solutions became time dependent (see Sect.5.2) and it increased very rapidly as concentration was increased. Apparent viscosity versus concentration in this region is not shown in Fig.5.1 since apparent viscosity is dependent not only on concentration and shear rate, but also on shearing time.

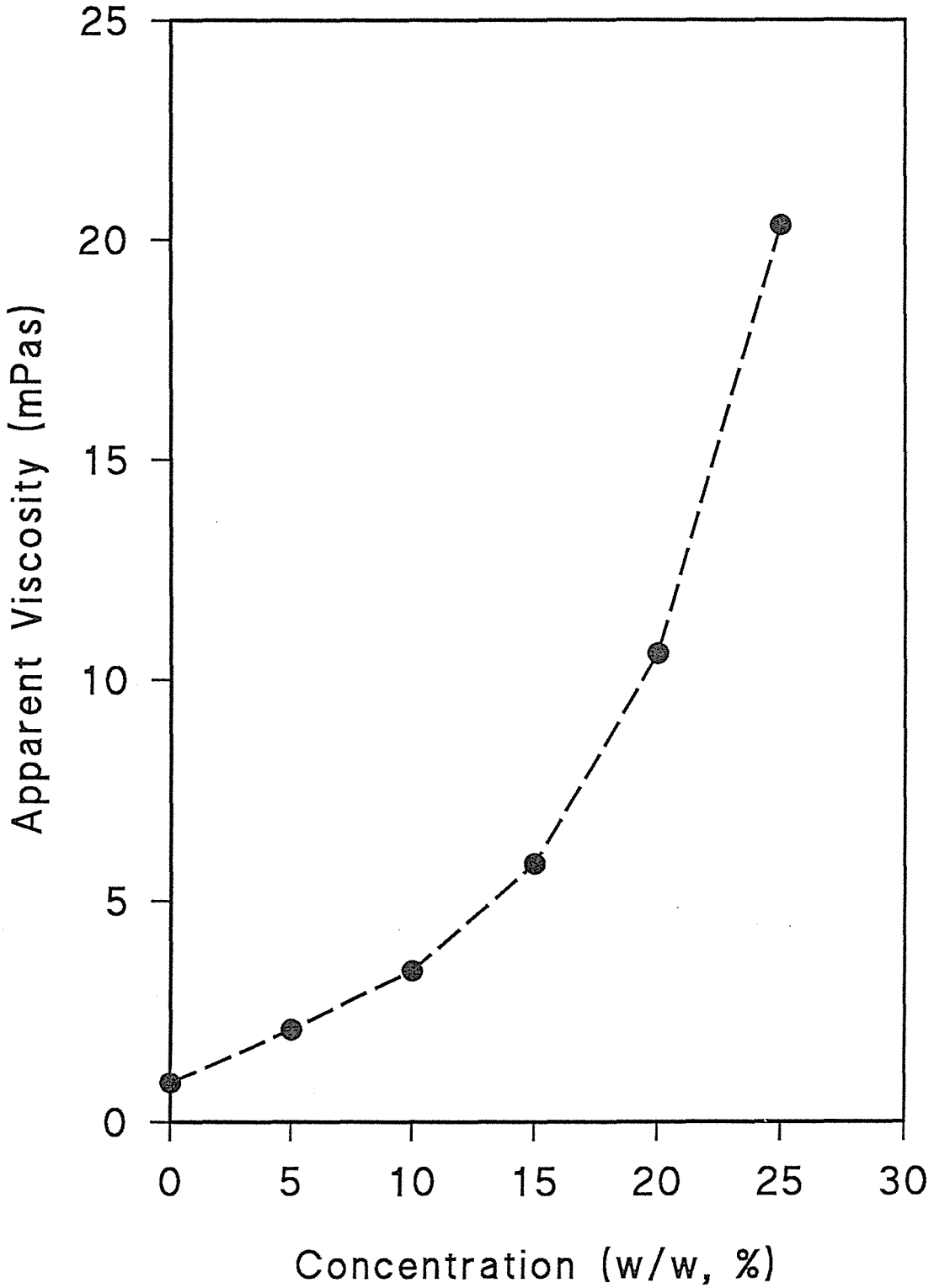


Fig. 5.1—Viscosity of Alacen 475 solutions at 463 (1/s) and 25°C as a function of concentration.

Theoretically, when a suspended material exists as rigid spheres with no hydrodynamic interaction between the particles, the flow property of the suspension is Newtonian and its viscosity is dependent only on the volume fraction of the suspended spherical particles. In such a case, the viscosity can be expressed by Einstein's equation (Pradipasena & Rha, 1977a; Lee & Rha, 1979; Prentice, 1984):

$$\eta_s = \eta_o(1 + a\Phi)$$

where η_s = viscosity of the dispersed system.
η_o = viscosity of the surrounding liquid.
a = constant, 2.5 for a small spherical rigid particle which carries no electrical charge.
Φ = volume fraction occupied by the dispersed particles.

However, the dispersion of whey protein molecules cannot be treated simply as a case like this. Whey protein molecules may have various geometrical shapes due to the association and dissociation of molecules (Timasheff, 1964). They are also quite flexible and cannot be considered as rigid spheres. Protein-water interactions may influence swelling, solubility and the hydrodynamic hydration spheres surrounding the molecules (Cheftel et al, 1985). Therefore, not only the volume fraction but also shape, flexibility, swelling and hydration of protein molecules may contribute significantly to the viscosity of the protein in solution. In addition to protein-water interactions, protein molecules in solution also interact with each other.

In the dilute region, the interactions between protein molecules are relatively small. The total viscosity effect may be the sum of the effects caused by each of the individual suspended molecules. Therefore, a linear relationship between viscosity and concentration was observed and the viscosity could be expressed by Einstein's

equation. As concentration increases, the volume of the dispersed phase increases, and so do the interactions of protein molecules. This corresponds to the semi-concentrated region defined previously. In this region, the disturbances of solvent flow produced by the suspended protein molecules are no longer independent, due to the presence of hydrodynamic interactions, hydrogen bonds, hydrophobic interactions and electrostatic interactions between the protein molecules. Thus 10 percent by weight seems to be a critical concentration. Below the critical concentration, the suspended protein molecules were kinetically independent and Einstein's equation was applicable. Above the critical concentration, deviation from Einstein's equation was observed and non-linearity between viscosity and concentration was apparent. In the concentrated region, intermolecular interactions between protein molecules increase strongly with increase in concentration, which may lead to the aggregation of protein molecules, e.g. the formation of protein aggregates. These concentrated protein solutions showed time dependent shear thinning behaviour (see Sect.5.2).

5.2 SHEAR THINNING BEHAVIOUR

The effects of shear rate on shear stress and apparent viscosity of Alacen 475 solutions at 25°C are shown in Fig.5.2, Fig.5.3 and Fig.5.4. At low WPC concentrations of 5-15% the solutions were Newtonian: apparent viscosity was independent of shear rate (Fig.5.3). At 20% apparent viscosity decreased slightly with shear rate at low shear rates (Fig.5.3), indicating slight shear thinning. At high protein concentrations of 30 to 40% shear rate sweeps produced hysteresis loops (Fig.5.3 and Fig.5.4) - indicating time dependent rheological behaviour. The effect of shear rate on apparent viscosity at high protein concentrations was also more marked, particularly at low shear rates. The WPC solutions therefore exhibited thixotropic behaviour at

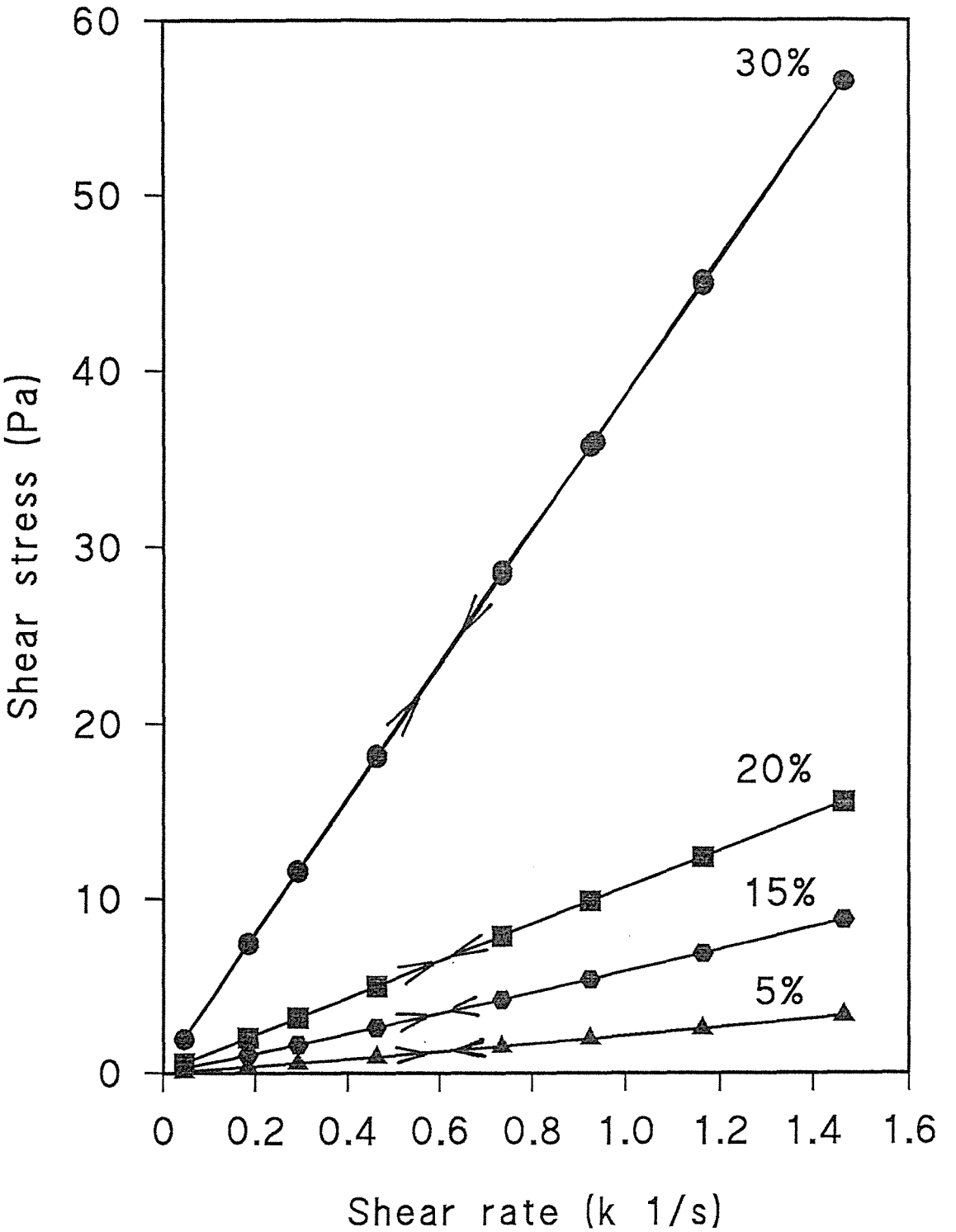


Fig. 5.2–Effect of shear rate on shear stress of Alacen 475 solutions at 25°C. The direction of the shear rate sweep is indicated by the arrow.

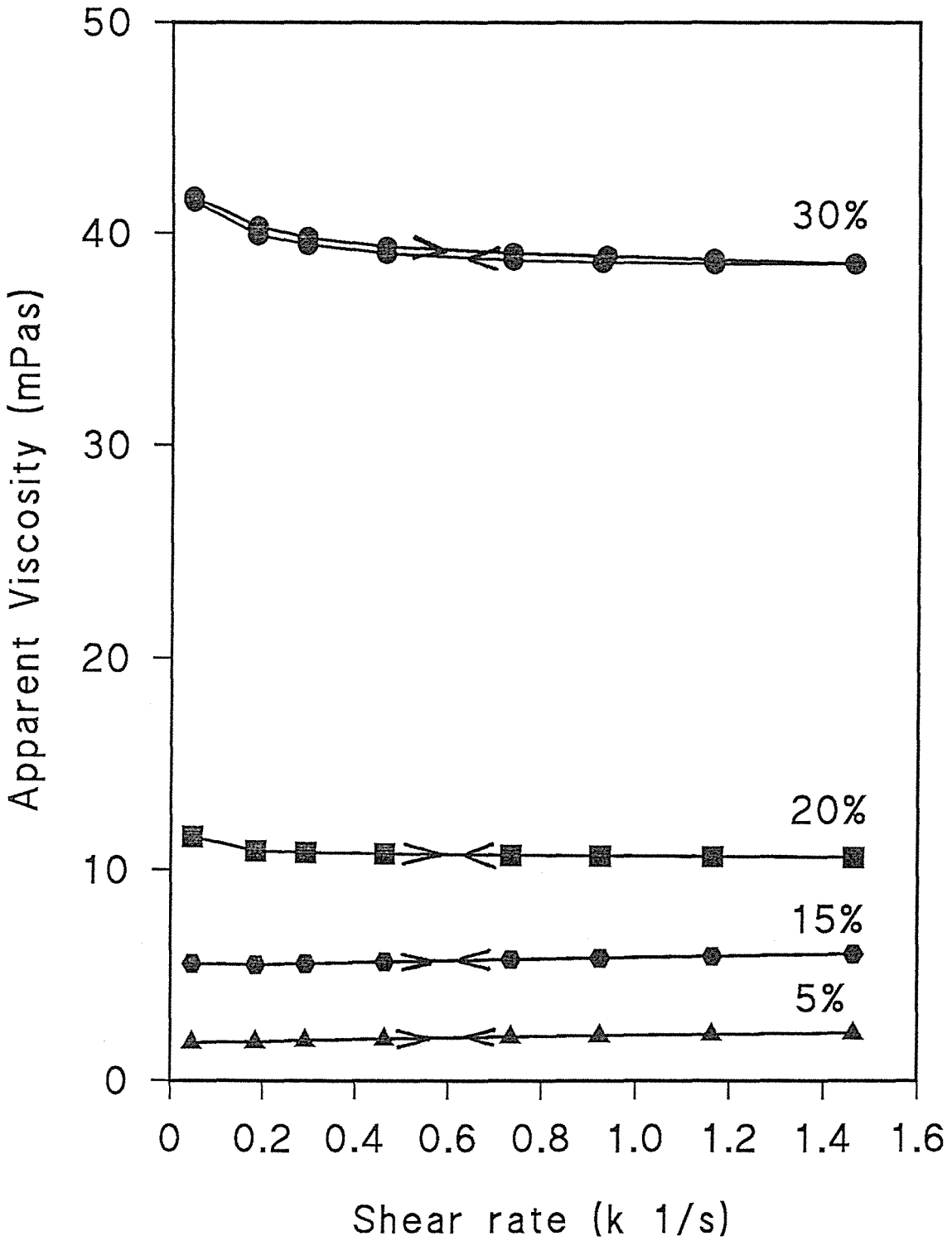


Fig. 5.3—Effect of shear rate on apparent viscosity of Alacen 475 solutions at 25°C (low concentration). The direction of the shear rate sweep is indicated by the arrow.

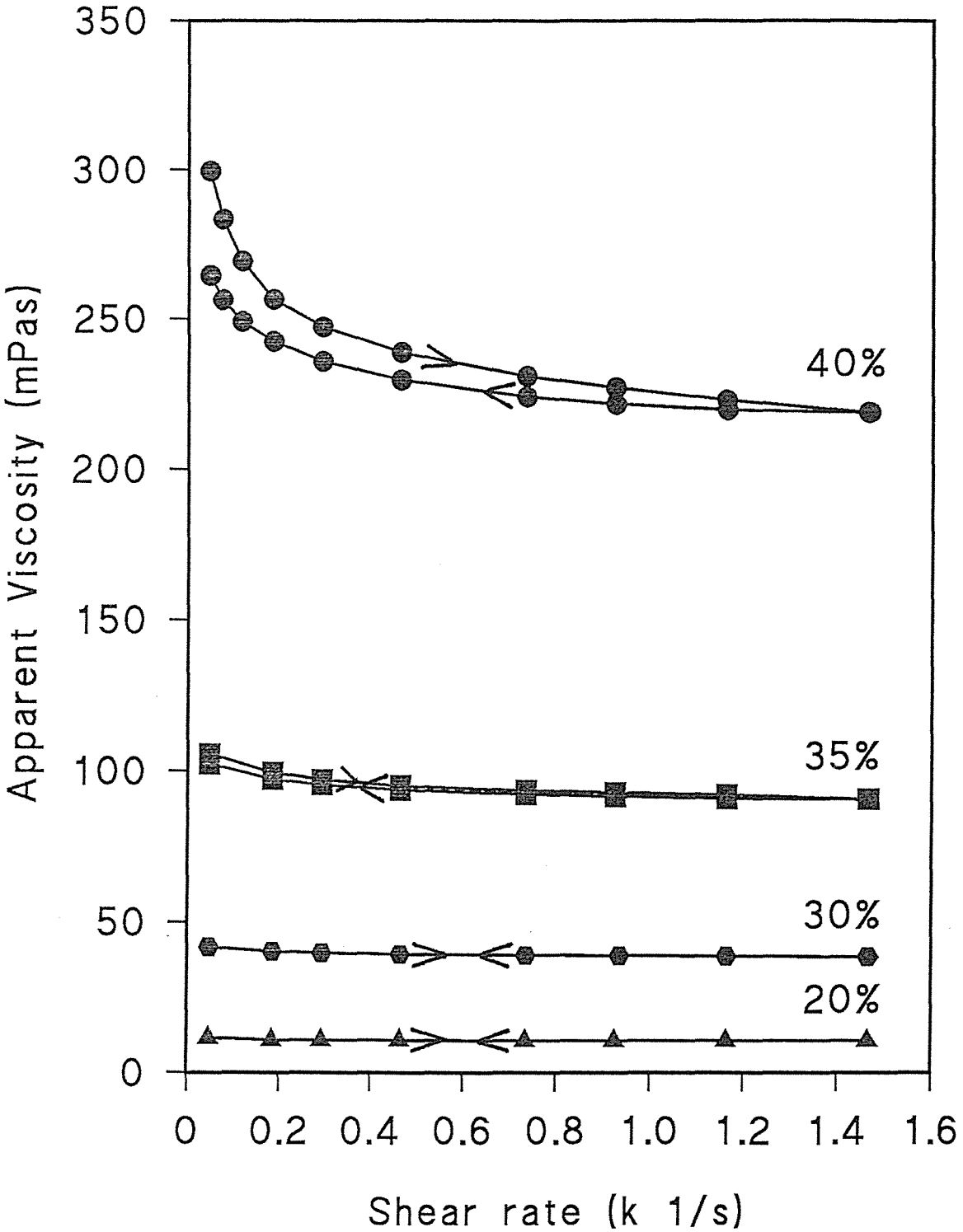


Fig. 5.4—Effect of shear rate on apparent viscosity of Alacen 475 solutions at 25°C (high concentration). The direction of the shear rate sweep is indicated by the arrow.

concentrations of 30% and above. Such shear thinning and time dependent behaviour of protein solutions is usually attributed to the following phenomena: (a) progressive orientation of molecules in the direction of flow and deformation or removal of the protein hydration sphere, and (b) rupture of weak bonds such as hydrogen bonds resulting in dissociation of protein aggregates or networks (Tung, 1978; Cheftel et al., 1985). Of these phenomena, the latter was probably dominant for WPC solutions since shear thinning was more marked at high protein concentrations and was not observed below 20% WPC.

5.3 TIME DEPENDENCE OF STRUCTURE BREAKDOWN AND RECOVERY

Fig.5.5 illustrates the time dependence of structure breakdown and recovery for a 40% WPC solution. During initial shear at 147 s^{-1} the shear stress decreased with shearing time and then reached a constant value after a short time (about 5 minutes). This behaviour might be attributed to two processes. Firstly, the protein-protein linkages at the high concentration would be broken down during shear. The rate of structure breakdown would be dependent on the number of structural linkages present and this would decrease with time. Secondly, the protein-protein linkages might be rebuilt again by Brownian motion and molecular collisions, and this simultaneous rebuilding of linkages and structure would increase with time because the number of possible new structural linkages would increase. Finally, a dynamic equilibrium would be established at 147 s^{-1} when rate of structural build-up equalled rate of break down. The shear stress at 147 s^{-1} would be expected to reach a constant value - as Figure 5.5 shows it does.

