Chapter 1: Introduction and literature review

1.1 Background and Significance

1.1.1 Definition of chitosan

Chitosan is an organic polysaccharide derived by deacetylation of chitin (Dee *et al*, 2001, Singla & Chawla, 2001), the second most abundant natural polymer found in nature after cellulose (No and Meyers, 1989), and is found in crustacean shells (Yanga *et al*, 2000, Khan *et al*, 2002), such as crab and shrimp. Chitosan is composed of β-(1$\rightarrow$4)-2-amino, 2-deoxy-D-glucopyranose (glucosamine unit) as shown in Figure 1.1. The chemical structures of chitin and chitosan are similar to that of cellulose. The only difference among the three polysaccharides is the acetamide group at the C-2 position of chitin and the amine group in chitosan instead of the hydroxyl group found in cellulose (Kurita, 1998).

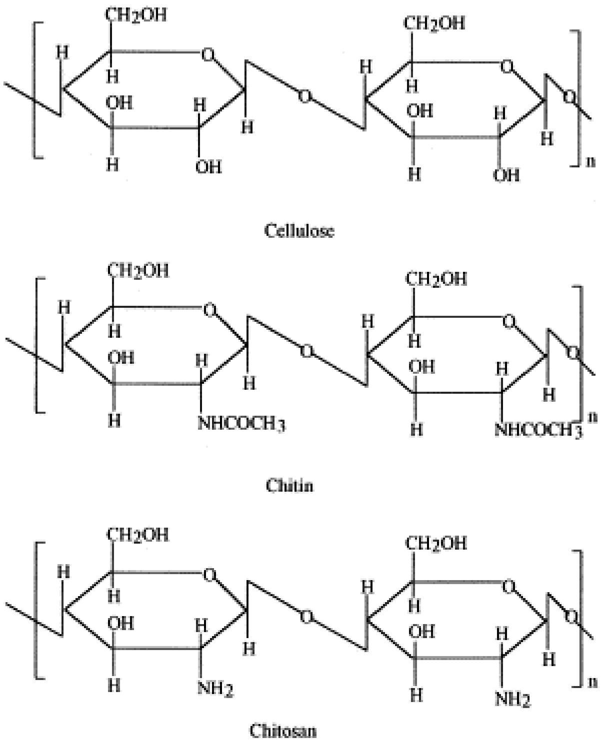


Figure 1.1: Structures of cellulose, chitin and chitosan

1.1.2 A brief history

The first reference to chitin in scientific literature was in 1811 when Henri Braconnot, a professor of natural history in France, separated chitin from a fungus (Khor, 2001). Odier in 1823 (Odier, 1823) managed to isolate the same material from the exoskeletan of a beetle and named this substance chitin from Greek meaning tunic or envelope. In 1859, chitosan was discovered by a French scientist Rouget (Rouget, 1859), who boiled chitin in potassium hydroxide rendering a new substance soluble in organic acids. He named it modified chitin. However, 35 years later, this modified chitin was named ‘chitosan’ by Hoppe-Seyler in 1894 (Hoppe-Seyler, 1894). The structure of chitosan was not resolved until 1950 (Khor, 2001). The first books on chitin were published in 1951(Muzzarelli, 1977). In 1963, K.M. Rudall described the crystallographic structure of chitin for the first time (Rudall, 1963).

Due to recent increased understanding of its functionality and tremendous potential applications in several fields, chitosan has received increased attention over the last three decades as one of the promising renewable polymeric materials (Majeti & Kumar, 2000, Sinha *et al*, 2004).

1.2 Physicochemical characteristics of chitosan

The physical (Mima *et al,* 1983), chemical (Miyoshi *et al*, 1992) and biological properties (Nishimura *et al,* 1984) of chitosan depend on two parameters: the degree of deacetylation (DD) and the molecular weight distribution both of which depend on the species and the method of preparation. The variation in preparation methods of chitosan results in differences in its DD, the distribution of acetyl groups, the viscosity and its molecular weight (Berger *et al*, 2005, Chen & Hwa, 1996).