When the shear rate was increased quickly from 147 s^{-1} to 734 s^{-1}, the shear stress decreased with time to a new steady value after about 5 minutes. It may be supposed that the new higher shear rate of 734 s^{-1} disrupted the dynamic

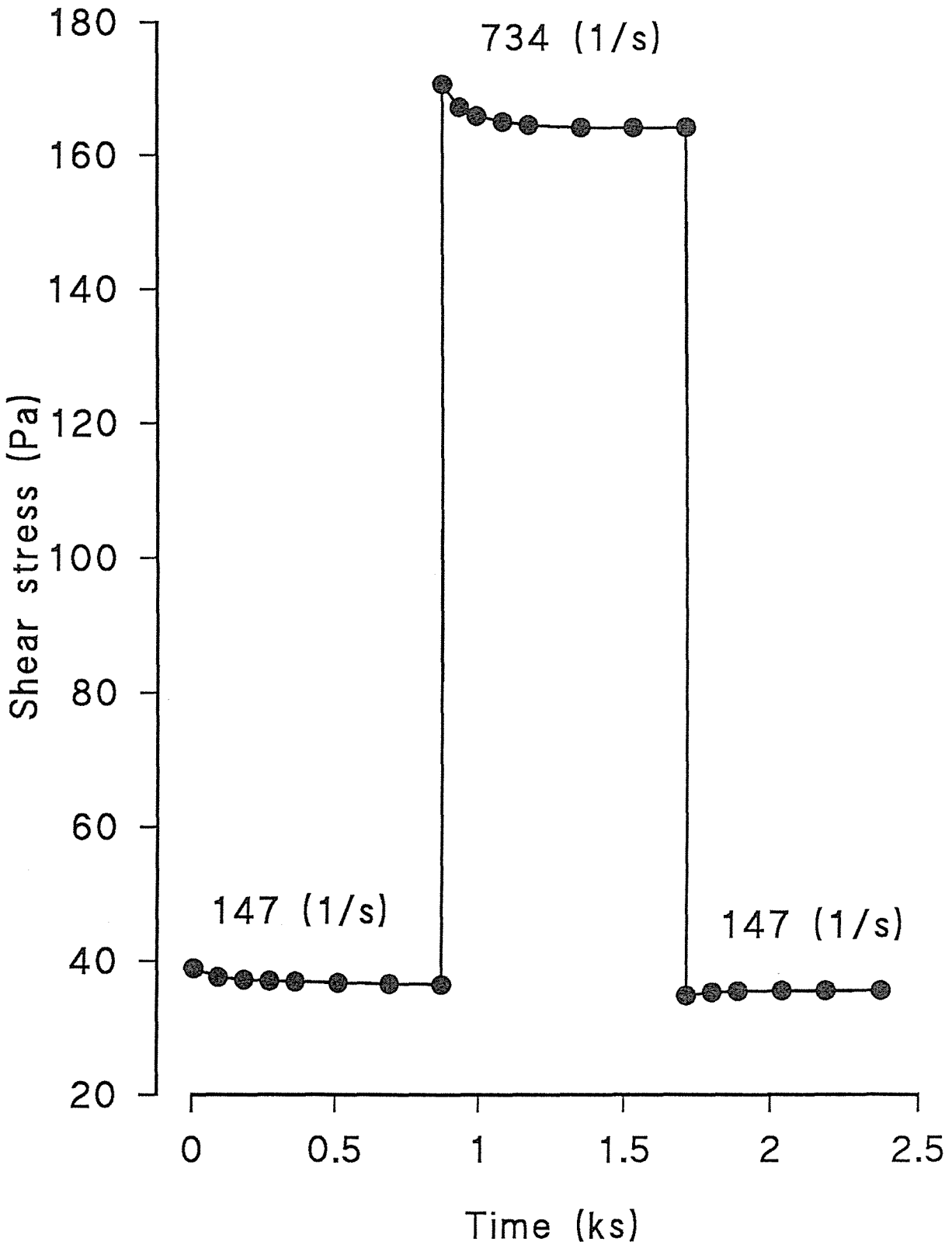


Fig. 5.5—Time dependence of structure breakdown and recovery for a 40% Alacen 475 solution at 25°C. The sample was sheared successively at 147, 734 and 147 (1/s).

equilibrium set up at 147 s^{-1} because it caused a greater rate of structure breakdown, but that a new equilibrium became established by the mechanism described above. When the shear rate was suddenly decreased from 734 s^{-1} back to 147 s^{-1} the shear stress increased slightly with time at 147 s^{-1} (Fig.5.5). This may indicate recovery of structure by Brownian motion from the equilibrium structure formed at 734 s^{-1}. However, any such structure recovery appears to have been slow because the shear stress increased only slowly with time at 147 s^{-1}.

In order to evaluate structure recovery at zero shear rate after shearing, oscillatory measurements were applied. A sample of 40% Alacen 475 solution was presheared at 734 s^{-1} for 10 minutes at 25°C and then the time dependence of structure recovery was monitored immediately after shearing by an oscillation test at a frequency of 1 Hz and a shear strain amplitude of 0.0618 (angular amplitude = 0.00618 radian). Another sample of 40% Alacen 475 solution, which had not been presheared, was also tested by an oscillatory method for comparison. The results are illustrated in Fig.5.6. Structure recovery at rest after shearing was apparent as indicated by the increase in storage modulus (G') and dynamic viscosity (η') with rest time. However, the rate of structure recovery evidently decreased with rest time since the slopes of the curves (dG'/dt and $d\eta'/dt$) decayed with time. After 12,000 seconds the broken structure had not recovered completely and structure recovery was still going on, indicating structure recovery at rest after shearing was a slow process.

5.4 TEMPERATURE DEPENDENCE

Fig.5.7 shows the viscosity-temperature relationships for Alacen 475 solutions at various concentrations. Rheological properties of WPC solutions were strongly temperature dependent. The viscosity, at first, decreased as the

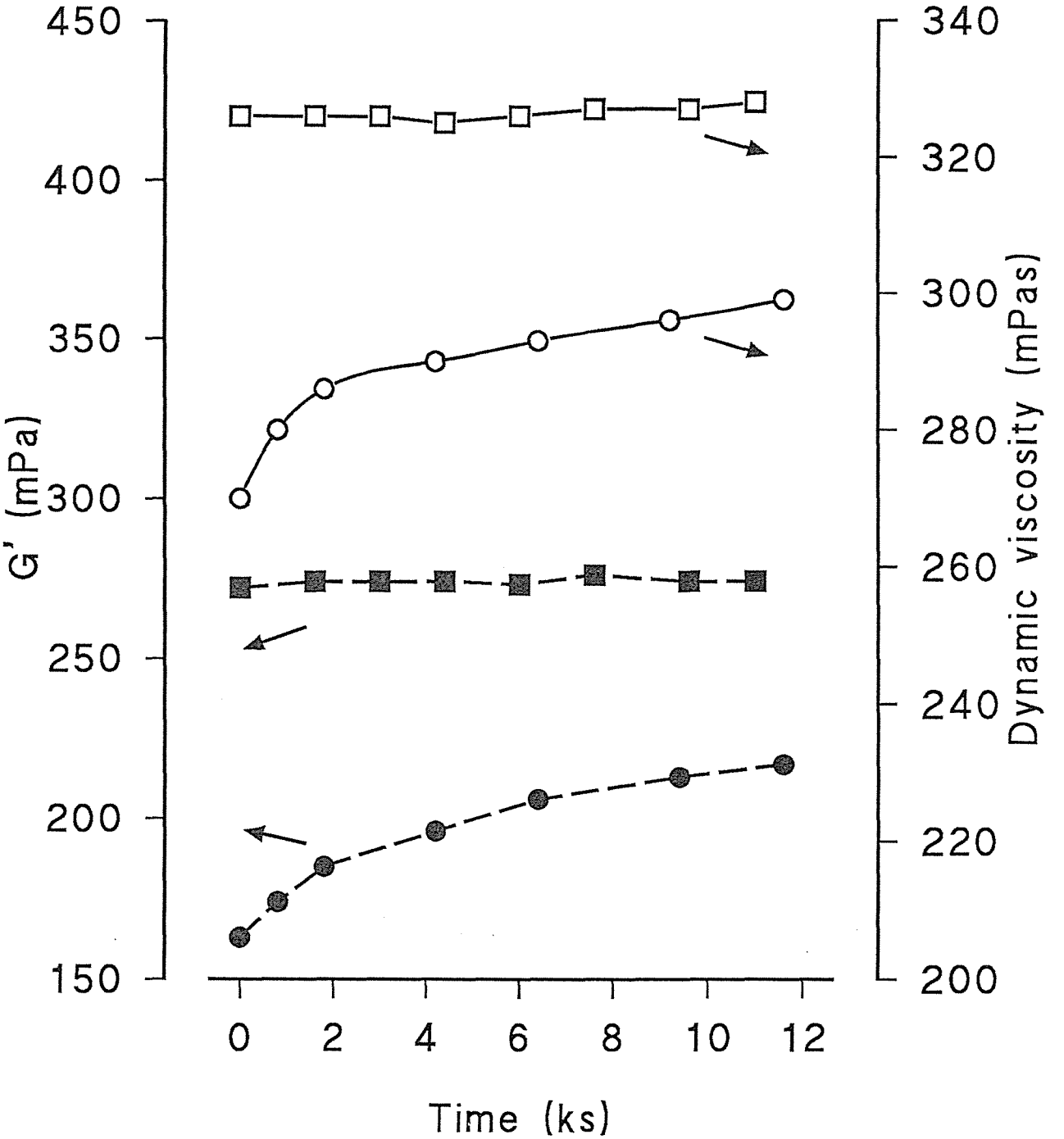


Fig. 5.6—Time dependence of structure recovery for a 40% Alacen 475 solution at 25°C and 1 Hz. (●, ○) The sample was presheared at 734 (1/s) for 10 minutes. (■, □) The sample was not presheared.

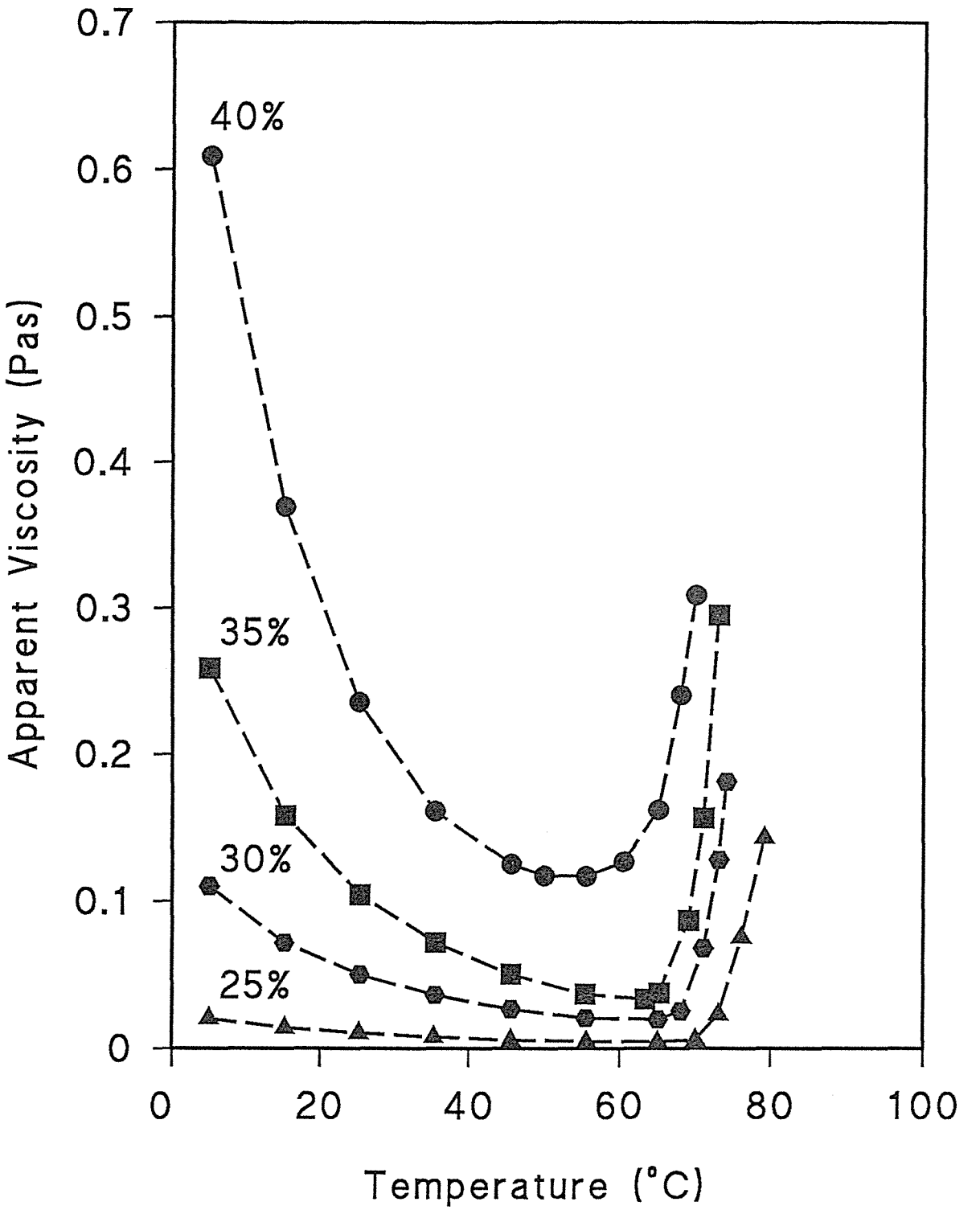


Fig. 5.7–Effect of temperature on apparent viscosity of Alacen 475 solutions at 463 (1/s). Temperature was increased at 1°C/min.

temperature increased and then increased rapidly with increase in the temperature. Three basic mechanisms may combine to affect the changes of viscosity with temperature in this case. Firstly, the viscosity of protein dispersions is a function of the intermolecular forces that restrict molecular motion. These forces depend upon the intermolecular spacings that are affected by changes of temperature (Holdsworth, 1971). As temperature increases, so do intermolecular spacings, and thus, viscosity decreases (Holdsworth, 1971).

Secondly, temperature-induced changes of protein structure occur as the temperature increases, especially at higher temperatures (Dewit & Klarenbeek, 1983). Below 60^{o}C increase of temperature may lead to reversible physico-chemical changes such as partial unfolding of protein molecules and changes of protein hydration (Pfeil, 1981; Dewit & Klarenbeek, 1984). Consequently, the axial ratio and hydrodynamic volume of protein molecules may increase. Protein-protein hydrophobic interactions also increase with increasing temperature due to exposure of reactive hydrophobic groups, leading to the formation of some protein aggregates. These changes with increasing temperature could result in increase in viscosity of WPC solutions.

Thirdly, protein aggregates formed by intermolecular interactions may be disrupted by shearing.

Obviously, the first and third mechanisms dominated at first (i.e. at lower temperature) and the viscosity decreased initially with temperature (Fig.5.7). As the temperature continued to increase, the first and third mechanisms balanced the second one, and the minimum viscosity of WPC solutions was obtained. The temperature at which this occurred decreased with increase in concentration as shown in Fig.5.7. After the minimum points of viscosity the second mechanism may be dominant and the viscosities increased with

increasing temperature. Actually, above 60°C the changes of protein structure are mainly irreversible (Dewit & Klarenbeek, 1983) and protein-protein hydrophobic interactions increase strongly with increasing temperature. Above 70°C the content of free -SH groups in whey protein increases rapidly (Lyster, 1964), and so does the formation of intermolecular disulphide bonds. Therefore, protein gelation occurred and the apparent viscosity increased markedly with increasing temperature as illustrated in Fig.5.7.

The effect of temperature on the apparent viscosity of 20% Alacen 475 solutions is shown in Fig.5.8. The apparent viscosity was independent of shearing time at 25°C. A slight decrease of apparent viscosity with shearing time occurred at 40°C. Time dependent shear thinning was observed at 50°C: the apparent viscosity first decreased quickly with time and then reached a constant value after a short time. At 60°C apparent viscosity increased slowly with shearing time, while at 70°C apparent viscosity increased dramatically with shearing time and the formation of a protein gel could be observed. It is clear that two opposing processes were occuring in these experiments and that one at least of these processes was highly temperature dependent. It is postulated that the two processes were structure breakdown by shear and structure build-up by protein-protein interactions. The structure breakdown by shear is presumably very similar to that described in Sect.5.3 above. Structure build-up might occur by the following mechanisms. Protein-protein interactions are known to be highly temperature dependent. An increase in temperature generally increases the unfolding of protein molecules, which increases exposure of reactive groups such as hydrophobic groups and -SH groups. Protein-protein hydrophobic interactions increase strongly with increasing temperature and the formation of disulphide bonds is also promoted by heating. Hydrophobic interactions and disulphide bond formation would lead to an increase in

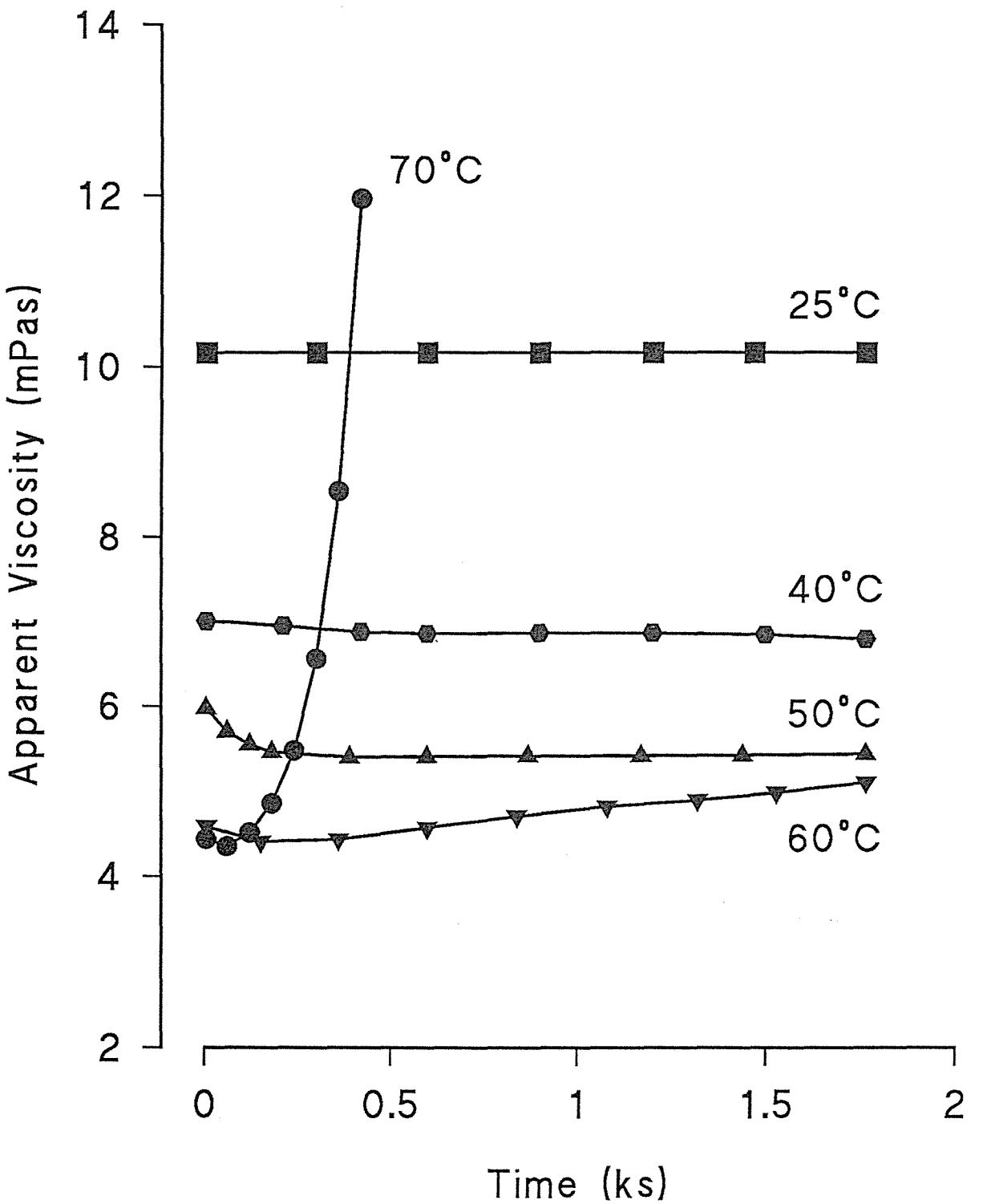


Fig. 5.8—Apparent viscosity of 20% Alacen 475 solutions as a function of time during steady shear at 147 (1/s) and at different temperatures.

apparent viscosity and could also lead to the formation of protein aggregates or protein networks which would further increase apparent viscosity. Structure breakdown by shear clearly dominated at low temperature whereas structure formation by protein-protein interactions dominated at high temperature. These results thus support the interpretation of the results shown in Fig. 5.7. Similar results were also obtained for 30% Alacen 475 solutions as illustrated in Fig.5.9.