1.2.1 Solubility

Chitosan is an amorphous solid and almost insoluble in water. Intermolecular hydrogen bonds, which can be formed in the molecules of chitosan, make it difficult to dissolve in water, alkali and common organic solvents. However, it is soluble in dilute aqueous weak organic acid solutions such as formic, acetic, and propionic acids. This is prompted by the presence of amino groups in its molecular structure, which are protonated in the aqueous acid solution making it soluble (Hamdine et al, 2005):

$$-NH_2 + H_3O^+ \leftrightarrow -NH_3{}^+ + H_2O$$

Hence, in the preparation of chitosan solution, aqueous organic acids solutions are always used as solubilizing agents. The solubility of chitosan in dilute acids depends on the DD in the polymer chain and the molecular weight. It is well recognized that higher molecular weight and lower DD have a tendency to make solubilization more difficult.

1.2.2 Degree of Deacetylation (DD)

The deacetylation process can be defined as the removal of acetyl groups from chitin molecules. Degree of deacetylation determines the content of free amino groups ($-NH_2$) in the polysaccharides and, therefore, can be used to differentiate between chitin and chitosan. It is one of the most important chemical characteristics that influences the physicochemical properties of chitosan and its appropriate applications (Muzzarelli, 1977, Li *et al,* 1992).

The degree of deacetylation (DD) has influence on the chemical, physical and biological properties of chitosan (Mima *et al*, 1983). Consequently, it is necessary to have an accurate and rapid method for the determination of the DD. Degree of deacetylation ranges from 56% to 99%, depending on the source of chitin (species) and the method of preparation (No & Meyers, 1995). Generally, chitin with a degree of deacetylation of 70% or above is known as chitosan (Li *et al*, 1992).

A number of methods for the determination of DD have been suggested in the literature, including infrared spectroscopy (Sanan *et al*, 1978), UV-spectrophotometry (Tan *et al*, 1998), nuclear magnetic resonance (Hiral *et al*, 1991), colloidal titration (Terayama,1952), and potentiometric titration (Broussignac, 1968, Ke & Chen, 1990). However, infrared spectroscopy method is considered as the rapidest technique among all.

The degree of deacetylation is affected by some factors, such as the concentration of the alkali, the reaction temperature, the reaction time and the particle size (No and Meyers, 1995). For desired solubility of chitosan, the deacetylation of chitin has to be achieved by at least 85% (No and Meyers, 1995).

1.2.3 Molecular weight

The molecular weight of chitosan varies with the raw material sources and the method of preparation. Several methods can be applied for the determination of its molecular weight, such as light scattering and gel permeation chromatography (GPC) (Jumaa *et al*, 2002, Methacanon *et al*, 2003). However, viscometry is the simplest, fastest and probably the most accurate determination method (Zhang & Neau, 2001).

Galed *et al*. (2005) have reported that chitosan is susceptible to a variety of degradation mechanisms including free radical depolymerization and acid, alkaline and enzymatic-catalyzed hydrolysis. During the preparation of chitosan, the degradation of chitosan polymer occurs by treatment with the concentrated acid and alkali. Accordin to Synoweicki and Al-Khateeb. (2003), the molecular weight of chitosan is affected by deproteinization conditions used for the isolaton of the chitinous substrate.

Chitosan's molecular weight distribution is influenced by several factors such as time, temperature, reagent's concentration and atmospheric conditions of the deacetylation reaction (Tolaimate *et al*, 2000). The DD process plays a significant role on the molecular weight of chitosan. The lower the degree of deacetylation, the higher is the molecular weight. The average molecular weight of chitosan is about 1.2×10^5 g mol^{-1} (Nagasawa *et al*, 1971).

1.2.4 Viscosity

Viscosity is an important factor in the conventional determination of the molecular weight of chitosan and its commercial applications. The viscosity of chitosan solution is affected by many factors, such as the degree of deacetylation, molecular weight, concentration, ionic strength, pH, and temperature (Li *et al*, 1992).

The viscosity of chitosan is found to decrease with an increased time of demineralization (Moorjani *et al*, 1975). The viscosity of chitosan in acetic acid tends to increase with decreasing pH, but decrease with decreasing pH in hydrochloric acid, giving rise to the definition of 'Intrinsic Viscosity' of chitosan which is a function of the degree of ionization as well as ionic strength. Bough *et al*. (1978) found that deproteinization with 3% NaOH and the elimination of the demineralization step in the chitin preparation decrease the viscosity of the final chitosan products. No *et al*. (1999) demonstrated that the viscosity chitosan is considerably affected by physical (grinding, heating, autoclaving, ultrasonication) and chemical (ozone) treatments, except for freezing, and decreases with an increase in treatment time and temperature. Chitosan solution stored at 4^{o}C is found to be relatively stable from a viscosity point of view (No *et al*, 1999). The effect of particle size on the quality of chitosan products was investigated by Bough *et al*. (1978) who reported that smaller particle size (1mm) results in chitosan products of both higher viscosity and molecular weight than those of either 2 or 6.4 mm particle size. They further enumerated that a larger particle size requires longer swelling time, resulting in a slower deacetylation rate. But, in contrast, Lusena and Rose (1953) reported that the size of chitin particle within the 20-80 mesh (0.841-0.177 mm) range had no effect on the viscosity of the chitosan solutions. However, reports that relate the viscosity of chitosan to the type of solubilizing organic acids solutions can be barely found in literature (Hamidine *et al*, 2005).

1.3 Rheology of Solutions

Rheology is defined as the science of the deformation and flow of matter (Rao, 1999; Steffe, 1992). It investigates the response of materials to applied stress or strain (Herh et al, 2000, Hackley & Ferraris, 2001). Rheological properties describe flow characteristics and textural behavior of substances. In fact, the success of a wide range of commercial products and industrial processes depends on meeting specific flow requirements. Rheology is an important tool in several fields including food industry, coating, personal care products and cosmetics, detergents, cement and medicine.

1.3.1 Fluids Flow Behavior

Fluids flow behavior can be divided into Newtonian and Non-Newtonian behavior.

Newtonian Behavior

In Newtonian fluids, the flow behavior is independent of the shear rate. It is only dependent on temperature and composition (Rao 1977a, b). This means that at a given temperature, the relationship between shear stress and shear rate is a straight line (Figure 2). It also means that the fluid's viscosity remains constant as the shear rate is varied (Figure 3). Some examples of Newtonian fluids include water, milk, filtered juices, honey and edible oils (Rao, 1999).

Non-Newtonian Behavior

A non-Newtonian fluid is defined as a fluid for which the relationship Force/Area is not constant. This behavior of fluid can be divided into time independent and time dependent. Time independent flow behavior may depend only on shear rate and not on the duration of shear, whereas time dependent flow behavior depends also on the duration of shear (Rao, 1999).

Time independent behavior

Shear-thinning behavior

Shear-thinning fluids are also referred to as pseudoplastic. With shear-thinning fluids, the curve (Figure 2) begins at the origin of the shear stress-shear rate plot but it concaves upward; that is, an increasing shear rate gives a less than proportional increase in shear stress (Rao, 1999). The viscosity (Figure 3) of the fluids decreases with increasing shear rate (Steffe, 1996). This type of fluids can be reversible (Howard, 1991). The reason for shear-thinning is probably due to breakdown of structural units in a food due to the hydrodynamic forces generated during shear (Rao, 1999). Most non-Newtonian foods exhibit shear-thinning behavior, including many salad dressings and some concentrated fruit juices.

Shear-thickening behavior

This type of behavior is also known as dilatant. In shear-thickening behavior, the curve (Figure 1.2) also begins at the origin of the shear stress-shear rate plot but it concaves downward; the increasing shear stress gives a less than proportional increase in shear rate (Rao, 1999). The viscosity (Figure 1.3) of a fluid increases with increasing shear rate Steffe, 1996). The reason for the shear-thickening fluids may due to the increase in the size of the structural units as a result of shear. Common examples of shear-thickening are candy compounds and corn starch in water (Rao, 1999).

Yield stress

The flow of some materials does not begin until a certain amount of force is applied to the fluid. This force is called yield stress (Rao, 1999). Steffe (1996) defined the yield stress as the minimum shear stress that is required to initiate flow. Bingham plastic and Herschel-Bulkley models (Figure 1.2) are examples of yield stress. When the shear rate-shear stress relationship is a straight line with a yield stress, the fluid is considered to follow Bingham plastic model. Examples of shear-thinning with yield stress behavior include tomato ketchup, mustard, and mayonnaise.