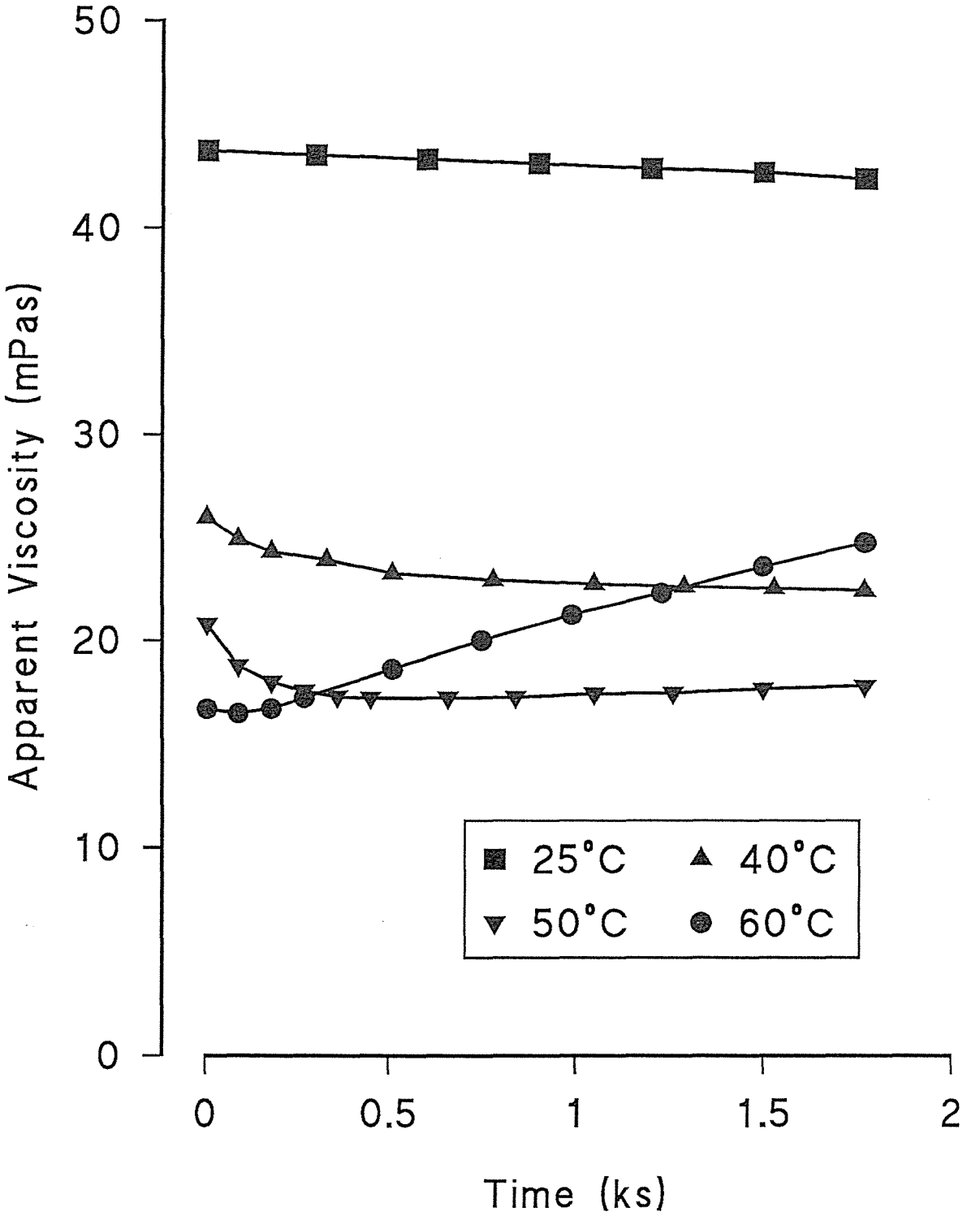


Fig. 5.9–Apparent viscosity of 30% Alacen 475 solutions as a function of time during steady shear at 147 (1/s) and at different temperatures.

CHAPTER 6

OSCILLATORY RHEOLOGICAL CHARACTERISATION OF HEAT-INDUCED GELATION OF WPC SOLUTIONS AND OF THE FORMED GELS

6.1 DEVELOPMENT OF GEL STRUCTURE DURING GELATION

6.1.1 Background Literature

Globular protein gel networks produced by heat are usually constructed from partially unfolded protein molecules still in corpuscular form (Barbu & Joly,1953; Tomb, 1970, 1974; Clark & Tuffnell, 1980; Clark et al, 1981; Hermansson, 1986, 1988; Koseki et al, 1989a, 1989b; Doi & Kitabatake, 1989). However, protein gel networks can be formed by either linear aggregations or by random aggregations.

The gelation of globular proteins by linear aggregations is described using the string of beads model proposed by Tomb (1974). In this model gels form as a result of association of partially unfolded protein molecules to produce linear strands, which is followed by interaction of these strands to give the gel mesh. Gels made of linear aggregations have been reported for β-lactoglobulin (Hermansson, 1988), serum albumin (Barbu & Joly, 1953; Clark et al, 1981), ovalbumin (Barbu & Joly, 1953; Doi et al, 1987; Koseki et al, 1989a, 1989b), insulin, lysozyme, ribonuclease (Clark et al, 1981) and soybean glycinin (Nakamura et al, 1984).

Gels formed by random aggregations of protein molecules have been observed for gels of casein (Dickinson & Stainsby, 1986), myosin heated at high ionic strength (Hermansson et al, 1986), β-lactoglobulin and whey protein concentrate made at high temperature, and ovalbumin at high ionic strength and a pH near the isoelectric point (Doi et al, 1987; Doi &

Kitabatake, 1989; Kitabatake et al., 1989; Koseki et al., 1989a, 1989b).

Whether protein gels are produced by linear aggregations or by random aggregations is dependent on electrostatic repulsion of protein molecules, which in turn is controlled by pH and ionic strength (Barbu & Joly, 1953, Hermansson, 1988; Doi & Kitabatake, 1989). If the repulsion energy barrier against random aggregations is sufficiently high the molecules can arrange themselves into strands and a fine stranded network is obtained because of a reduced energy barrier near the ends of a growing strand (Clark & Lee-Tuffnell, 1986; Hermansson, 1986; Doi & Kitabatake, 1989). As pH approaches the isoelectric point or the ionic strength is increased, the electrostatic repulsion energy barrier is reduced because of the reduction of the charge protein molecules carry. Then, random aggregations tend to occur. For example, depending on pH values, β-lactoglobulin can form a transparent fine stranded gel structure by linear aggregations as well as an opaque aggregated structure by random aggregations (Hermansson, 1988). Ovalbumin can form a clear gel by linear aggregations as well as a turbid gel and even a coagulum by random aggregations, this behaviour being governed by pH and ionic strength (Doi et al., 1987; Doi & Kitabatake, 1989; Kitabatake et al., 1989; Koseki et al., 1989a, 1989b).

However, it is difficult to define how random an aggregation is. It appears that there are many intermediate stages between completely linear aggregations and completely random aggregations. As the repulsion energy barrier of the molecules is gradually reduced, the degree of linear aggregations decreases while the degree of random aggregations increases. Hermansson(1982b) found that blood plasma gels constructed at different temperatures and pHs were composed of some large aggregates(random aggregates) together with regions of ordered linear strands between

them. It was also observed that between a clear ovalbumin gel and an ovalbumin coagulum there was an intermediate called a turbid gel which consisted of some large random aggregates floating in a linear stranded network (Doi et al., 1987; Doi & Kitabatake, 1989; Kitabatake et al., 1989)

6.1.2 Structure Development During Gelation

The development of protein gel structure during the gelling process was followed rheologically. It soon appeared that the build-up of a protein gel structure might be monitored by the time dependence of the storage modulus G′, loss modulus G", and phase angle δ. (tanδ=G"/G′).

Fig.6.1 illustrates a typical case of which the continuous changes in dynamic rheological parameters(G′, G", η′, δ) as a function of time at 73°C for a 30% WPC solution represent the development in protein gel structure during heat-induced gelation. After heating at 73°C for about 180s G′, G", η′, began to increase while δ started to fall rapidly, indicating the beginning of protein gel formation. After this G" and η′ increased steadily until maximum values were attained. Then G" and η′ decreased up to the completion of the gelling process. The storage modulus G′ rose rapidly at first and then slowly until a plateau was reached.

To be able to understand the relationship between the change in dynamic rheological parameters and the development of the protein gel structure during the gelling process, it is necessary to know how the protein gel structure is formed and how the development of the protein gel structure contributes to the build-up or other change in rheological parameters. In the following paragraphs a possible mechanism for the development of whey protein gel structure is proposed.

Gelation of protein solutions involves a phase transition from a state of solution to a state which is an infinite gel

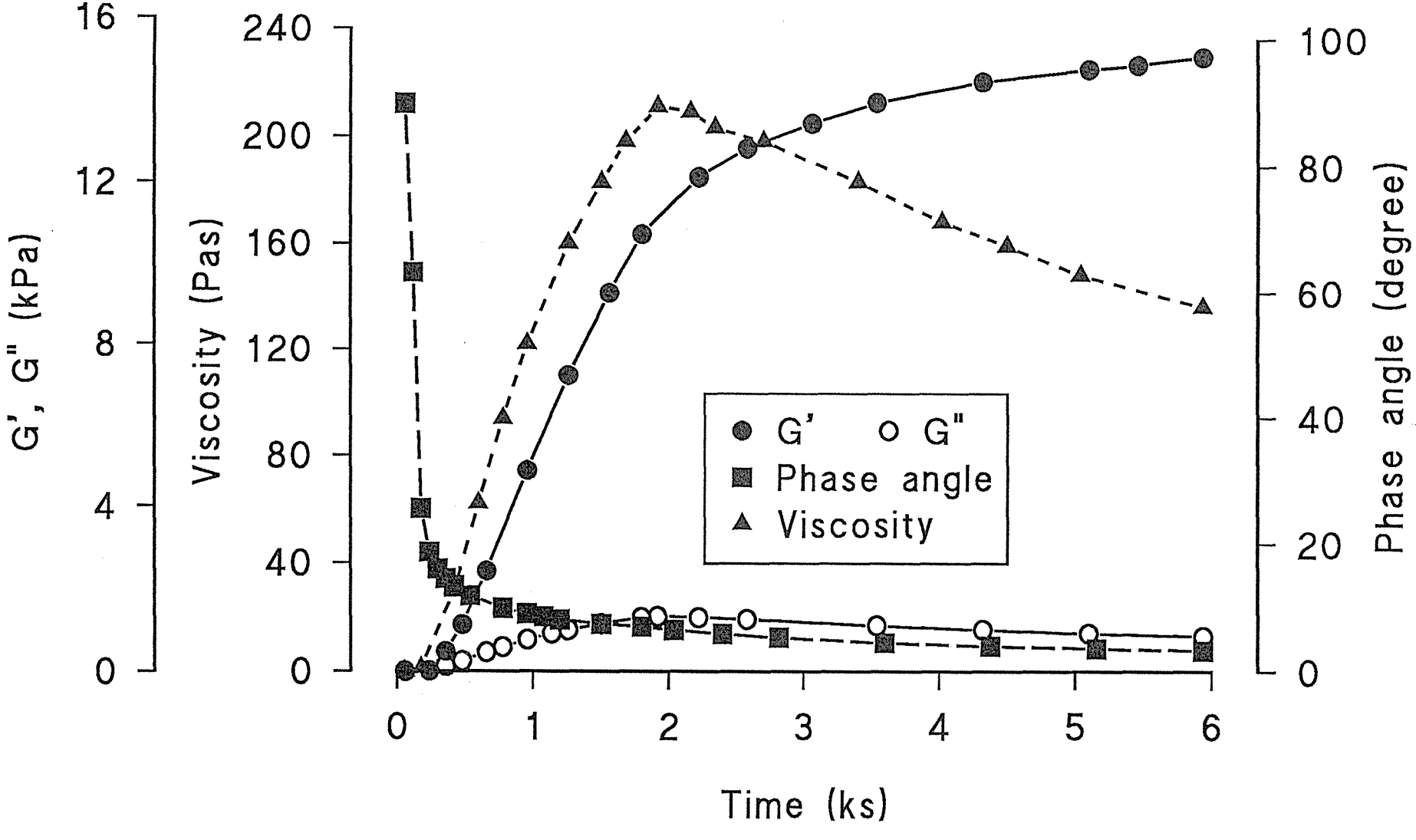


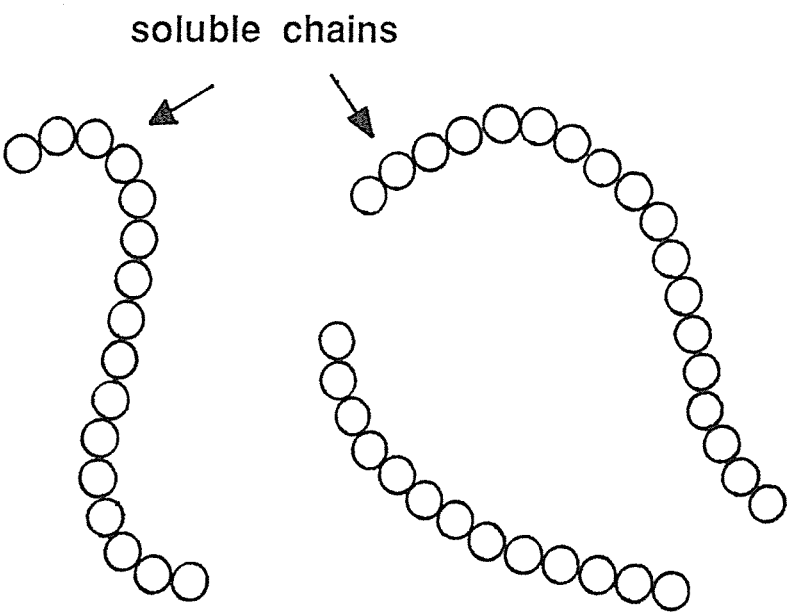
Fig. 6.1—Changes in storage modulus G', loss modulus G", phase angle, and dynamic viscosity during the thermal gelation of 30% Alacen 475 solution at 73°C.

network. The gelation process is characterized by the conversion factor (Flory, 1953), p. This is defined as the ratio of the actual number of bonds formed at a given moment to the maximum possible number of such bonds. This factor (p) is equal to zero and one at the beginning and at the end of gelation respectively. Thus for small p no gel is present whereas for p close to one a gel network exists. Therefore, there is in general a sharp phase transition at some intermediate critical point $p = p_c$ where an infinite gel network starts to appear. For $p<p_c$, there is a sol that is an assembly of finite aggregates formed from protein molecules. For $p>p_c$, there is a sol embedded in a gel that is an infinite network (i.e., it has the size of the container). The point $p = p_c$ is the gel point.

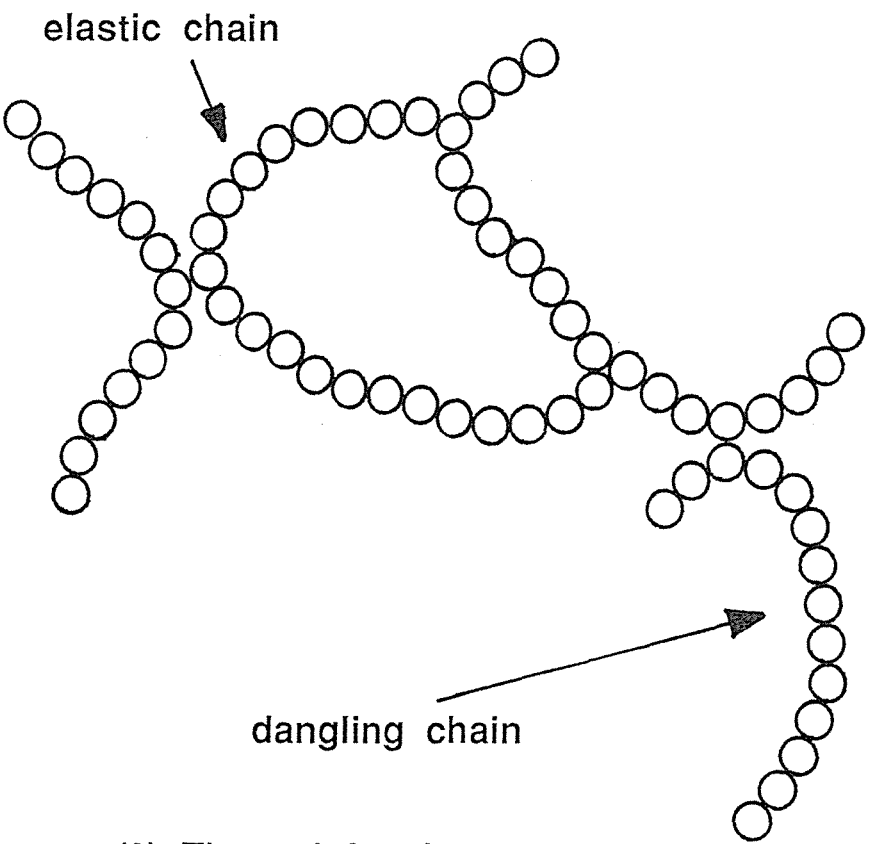
During heat-induced gelation of WPC solutions a sol is formed at first. Then the gel point is reached at which a gel network starts to appear in a sol, and G′ and G" begin to rise rapidly. Beyond the gel point a sol fraction coexists with a gel fraction, and the sol is trapped in the interior of the gel.

Bibbo and Valles (1982, 1984) described how a synthetic polymer network after the gel point was composed of elastic chains, pendant chains and soluble materials. They stated that the elastic chains were those joined by both ends to the infinite gel structure, the pendant chains were those joined by only one end to the gel and the soluble materials were those not attached to the gel.

Similarly, it is proposed that for whey proteins after the gel point there are three different kinds of chain elements in the gel fraction and the sol fraction. This is shown in Fig.6.2. The first type of chains are the elastic chains joining by both ends to the infinite gel structure. The second type of chains are the dangling ones, which are joined by only one end to the gel. Finally we have the



(1) The sol fraction.



(2) The gel fraction.

Fig.6.2-The sol fraction and the gel fraction.

soluble chains, which are not attached to the gel and remain in the sol fraction. However, it should be borne in mind that the chain here is probably made up of nearly globular protein molecules connecting end to end with each other in a linear fashion, which is certainly different from the singular molecular chain defined by Bibbo and Valles (1982, 1984). Barbu & Joly (1953) examined the linear aggregation of horse serum albumin and egg albumin molecules by various methods and concluded that the length of a linear aggregate (a chain) or the number of molecules in one chain was dependent on protein concentration, pH, ionic strength and temperature.

As can be seen from Fig.6.2, the gel fraction is made up of elastic chains and dangling ones while soluble chains make up the sol fraction. It should be remembered that the sol fractions are trapped in the holes of the gel.

Bibbo and Valles (1984) studied the contribution of dangling chains (or pendant chains) on the loss modulus of a model silicone network and concluded that dangling chains had much more potential for energy dissipation than the elastic chains or the sol fraction. In the case of this study, therefore, it may be possible to infer that the loss modulus G" is largely contributed by dangling chains, although contributions of the weak gel structure and the sol fraction to G" may also exist. Dangling chains contribute to the gel fraction, but they do not contribute to the elasticity (Essam, 1980; Staufer et al, 1982). Therefore, G′ is contributed by elastic chains though a very minor contribution from the sol fraction may not be excluded.

From the gel point to the completion of the gelation, the gel fraction increases continuously from zero to one while the sol fraction decays from one to zero. Beyond the gel point G" rose initially with time as illustrated in Fig.6.1. As discussed previously the value of G" is proposed to be

mainly proportional to the number of dangling chains on the gel network. In order to simplify the analysis the contributions of the weak gel and the sol fraction to G" were neglected. Therefore, the number of dangling chains on the gel network increased with time until the maximum of G", but their rate of increase was probably the net effect of two opposing processes: the creation of dangling chains from the sol fraction and the vanishing of these to form elastic ones. Obviously, creation of dangling chains from the sol fraction was predominant.

As the sol fraction decreased and the number of dangling chains on the gel network increased, the formation rate of dangling chains decreased while their vanishing rate increased. For this reason the build-up of G" tended to slow down (Fig.6.1). Finally, when the formation rate of dangling chains equalled their vanishing rate, the maximum number of dangling chains on the gel network was attained and G" reached a maximum point as shown in Fig.6.1.

After the maximum number of dangling chains was reached, the vanishing rate of dangling chains exceeded their formation rate, hence the number of dangling chains on the gel network started to decrease. This was reflected by the decrease of G" after the maximum point. The vanishing of the sol fraction also contributed to the decrease of G". The decrease in G" from a maximum is exihibited by most synthetic polymer systems and by some biopolymer systems (Clark & Ross-Marphy, 1987). The maximum of G" correlated with the maximum in the weight fraction of dangling chains on the silicone network in the experimental work of Bibbo and Valles(1984). Eventually, the sol fraction and dangling chains on the gel network would disappear altogether if gelation proceeded far enough, and G" would reach a constant minimum value, which it tends to do (Fig.6.1).

As illustrated in Fig.6.1, beyond the gel point the rapid build-up of G′ was due to the efficiency of formation of

elastic chains (from soluble chains in the sol fraction and dangling chains on the gel network). As gelation proceeded, the storage modulus G′ increased steadily because of the continuous increase of elastic chains in the gel fraction. After the maximum of G", G′ tended to rise more and more slowly (Fig.6.1). This might be due to the decrease of the dangling chains on the gel network as well as the vanishing of the sol fraction after the maximum of G" causing the formation of elastic chains from the dangling chains and the sol fraction to become slower and slower. Finally, G′ would reach a plateau value as the maximum number of elastic chains was attained, corresponding to the completion of gelation: the gel fraction rose to one while the sol fraction decayed to zero.

6.1.3 Concentration Dependence

The effect of concentration on G′ and G" is illustrated in Fig.6.3 and Fig.6.4. The concentration dependence of protein gel structure development was reflected by changes in G′ and G" with time. The storage modulus G′ rose faster with time at higher protein concentrations (Fig.6.3), showing that the formation rate of elastic strands was higher at higher concentrations. As can be seen in Fig.6.4, at higher concentration G" increased more rapidly with time after the gel point until a maximum value was reached. Then G" decreased more quickly at higher concentrations up to the completion of the gelling process for WPC solutions of 25% to 40%. The maximum point of G", which might correlate with the maximum number of dangling chains on the network, occurred earlier and had a higher value at higher concentration. For 20% WPC solution G" increased slowest and had not reached a maximum value after 6000s.

It is considered that the formation rate of dangling chains and elastic chains was dependent on concentration and was higher at higher concentrations. Soluble chains, dangling

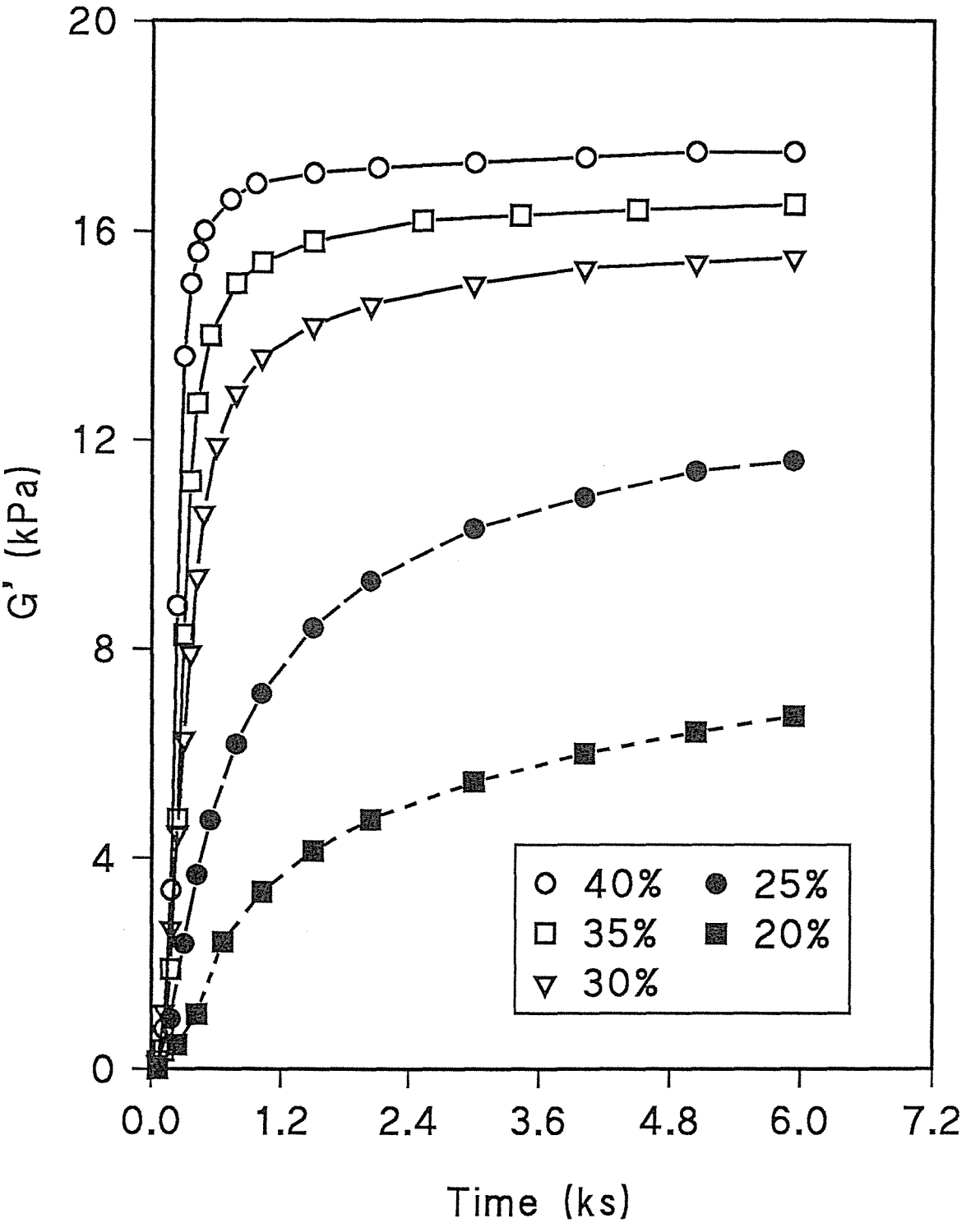


Fig. 6.3–Storage modulus(G') as a function of time during thermal gelation of Alacen 475 solutions at 80°C.

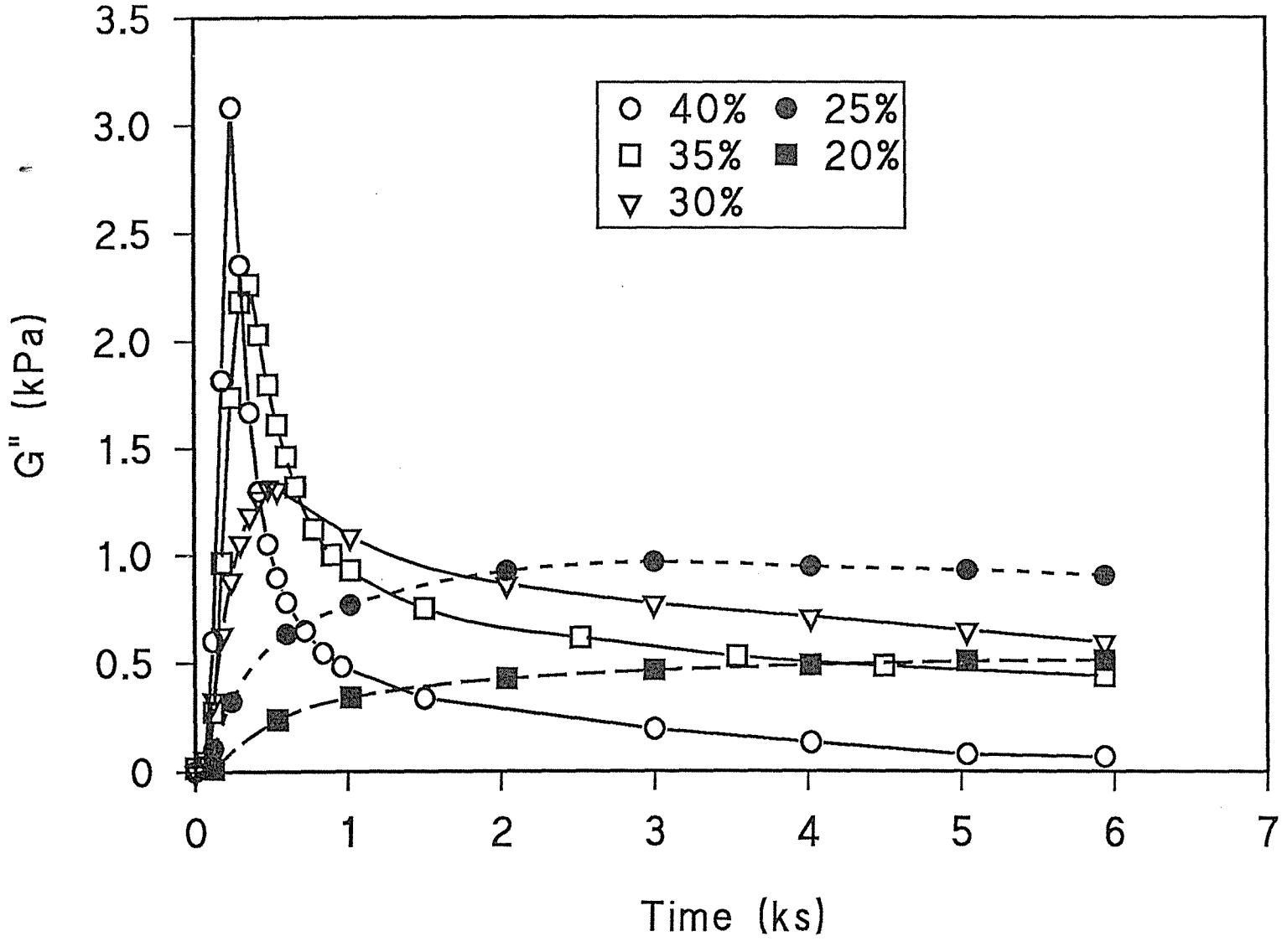


Fig. 6.4–Loss modulus(G") as a function of time during thermal gelation of Alacen 475 solutions at 80°C.

and elastic chains were formed more readily at higher concentrations because of the higher density of protein molecules and hence the greater probability of intermolecular contact. Before the maximum values of G" the increase of dangling chains on the gel network was more rapid and more pronounced at higher concentrations. After the maximum values dangling chains and the sol fraction vanished more rapidly at higher concentrations. The maximum number of dangling chains on the network achieved during the gelling process, presumably, increased with increasing concentration.

6.1.4 Temperature Dependence

The storage modulus G′ and loss modulus G" as a function of time at temperatures of 70^{o} to 80^{o}C for a 30% WPC solution are illustrated in Fig.6.5 and Fig.6.6, respectively. As can be seen in Fig. 6.5, G′ rose fast at first, particularly at high temperatures, showing that elastic chains began to form rapidly and that their formation rate was more pronounced at higher temperatures. The loss modulus G" also rose quickly at first, especially at high temperatures (Fig.6.6). As already discussed in Sect.6.1.2, the increase of G" is largely due to the build-up of dangling chains on the gel network, which is the net result of the creation of new dangling chains from the sol fraction as well as disappearance of existing ones to form elastic chains. The build-up of dangling chains in the gel fraction proceeded faster at higher temperatures beyond the gel point as indicated by the fact that the rate of increase of G" was more pronounced at higher temperatures. Consequently, the maximum number of dangling chains which correlatd with the maximum in G" was attained sooner at higher temperatures. After the maximum values of G" the dangling chains on the gel network vanished gradually and their vanishing rate increased with increasing temperature.

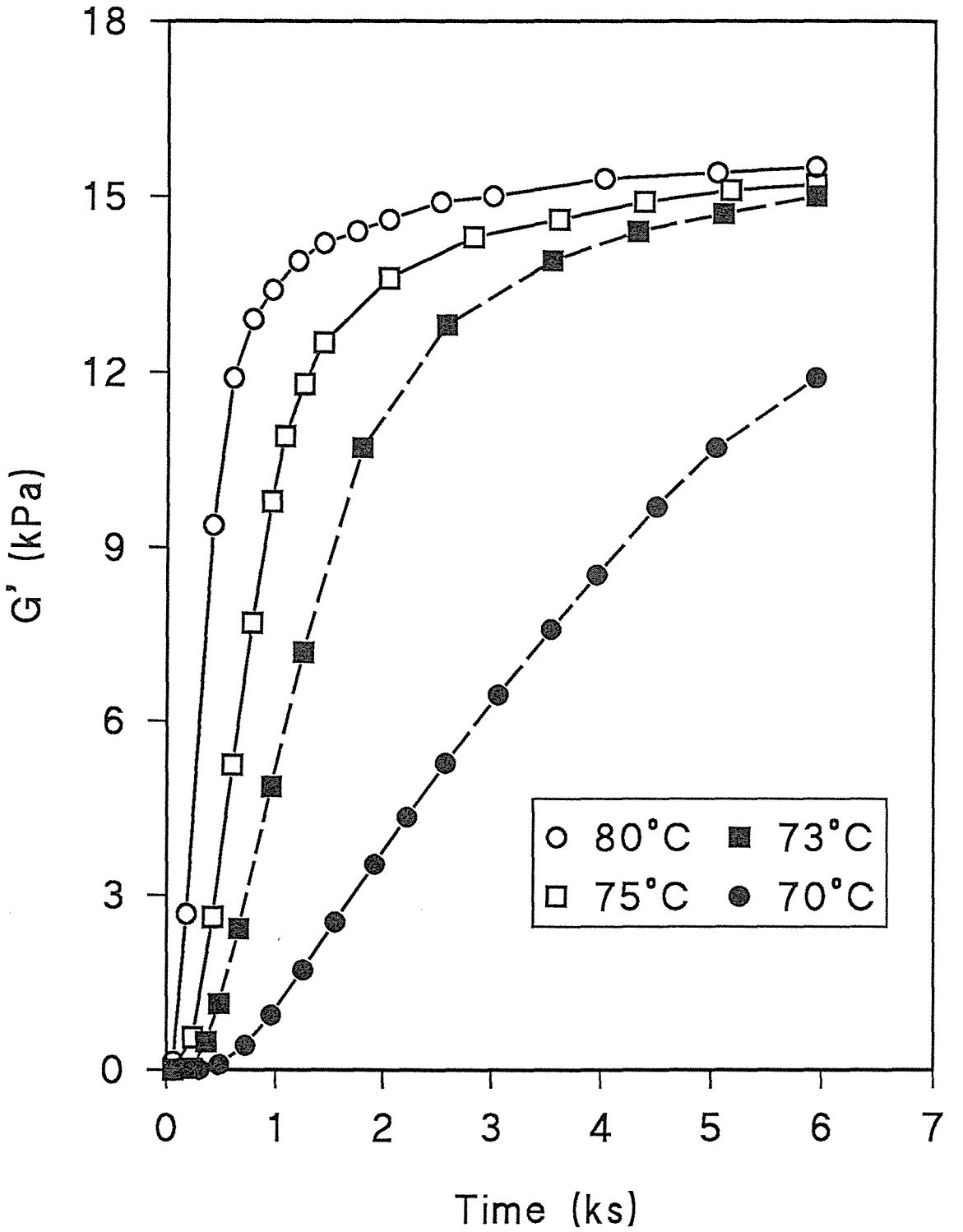


Fig 6.5–Storage modulus(G') as a function of time during thermal gelation of 30% Alacen 475 solutions at different temperatures.

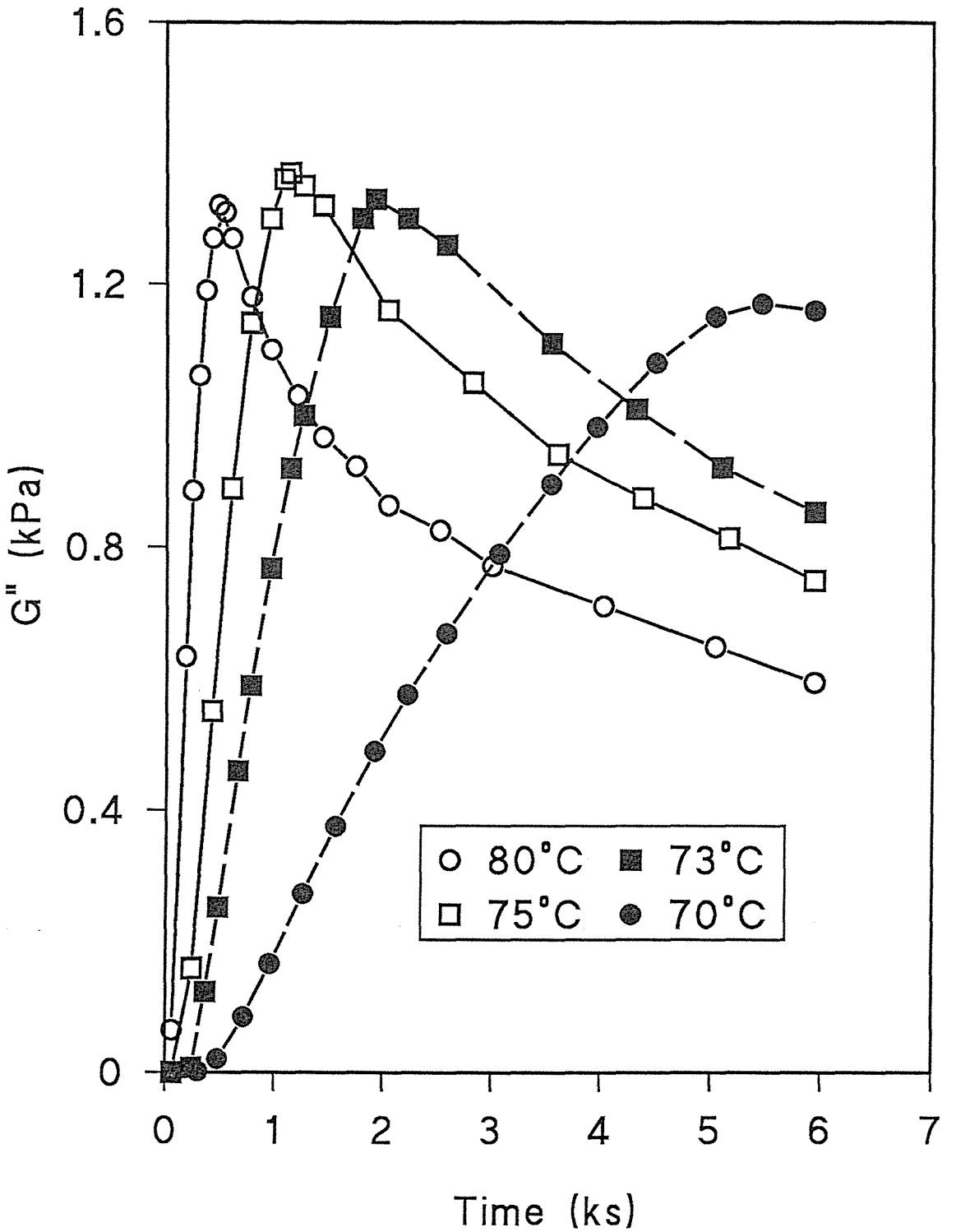


Fig 6.6–Loss modulus(G") as a function of time during thermal gelation of 30% Alacen 475 solutions at different temperatures.

All these results showed that in the temperature range 70°-80°C the effect of temperature on the development of the protein gel structure was largely kinetic: the gelling process including protein molecules unfolding and aggregating to form chains, and the chains cross-linking to give a gel mesh, proceeded faster at higher temperatures. In this temperature range (70°C-80°C), if the gelation proceeds to completion the final protein gels may have similar structures since G′ approaches similar values regardless of gelling temperature, and so does G".

Once the temperature was raised above 80°C, the situation was different. This is shown in Fig.6.7. The storage modulus G′ decreased with increasing temperature. As discussed previously in Sect.6.1.1, electrostatic repulsion is responsible for linear aggregations because of a reduced repulsion energy barrier near the ends of a growing chain. The increase of temperature would increase the kinetic energy of protein molecules and intermolecular interactions might also be enhanced. In this case it is suggested that partially unfolded protein molecules might have kinetic energies and intermolecular attractive potential energies predominant compared to the repulsion energy barrier, and that random aggregations would tend to occur. In this circumstance, some protein molecules which possessed high kinetic energies and strong intermolecular attractions might form coarsely random aggregates, and would therefore exclude themselves from contributing to the formation of linear aggregates. The protein gels would then have a lower density of elastic chains and consequently lower G′ at higher temperatures (above 80°C). It is also well recognised that protein gels of maximum strength are formed only when a critical balance of protein-protein interactions and protein-solvent interactions is attained. If protein-protein interactions are far stronger than protein-solvent interactions, a precipitate rather than a gel tends to form. In this experiment, presumably, protein-protein interactions

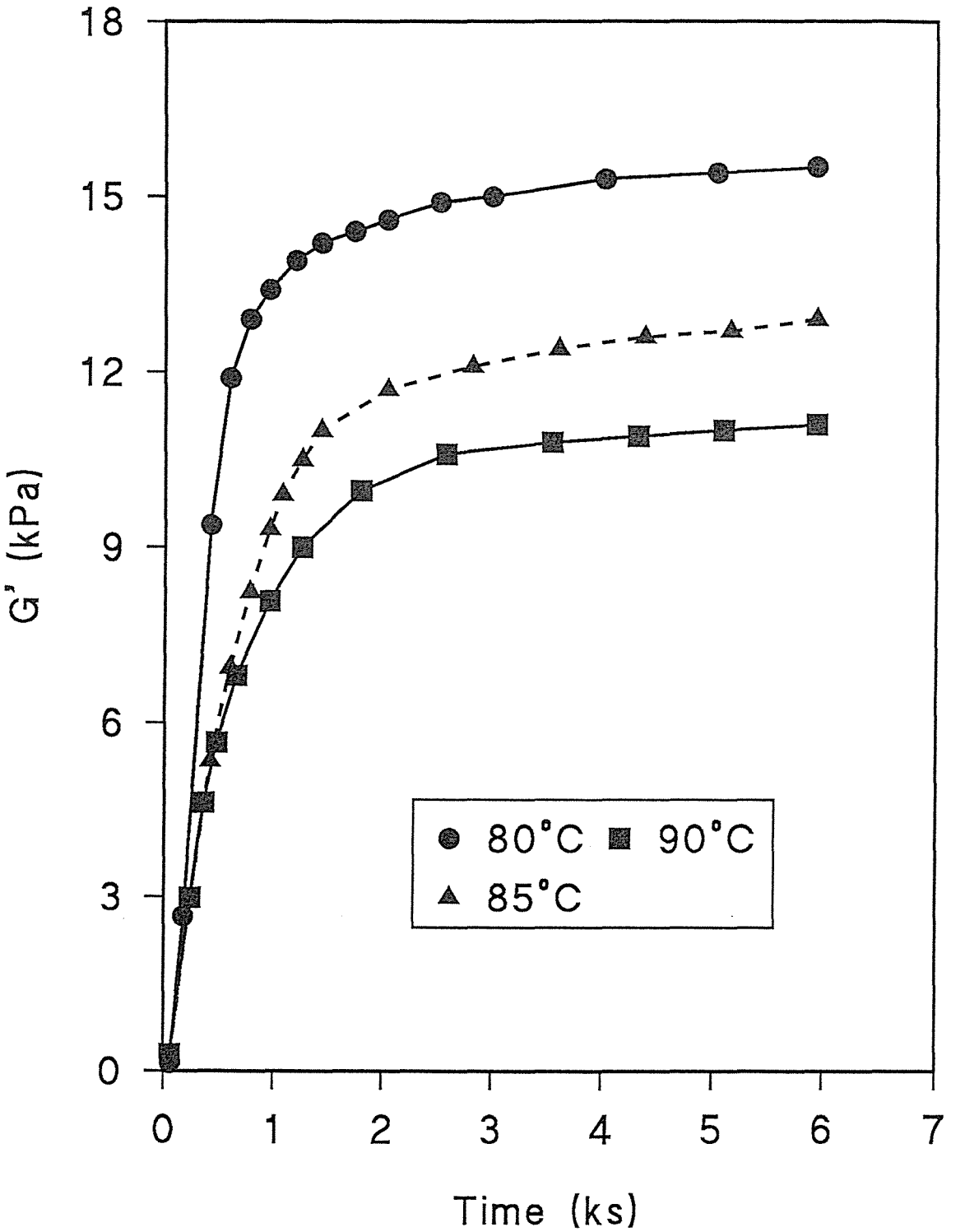


Fig 6.7–Storage modulus(G') as a function of time during thermal gelation of 30% Alacen 475 solutions at different temperatures.

induced by temperature above 80°C were predominant compared to protein-solvent interactions, which resulted in the formation of a coarse and fragile gel network with less strength and less elasticity.

Similar results were obtained for the gelation of 20% WPC solutions at temperatures of 75°C to 90°C. This is shown in Fig.6.8 and Fig.6.9. The gel formation at 80°C showed a more pronounced increase in G′ than at the 75°C, indicating the elastic chains were formed faster in the 80°C gel network. This may be attributed to kinetic factors including more protein molecules unfolding and increased disulfide cross-linking at the higher temperature. The final protein gels made after heating for 6000s showed a decrease in G′ as the temperature was increased from 80°C to 90°C (Fig.6.8). The formation of local aggregate particles due to random aggregations might be responsible for this decrease in G′ with temperature since some protein molecules would have contributed to the formation of random aggregates and would not be available for forming elastic chains. The build-up of dangling chains with temperature as reflected by the increase in G" was also controlled by two factors: kinetic effects and random aggregations. The build-up of dangling chains was faster at higher temperature and the maximum number of dangling chains decreased with increasing in the degree of random aggregations, i.e. as temperature increased.

6.2 VISCOELASTIC PROPERTIES OF THE FORMED GELS

6.2.1 Concentration Dependence

G′, G" and phase angle versus concentration for protein gels made at 80°C after heating for 6000s are shown in Fig.6.10. The storage modulus G′ of protein gels increased with increasing WPC concentration. At higher concentrations the protein gels obtained are considered to have had a higher

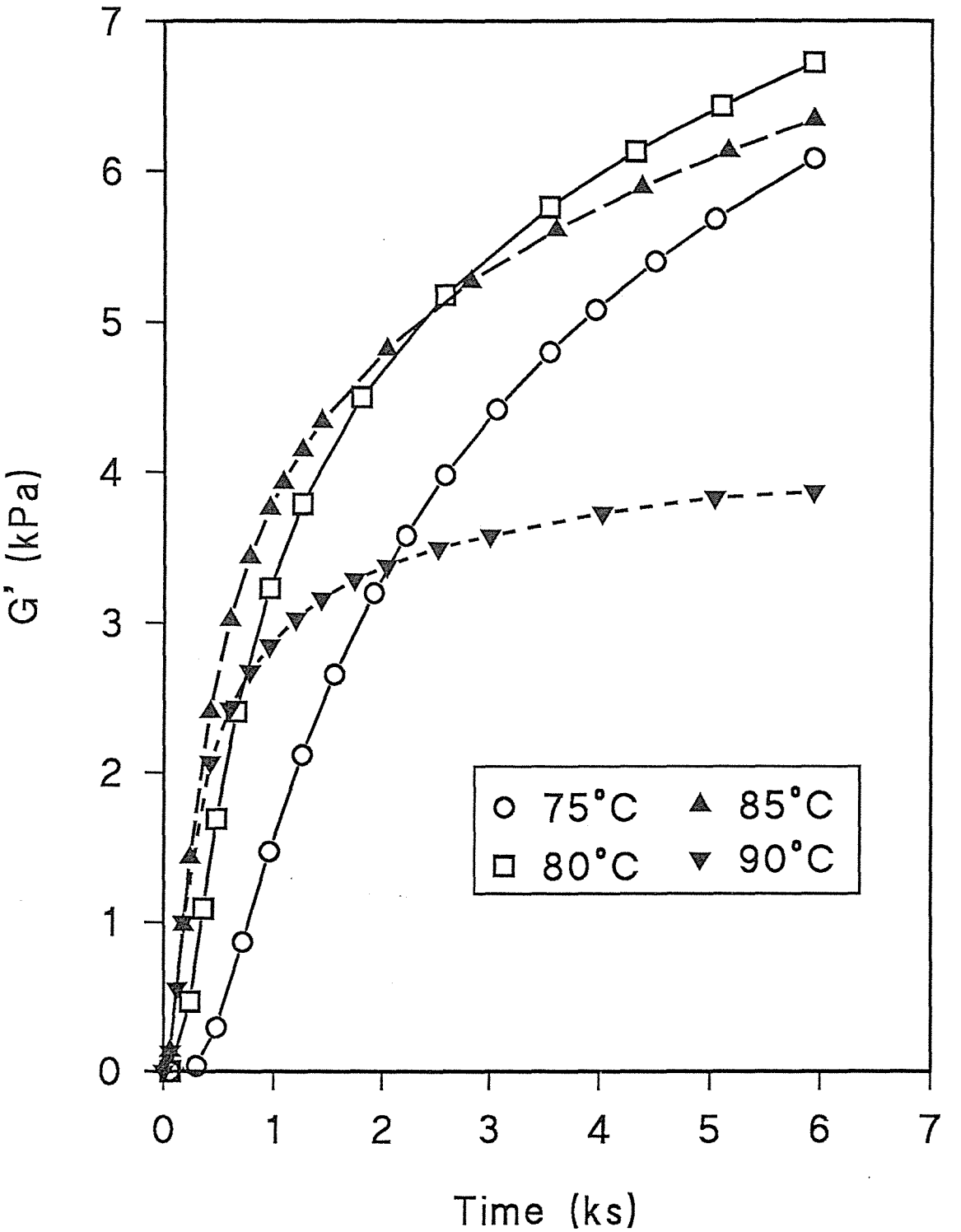


Fig 6.8–Storage modulus(G') as a function of time during thermal gelation of 20% Alacen 475 solutions at different temperatures.

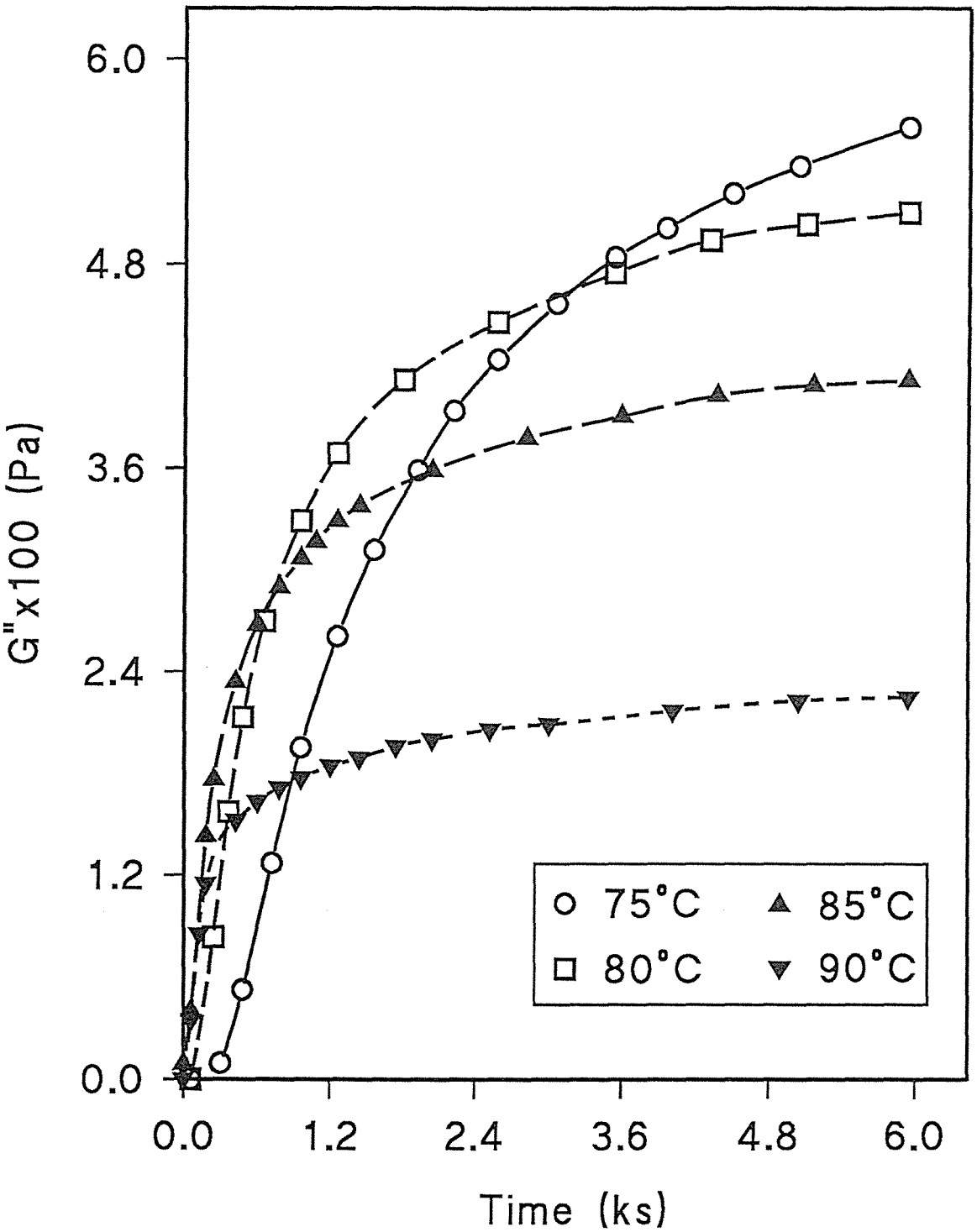


Fig 6.9—Loss modulus(G") as a function of time during thermal gelation of 20% Alacen 475 solutions at different temperatures.

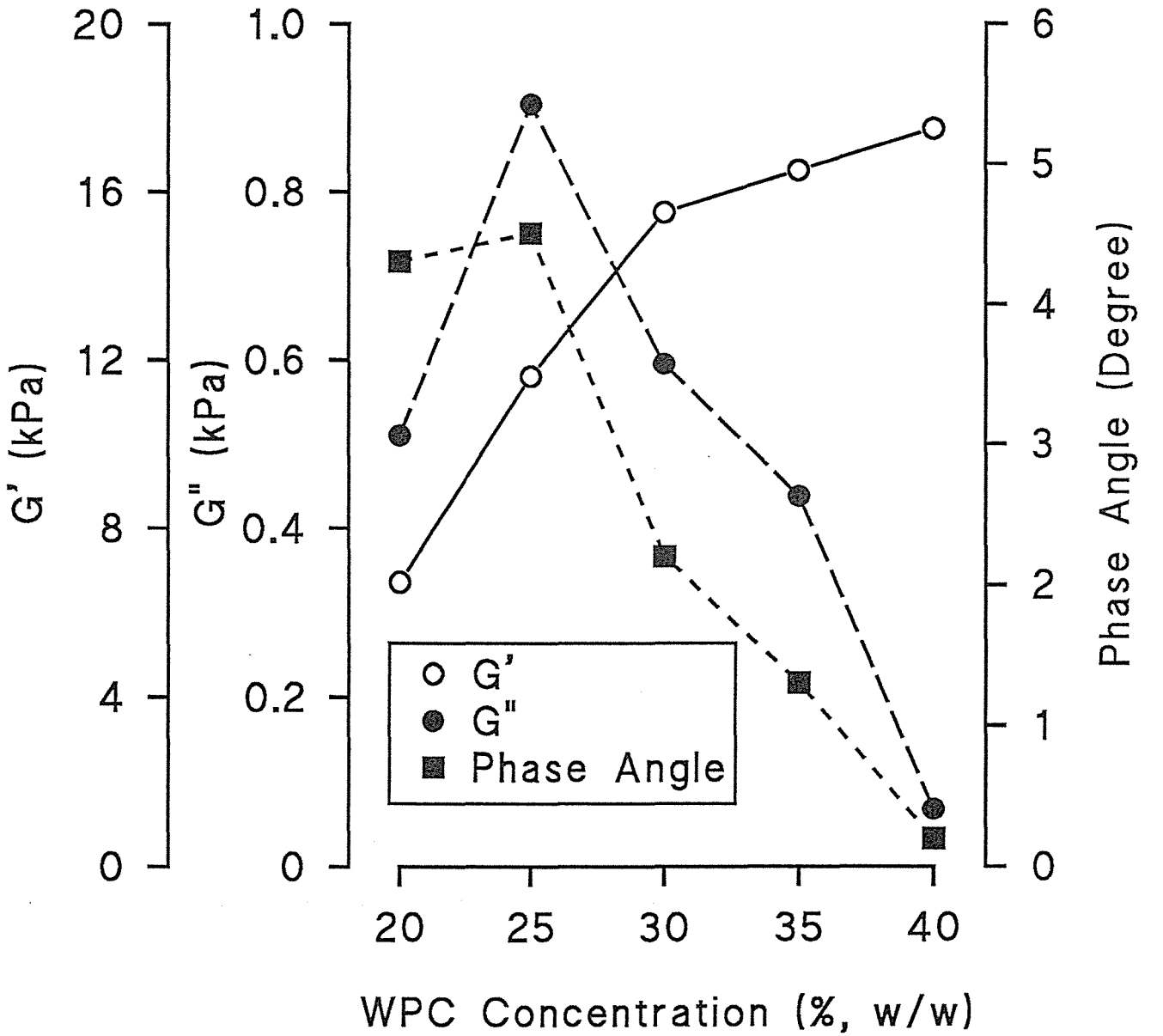


Fig. 6.10—Storage modulus G'(○), loss modulus G"(●) and phase angle(■) vs concentration for Alacen 475 gels made at 80°C after heating for 6000s.

density of elastic chains and more intermolecular protein interactions such as hydrogen bonds, hydrophobic interactions and disulphide bonds. Therefore, protein gels formed at higher concentrations were stronger than those made at lower concentrations.

Measurements of phase angle for gels at different concentrations revealed that the 25% gels, though stronger, were actually less elastic than 20% gels (Fig.6.10). This happened because the gelling process for 20% and 25% gels had not gone to completion (Fig.6.4), and 25% gels had more dangling strands on the network than 20% gels (Fig.6.10). It is probable that 25% gels would be more elastic than 20% gels after the gelation is completed, but it takes a long time for the gelation to be completed. Above the concentration of 25%, actually, the gelation had gone to completion as shown in Fig. 6.3 and Fig.6.4, and the loss modulus G" and phase angle for final protein gels decreased with increasing concentrations as illustrated in Fig.6.10, showing that the final protein gels possessed more elasticity at higher concentrations.

6.2.2 Temperature Dependence

Fig.6.11 shows G′, G" and phase angle as a function of temperature for 30% protein gels obtained after heating for 6000s. G′ increased whereas G" and phase angle decreased with increasing temperature from 70^{o}C to 80^{o}C. Then G′ decreased whereas G" and phase angle increased in the temperatures of 80^{o}C to 90^{o}C.

Below 80^{o}C protein gels made at higher temperatures probably possessed more elastic strands, less dangling ones and a lower sol fraction, and more intermolecular hydrophobic and disulphides bonds because of more protein unfolding and the gelation proceeding faster at higher temperatures. Therefore, protein gels were both stronger and more elastic at higher temperatures.

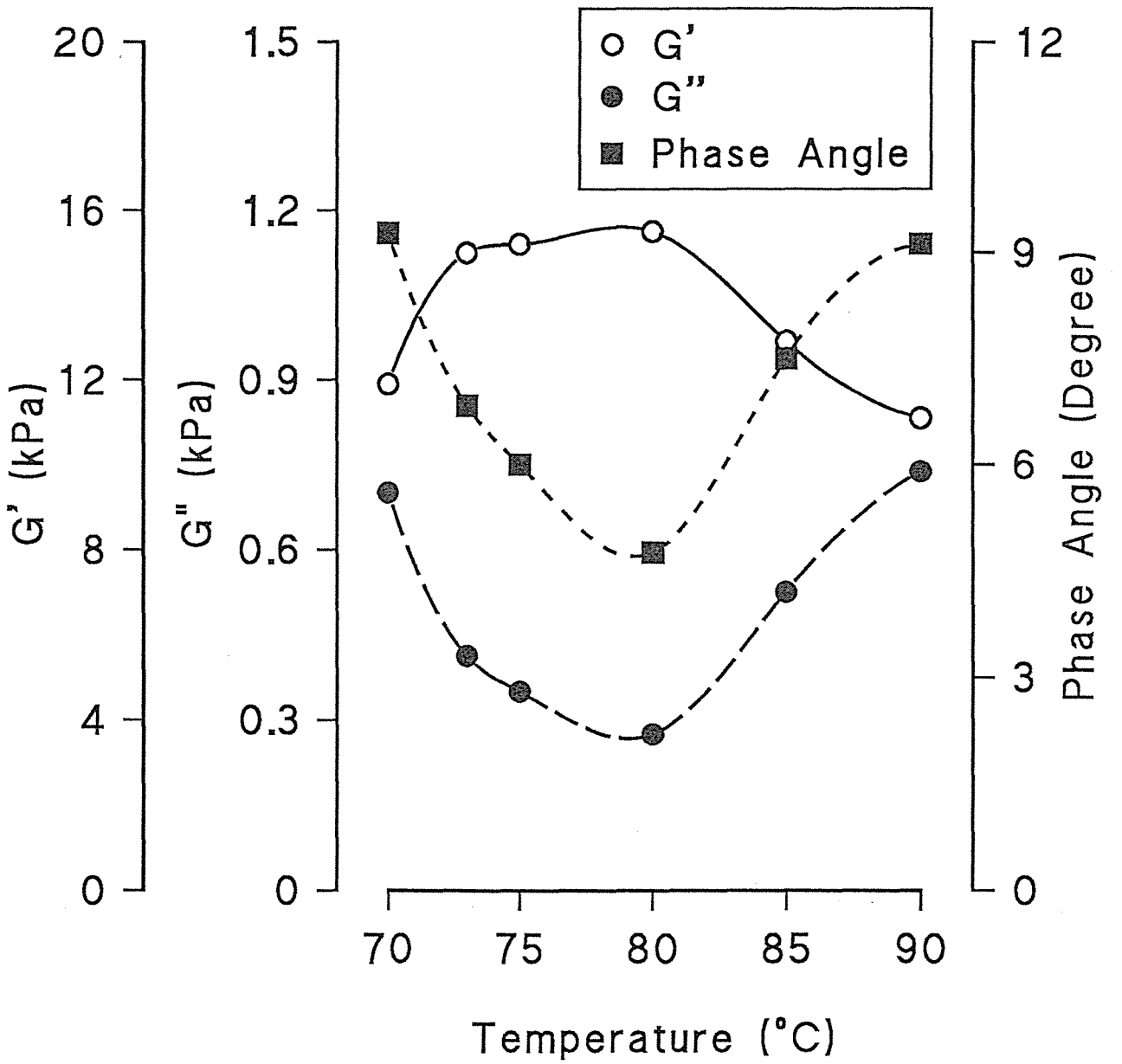


Fig. 6.11—Storage modulus G'(○), loss modulus G"(●) and phase angle(■) vs temperature for 30% Alacen 475 gels made after heating for 6000s.

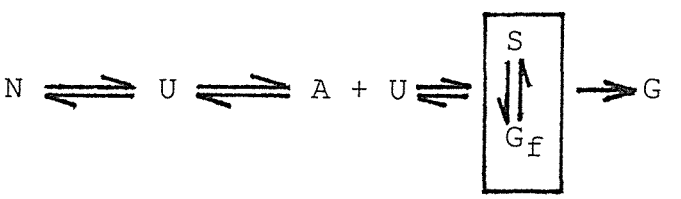
Above 80°C, however, protein molecules might have kinetic energies and intermolecular attractive potential energies predominant compared to the repulsive potential energy barrier, and random aggregations might have occurred. Gels containing some coarsely random aggregates would have less density of elastic chains, and would be weaker and less elastic. The degree of random aggregations probably increased with temperature in the range 80°C-90°C. Protein gels formed at higher temperatures (above 80°C), therefore, might have more random aggregates and less elastic chains in the gel network, imparting less strength and less elasticity - thus exhibiting greater G" and greater phase angle (i.e. more liquid-like behaviour).

6.3 TEMPERATURE, CONCENTRATION AND TIME DEPENDENCE OF THE LIQUID-GEL TRANSITION

6.3.1 Constant Temperature Experiments

The change of storage modulus(G') with time for 10% and 30% Alacen 312 solutions near the beginning of gel formation is illustrated in Fig.6.12 and Fig.6.13 respectively. The effect of temperature on the commencement and the rate of gel formation was very significant. As the temperature was increased the delay before gel formation began became less, but the initial slope (dG'/dt) increased. At 67°C for 10% and at 60°C for 30% WPC solutions, the transition from liquid to gel had not commenced even after 6000s.

It is clear that the pronounced temperature dependence of the onset and the initial rate of gelation is mainly due to kinetics. The gelling process of protein may be simply illustrated as:



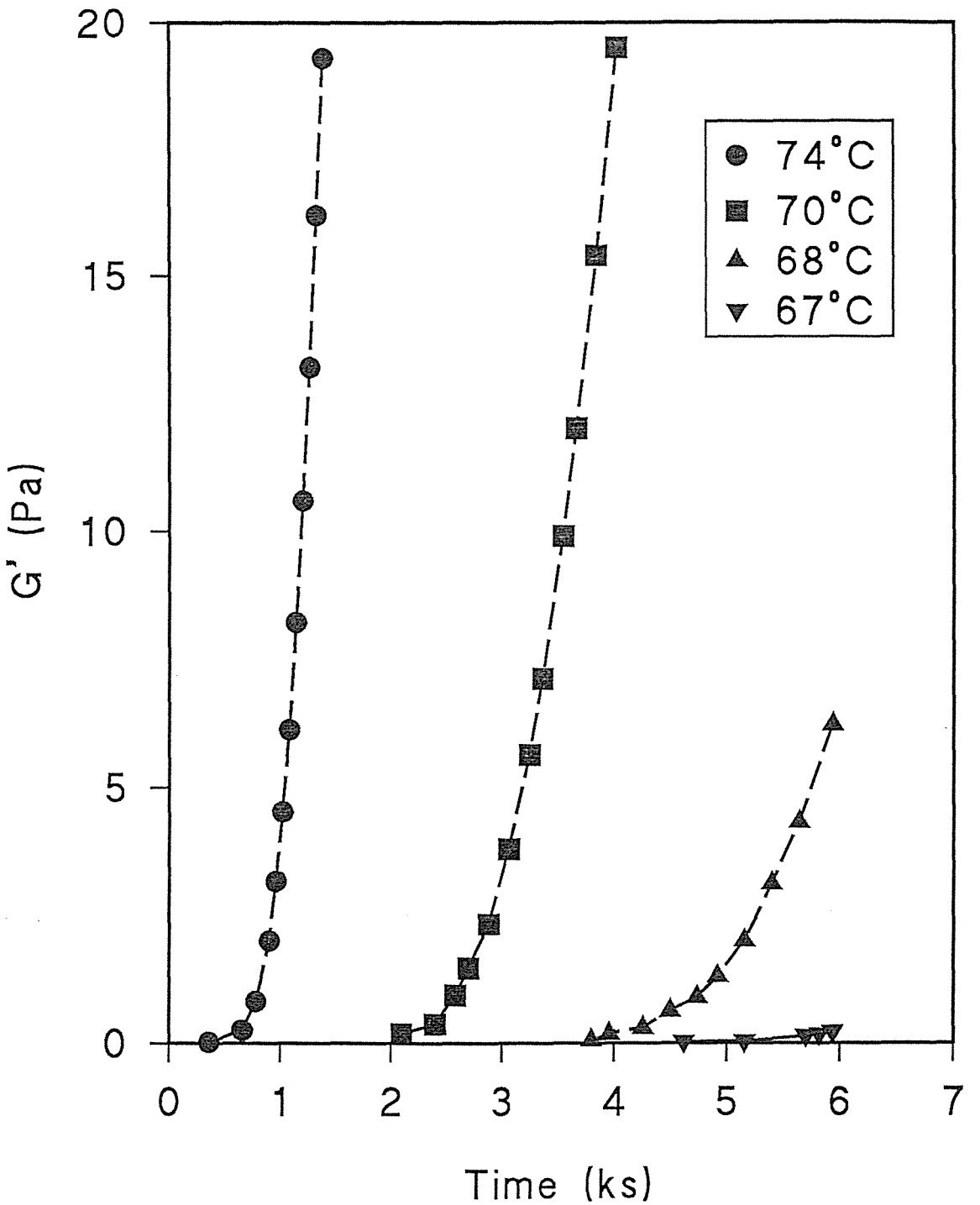


Fig. 6.12–Storage modulus(G') as a function of time during the initial stage of gel formation for 10% Alacen 312 solutions at different temperatures.

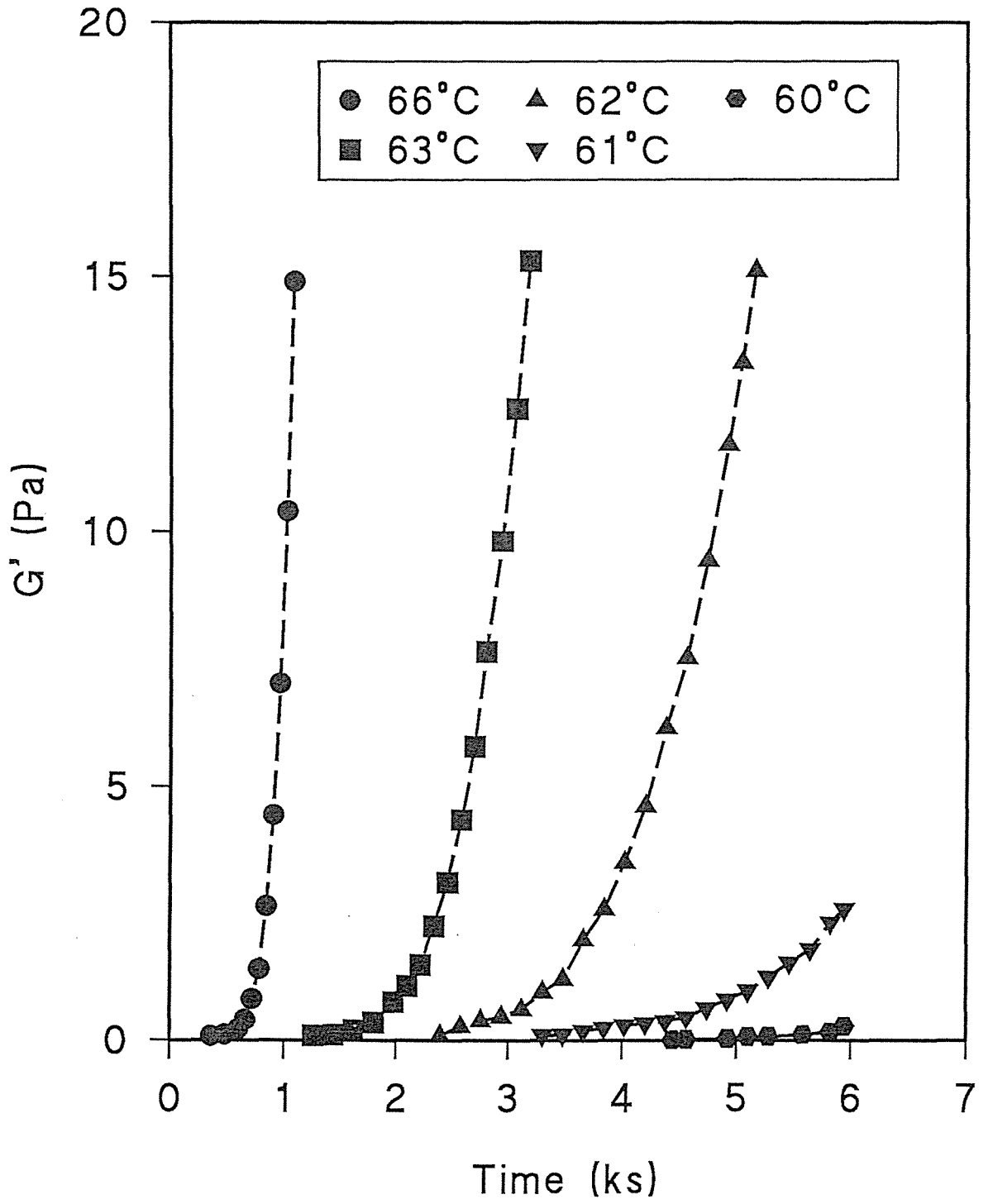


Fig. 6.13–Storage modulus(G') as a function of time during the initial stage of gel formation for 30% Alacen 312 solutions at different temperatures.

N --- native protein molecules
U --- partially unfolded protein molecules
A --- aggregates
S --- the sol fraction
G_f--- the gel fraction
G --- gels

Obviously, this is a four step mechanism for protein network formation. Whey protein molecule unfolding is considered to be mainly irreversible above 60^{o}C (Dewit & Klarenbeek, 1984). It is likely that the rate of all reactions shown above increases dramatically with increasing temperature. This automatically leads to the high temperature dependence for both the commencement of the rise in storage modulus (G') and the initial rate of its increase.

Similar results were obtained for Alacen 475 and Alacen 392 solutions at concentrations of 10% to 30%. However, as the concentration was increased to 40%, Alacen 475 solutions showed a different gelling behaviour from Alacen 312 and Alacen 392 solutions. Alacen 475 solutions began to gel at 30^{o}C whereas the gelling temperature was above 55^{o}C for both Alacen 392 and Alacen 312 solutions, as illustrated in Fig.6.14, Fig.6.15 and Fig.6.16. Below 40^{o}C the involvement of intermolecular disulphide bonds in gel formation can virtually be neglected (see Sect.6.3.3 below). The protein gels obtained for Alacen 475 solutions below 40^{o}C were actually very soft as would be expected because no intermolecular disulphide bonds were involved in gel formation. Formation of soft protein gels at temperatures of 30^{o}C to 40^{o}C for Alacen 475 solutions (see Fig.6.16) may largely result from intermolecular non-covalent bonds such as hydrogen bonds, hydrophobic interactions & electrostatic interactions. These intermolecular bonds are influenced by factors such as pH, ionic strength, protein concentration, method of protein preparation, prior treatment and

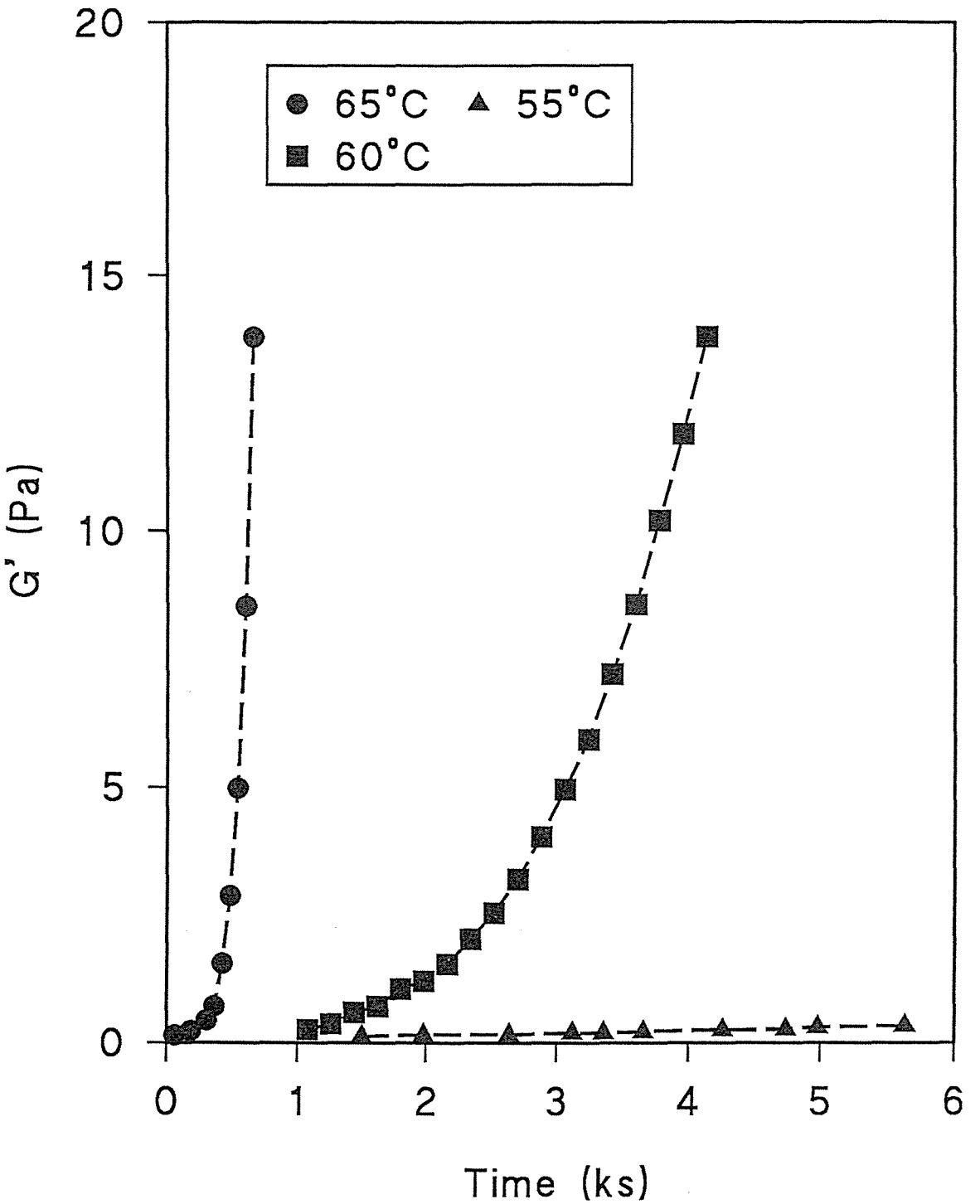


Fig. 6.14—Storage modulus(G') as a function of time during the initial stage of gel formation for 40% Alacen 312 solutions at different temperatures.

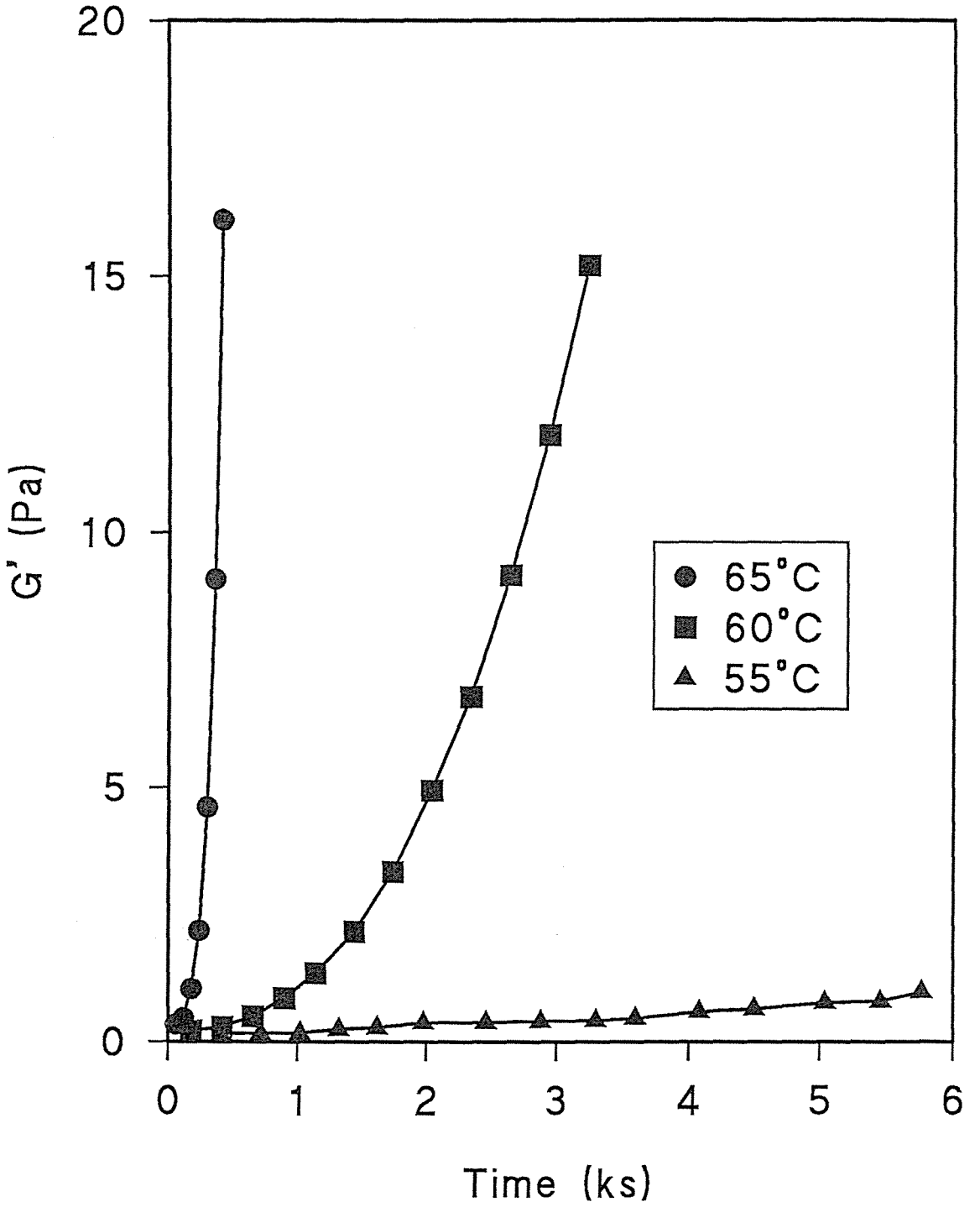


Fig. 6.15—Storage modulus(G') as a function of time during the initial stage of gel formation for 40% Alacen 392 solutions at different temperatures.

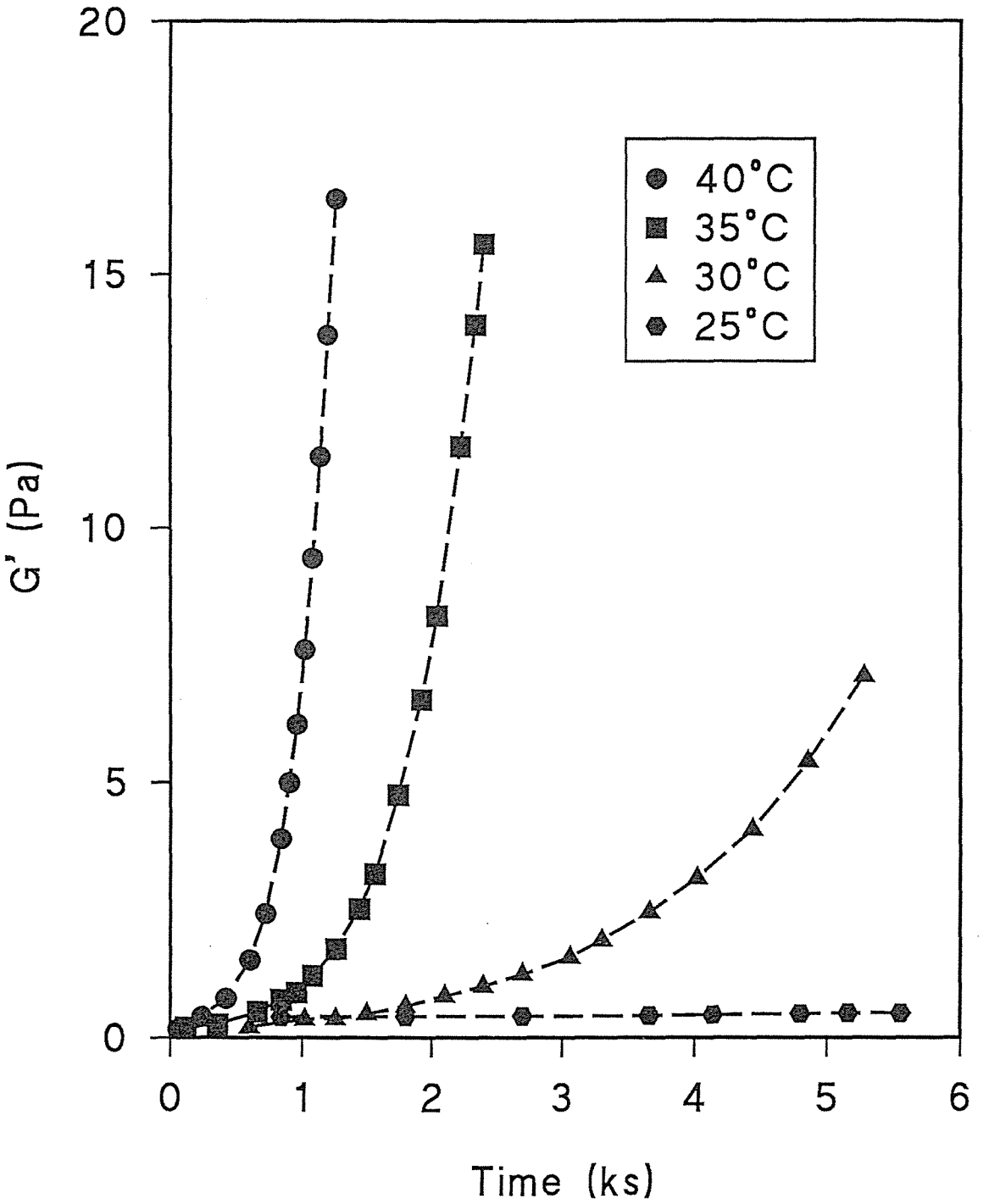


Fig. 6.16—Storage modulus(G') as a function of time during the initial stage of gel formation for 40% Alacen 475 solutions at different temperatures.

composition. It is not possible to judge which of these factors is mainly responsible for the gelation of Alacen 475 solutions at much lower temperatures than Alacen 312 and Alacen 392 solutions until more information, especially about methods of manufacturing these three WPCs, is available.

The effect of concentration on the liquid-gel transition temperature for three WPC solutions (Alacen 475, Alacen 392 and Alacen 312) is illustrated in Fig.6.17. At the concentration of 10% Alacen 475 solutions had the highest gelling temperature while Alacen 312 had the lowest temperature. Above 20% all three WPC solutions had almost the same gelling temperature except for Alacen 475 solutions which exhibited a dramatic drop in gelling temperature above 30%.

6.3.2 Constant Concentration Experiments

Fig.6.18 shows traces of storage modulus (G') and phase angle as a function of time for protein concentrations of 20% and 30% at 66^{o}C. The effect of concentration on both the onset and the initial rate of gelation is marked as indicated by the concentration dependence of the commencement of the rise in G' and the initial slope (dG'/dt). This behaviour was attributed to kinetic factors. The partially unfolded protein molecules forming into chains and the joining of chains to give a gel mesh proceeded faster at higher concentrations because of higher density of protein molecules and hence greater probability of intermolecular contact.

6.3.3 Temperature-Scanning Experiments

Changes in storage modulus(G') as a function of temperature during thermal gelation of Alacen 312, Alacen 392 and Alacen 475 are illustrated in Fig.6.19, Fig.6.20 and Fig. 6.21. The

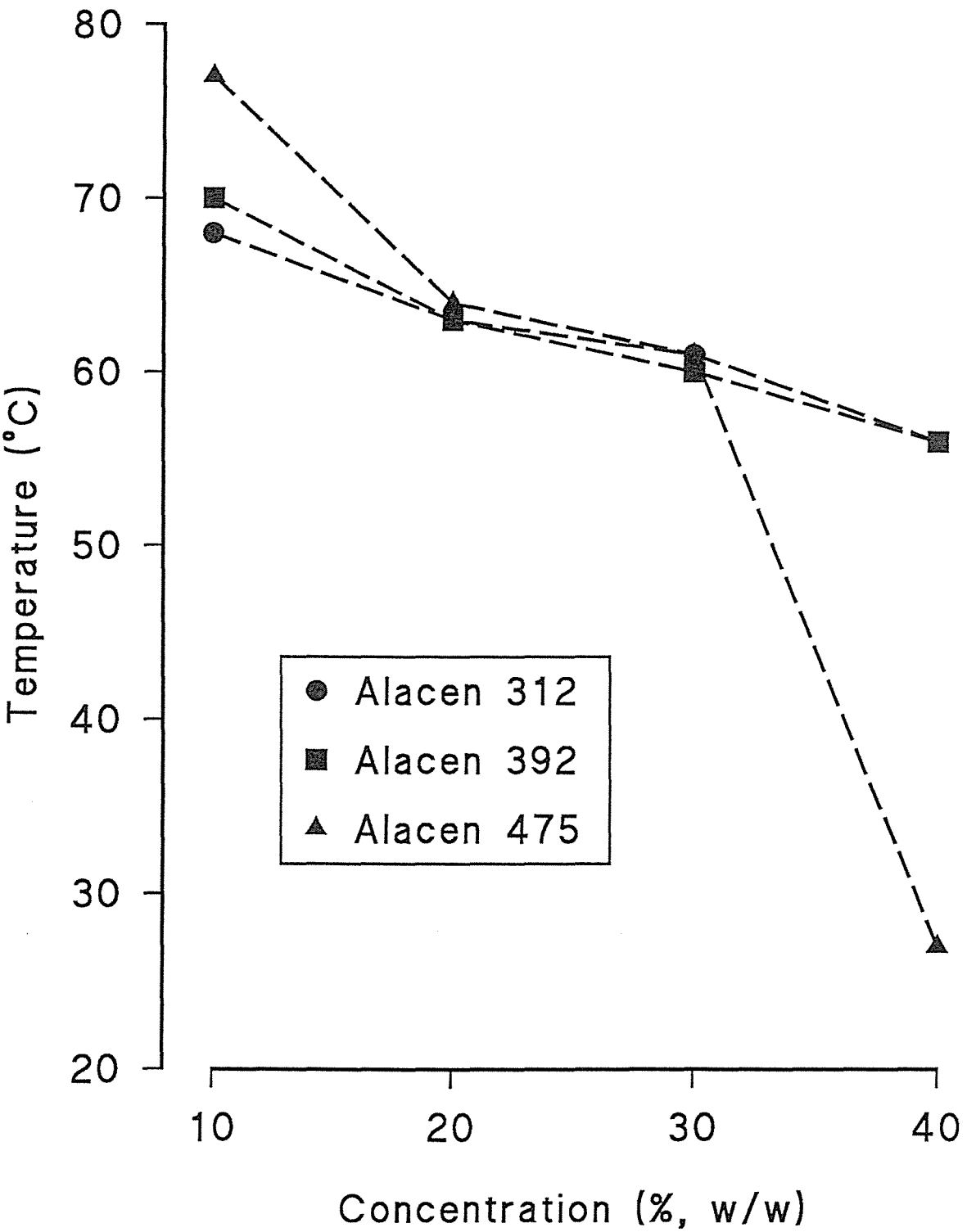


Fig. 6.17–Temperature for commencement of the liquid–gel transition as a function of concentration for three kinds of WPC solutions after heating for 6000s.

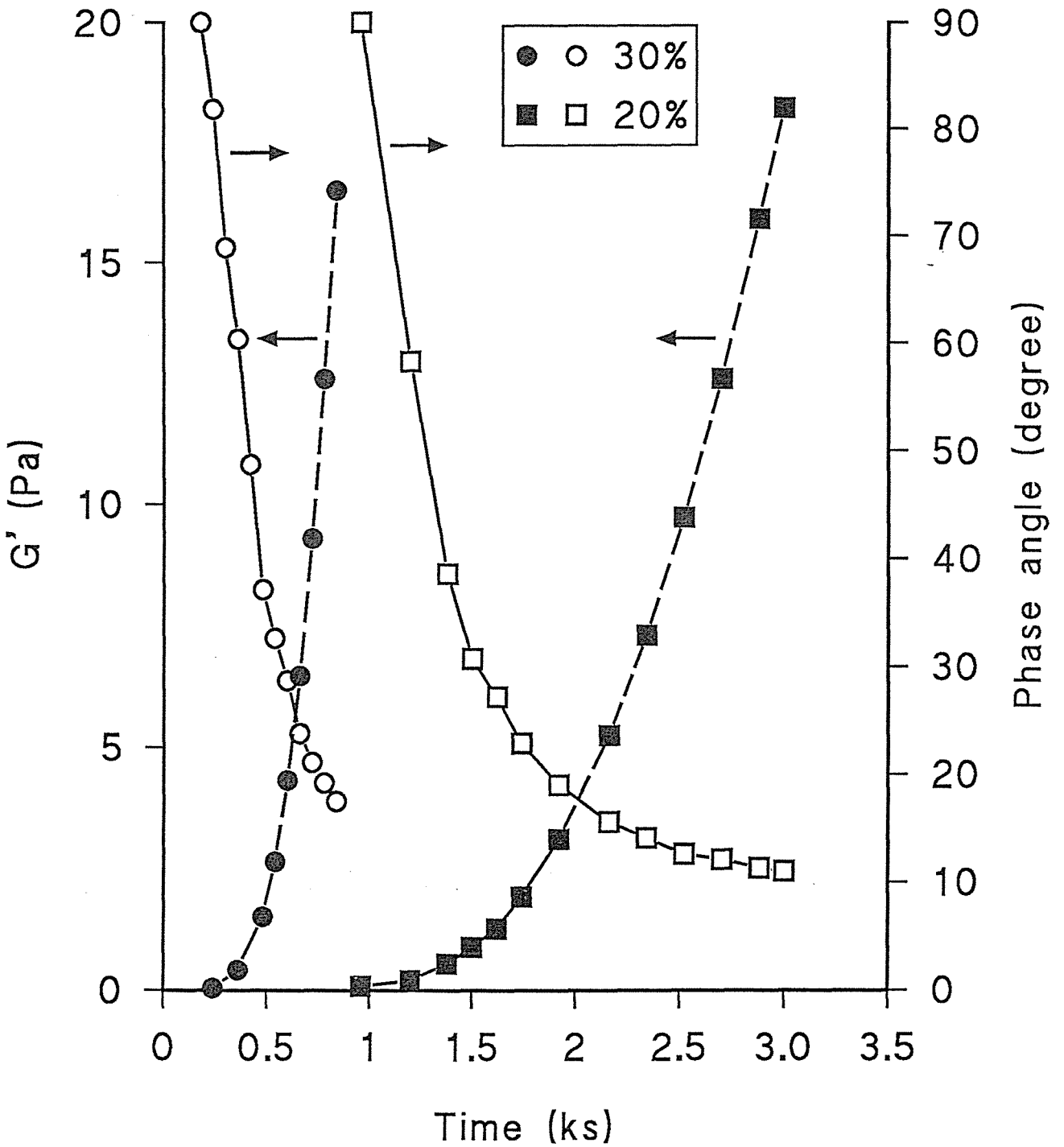


Fig. 6.18—Storage modulus(G') and phase angle as a function of time during thermal gelation of 20% and 30% Alacen 392 solutions at 66°C.

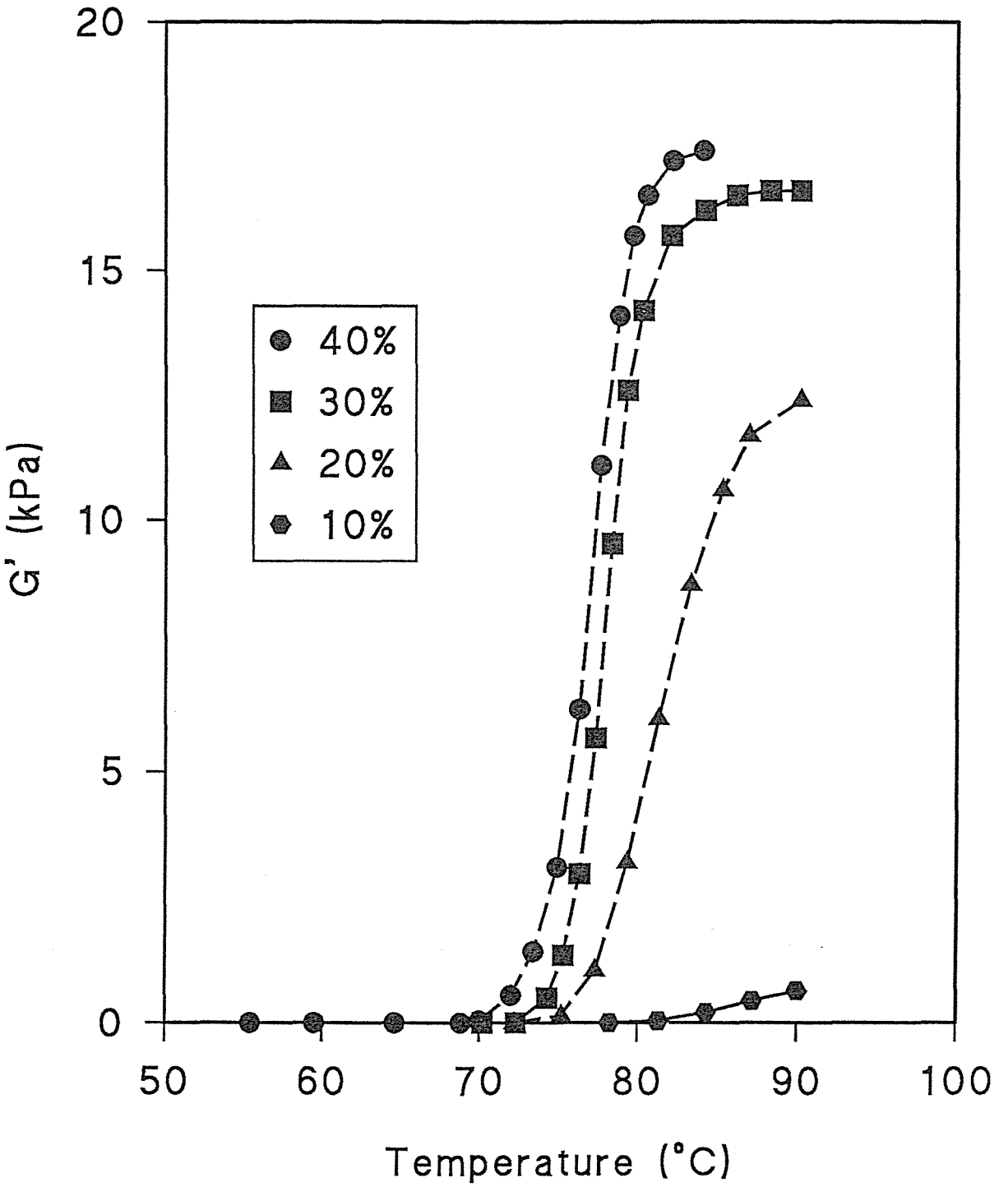


Fig. 6.19–Storage modulus (G') as a function of temperature during thermal gelation of Alacen 312 solutions at different concentrations. Temperature was increased at 1°C/min.

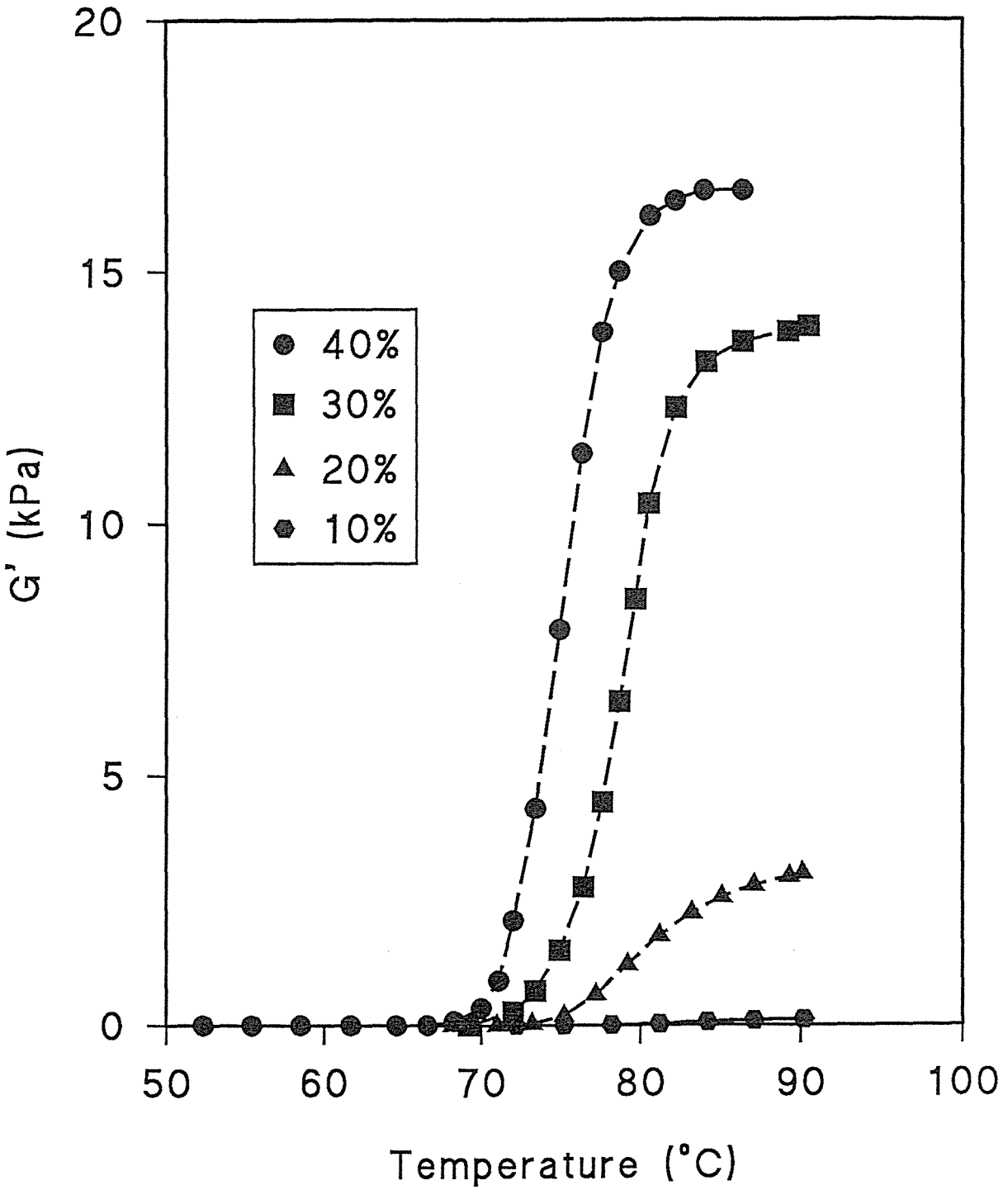


Fig. 6.20–Storage modulus (G') as a function of temperature during thermal gelation of Alacen 392 solutions at different concentrations. Temperature was increased at 1°C/min.

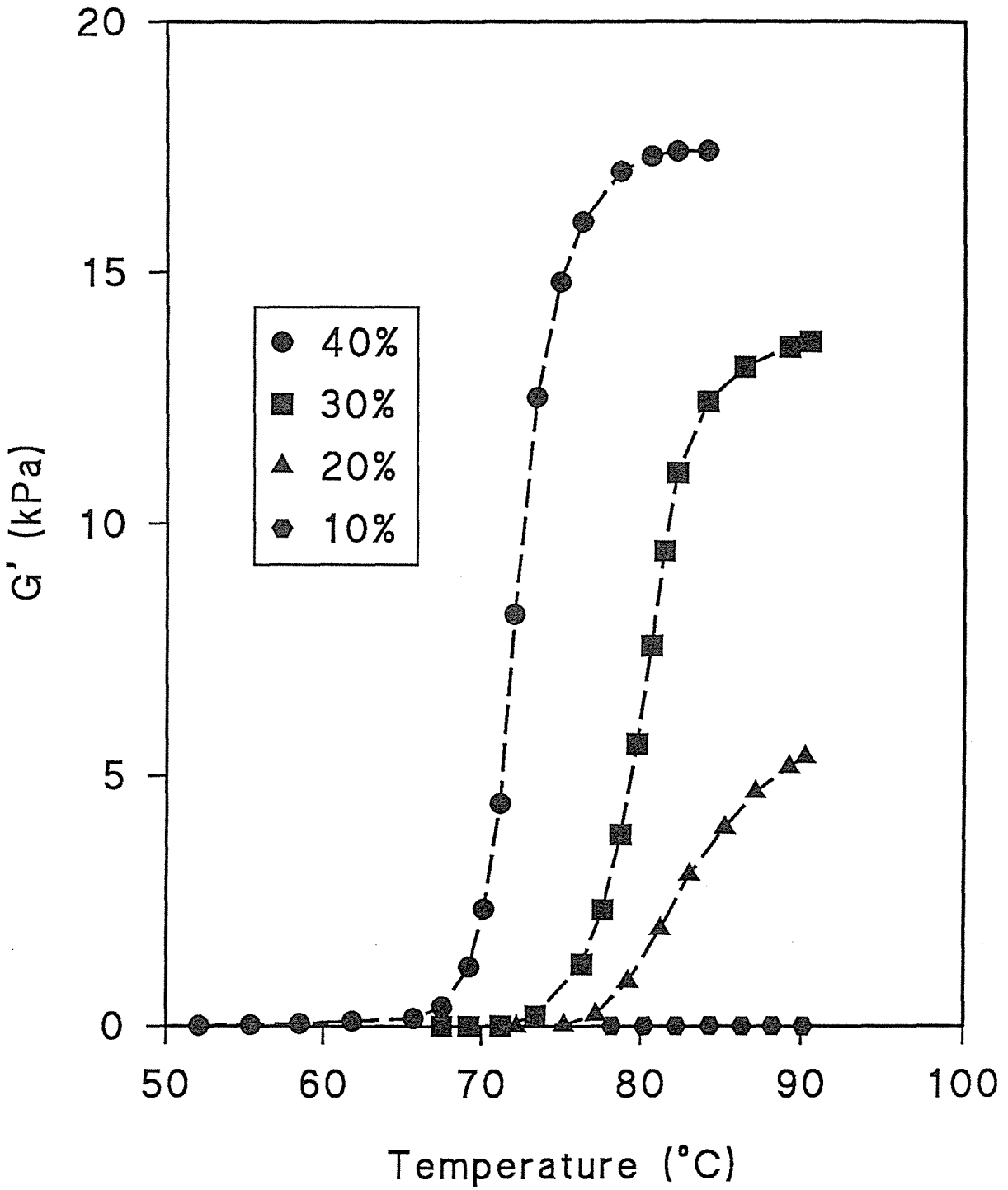


Fig. 6.21–Storage modulus (G') as a function of temperature during thermal gelation of Alacen 475 solutions at different concentrations. Temperature was increased at 1°C/min.

heating rate and the frequency used were 1°C/min. and 1Hz, respectively. For the three WPC solutions the temperature at which gelation started decreased with increasing protein concentration whereas the slope of the storage modulus versus temperature plot increased. This behaviour was also attributed to the kinetic factors discussed previously. The three WPC solutions did not gel at a protein concentration of 10% in these temperature-scanning experiments. The protein gels formed were stronger at higher concentrations as indicated by the higher values of G′ because of more intermolecular interactions such as hydrogen bonds, disulfide bonds and hydrophobic interactions being involved in the gel formation.

The comparison of changes in G′ during thermal gelation for three WPC solutions at 40% is shown in Fig.6.22 and Fig.6.23. As can be seen in Fig.6.22, Alacen 475 solution began to gel at a much lower temperature than either Alacen 312 or Alacen 392, which was consistent with the results obtained from the constant temperature experiments in Sect. 6.3.1. However, the temperature at which G′ exhibited a dramatic increase was similar for all three WPC solutions as illustrated in Fig.6.23. Actually, all three WPC solutions formed stronger gels only at temperatures above 70°C (Fig.6.23), although Alacen 475 solutions could form soft gels between 45°C and 70°C (see Fig.6.22 and Fig.6.23).

The -SH groups of whey proteins are located chiefly in β-lactoglobulin. In the native state they are masked, but on partial denaturation by heat they become free and highly reactive and are then involved in the formation of intermolecular disulphide bonds (Lyster, 1964). The content in free -SH groups is consistent with the degree of protein molecule unfolding. Lyster(1964) found that the content of free -SH groups in whey protein increased very slightly with temperature up to 70°C but showed a very marked increase above 70°C. It is interesting to note that, as shown in

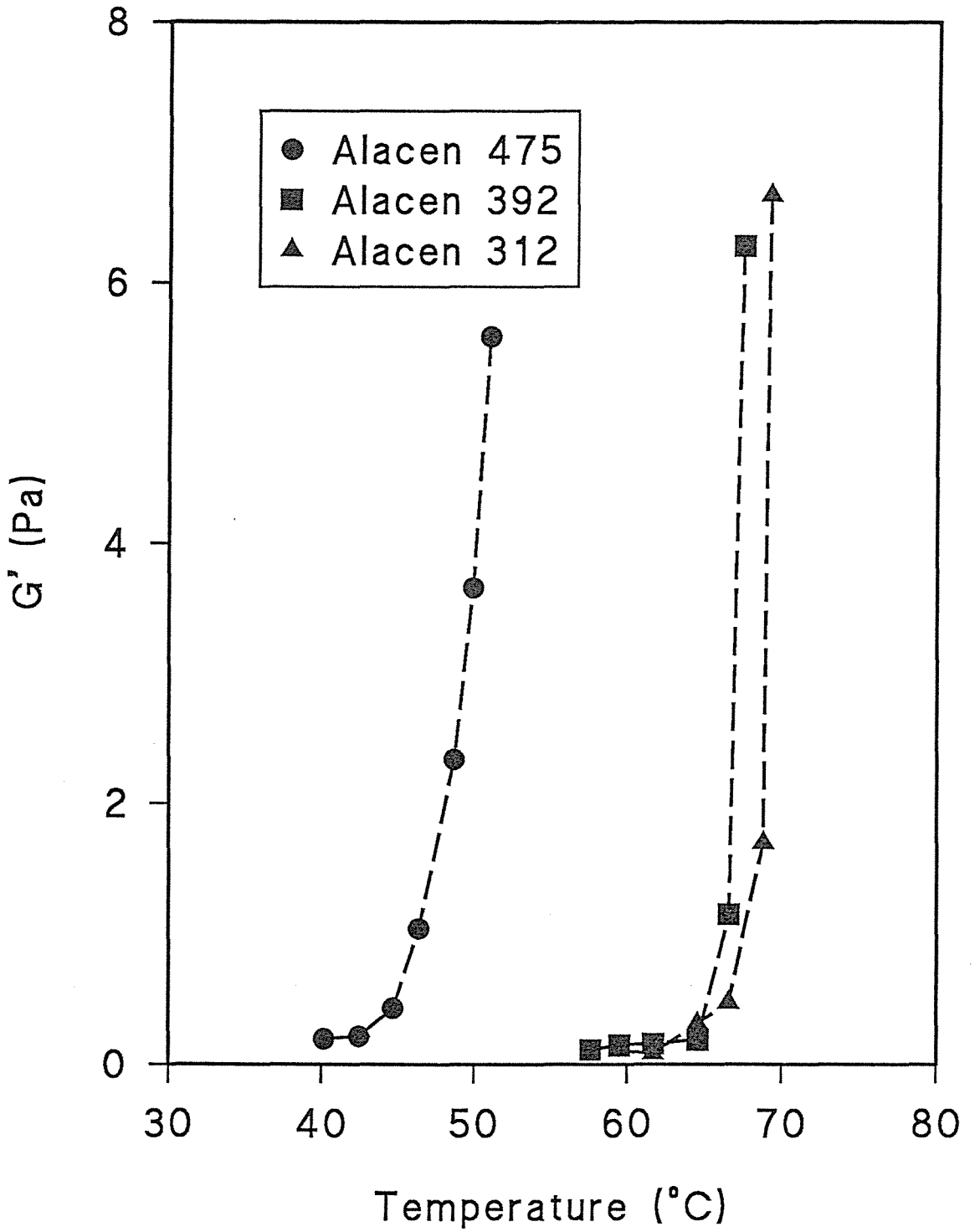


Fig. 6.22–Storage modulus (G') as a function of temperature during thermal gelation of three kinds of 40% WPC solutions. Temperature was increased at 1°C/min.

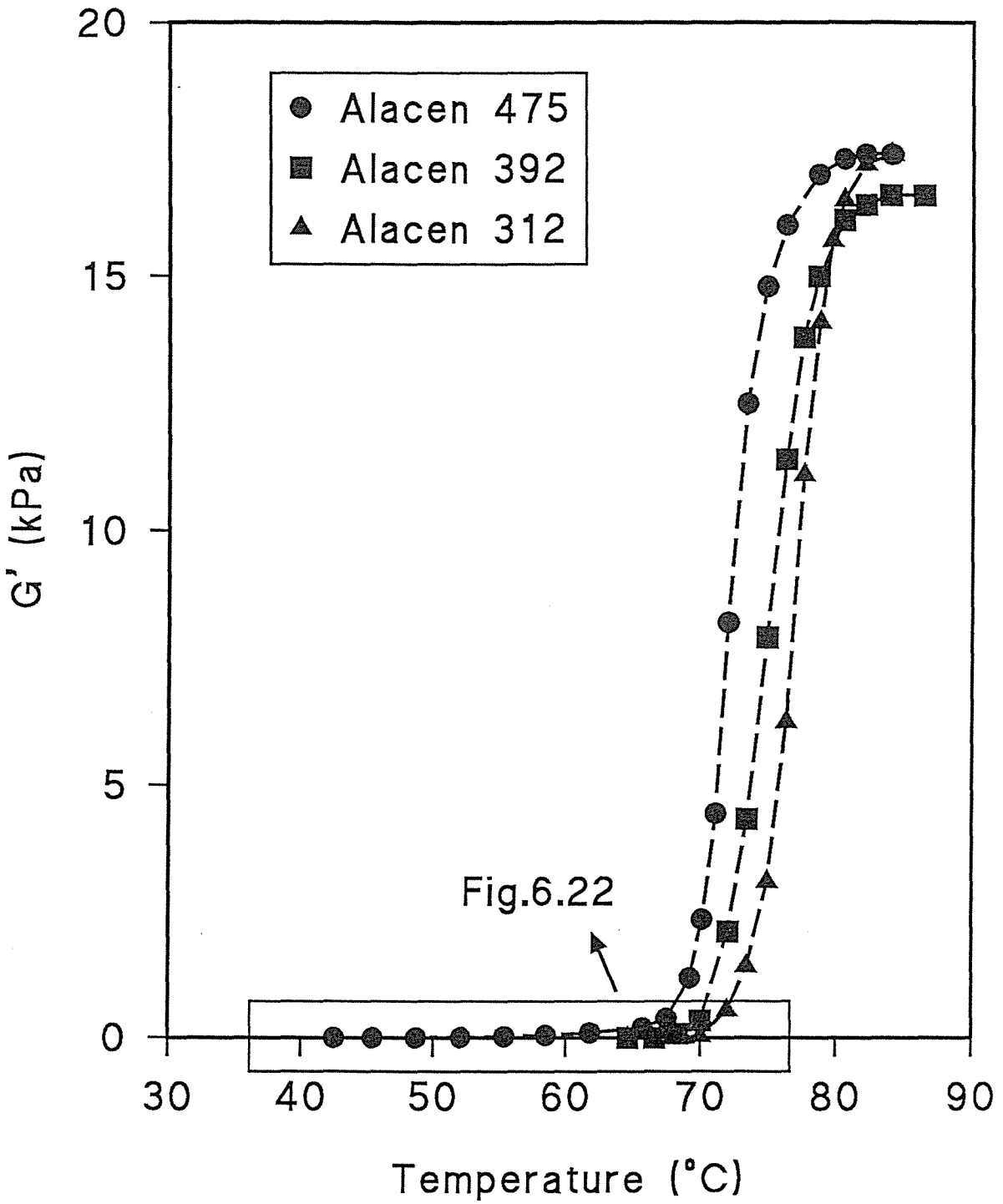


Fig. 6.23–Storage modulus (G') as a function of temperature during thermal gelation of three kinds of 40% WPC solutions. Temperature was increased at 1°C/min.

Figs.6.19 to 6.23, the storage modulus(G′) rose rapidly only above 70°C. This increase in G′ above 70°C must correlate with the involvement of intermolecular disulfide bond formation in the gel network. The presence of a large number of intermolecular disulphide bonds in the gel network above 70°C made the gel stronger and thermally irreversible.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

The WPC solutions were Newtonian at concentrations up to 15%, were slightly shear thinning at 20 and 25%, and were thixotropic at 30% and above. The apparent viscosities of WPC solutions increased with increase in concentration. The apparent viscosities of Alacen 475 solutions at concentrations less than 10 percent by weight could be calculated by the relationship $\eta_s = \eta_w(1+28C)$.

For 40% WPC solutions the structure broken down by shearing at a high shear rate (e.g., 734 s^{-1}) recovered slowly when the shear rate was suddenly reduced to either a low value (e.g., 147 s^{-1}) or to zero. The rate of both structure breakdown and structure recovery decreased with time.

The apparent viscosities of WPC solutions decreased at first with increase in temperature until minimum viscosities were attained, and then they increased rapidly with further increase in temperature, indicating gelation of the solutions. 20% and 30% WPC solutions thinned with shearing time at 40 and 50°C but thickened with shearing time at 60 and 70°C.

The storage modulus (G′) increased to a plateau value during WPC gel formation whereas the loss modulus (G"), and the dynamic viscosity (η') increased to a maximum and then decreased again. The rate of change in G′, G" and η' during heat-induced gelation was dependent on concentration and temperature and might be consistent with the rate of protein gel structure development. A gelation model was proposed to explain the formation of protein gel structure for globular protein solutions.

The storage modulus (G') of protein gels increased with increasing WPC concentration, showing that protein gels formed at higher concentrations were stronger than those made at lower concentrations.

The storage modulus (G') after gel formation from 30% Alacen 475 solution increased with increasing temperature up to 80°C and then decreased at 85°C and decreased further at 90°C. Protein gel properties might be controlled here by a balance between intermolecular attractive forces, intermolecular repulsive forces and kinetic effects (which could favour either aggregation or disaggregation). This balance influenced linear and random aggregation of protein molecules.

The liquid-gel transition or gel point was dependent on concentration and temperature. The pronounced temperature and concentration dependence of the onset and the initial rate of gelation was mainly due to kinetics. At the concentration of 10% Alacen 475 solutions had the highest gelling temperature while Alacen 312 had the lowest gelling temperature. Above 20% all three WPC solutions had almost the same gelling temperature except for Alacen 475 solutions which exhibited a dramatic drop in gelling temperature above 30%.

In temperature-scanning experiments, all three WPC solutions formed strong gels only at temperatures above 70°C. Alacen 475 solutions at a concentration of 40% formed soft gels between 45°C and 70°C.

In future work on this subject, it is recommended that the effect of pH and ionic strength on protein gel structure development and protein gel properties be explored. When the gelation mechanism is further understood qualitatively, mathematical models might be introduced to describe the gelling process quantitatively. The effects of pH and ionic

strength on the flow properties of WPC solutions are also worth investigating.

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NOMENCLATURE

Notation	Description
a	constant
A	aggregates
C	fractional weight concentration
G	gel
G_f	gel fraction
G′	dynamic storage modulus
G"	dynamic loss modulus
G^*	dynamic complex modulus
ΔG	free energy change
ΔH	enthalpy change
N	native protein molecules
S	sol fraction
ΔS	entropy change
t	time
T	temperature

Notation	Description
U	partially unfolded protein molecules
WPC	whey protein concentrate

Greek Letters

η	steady shear viscosity
η'	dynamic viscosity
η_o	viscosity of the surrounding liquid
η_w	viscosity of water
η_s	apparent viscosity of solution
Φ	volume fraction occupied by the dispersed particles
ω	angular frequency
δ	phase angle (loss angle)
γ_o	maximum strain
$\gamma(t)$	sinusoidally varying strain
τ_o	maximum stress
$\tau(t)$	sinusoidally varying stress