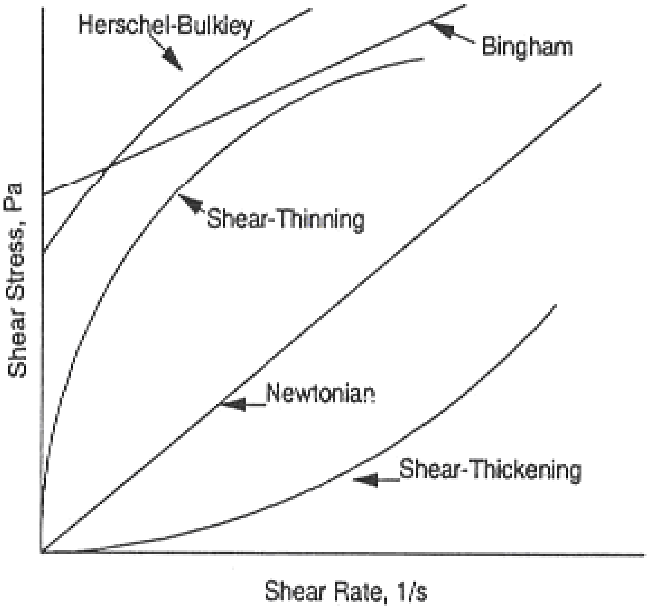


Figure 1.2: Relationship between shear stress and shear rate for typical time independent fluids (Steffe, 1996).

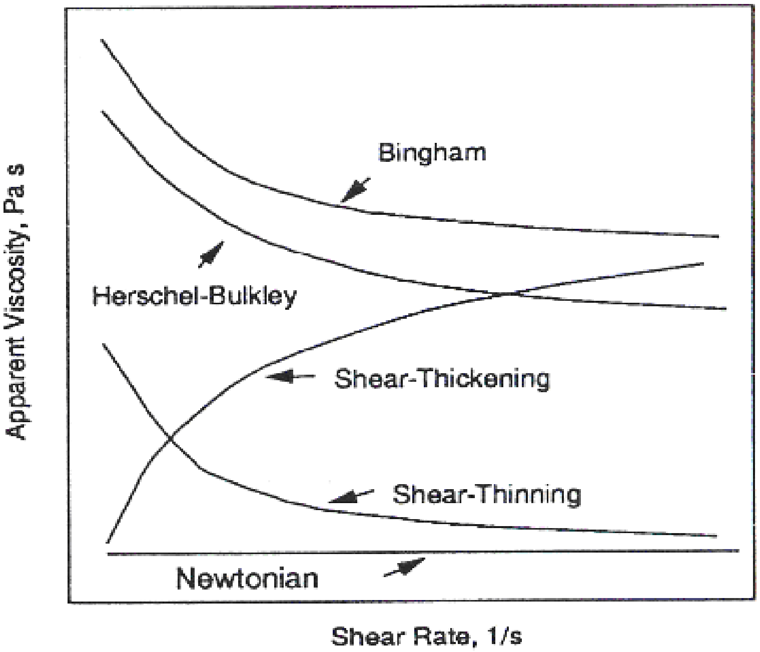


Figure 1.3: The relationship between apparent viscosity and shear rate for time-independent fluids (Steffe, 1996).

Time-dependent behavior

This type of fluids displays a change in viscosity with time under conditions of constant shear rate and temperature. It can be divided into two categories; thixotropic and antithixotropic (rheopectic).

Thixotropic behavior

Fluids that exhibit time-dependent shear-thinning behavior are said to have a thixotropic flow behavior (Rao, 1999). A thixotropic fluid undergoes a decrease in viscosity as a function of time while it is subjected to a constant temperature and shear rate (Rao, 1977a). When the hydrodynamic forces during shear are sufficiently high, the interparticle linkages are broken, resulting in reduction in the size of the structural units which means lower resistance to flow during shear (Mewis, 1979). Common examples of foods containing this type of behavior are salad dressings and soft cheeses.

Antithixotropic behavior

Time-dependent shear-thickening behavior is called antithixotropic behavior (Rao, 1999). It is the opposite of thixotropic behavior, in which the viscosity increases as a function of time at a constant temperature and shear rate (Rao, 1977a).

1.4 Chitosan films and membranes

It is well known that chitosan can be easily filmablized, especially, by a casting technique (Kanke *et al*, 1989, Bonvin & Bertorell, 1993), which makes it useful for many applications including skin care, cosmetics, contact lenses and in various other technologies. Chitosan film properties have been reported by numerous authors (Butler *et al*, 1996, Chen & Hwa, 1996, Singh & Ray, 1998, Bégin & Van Calsteren, 1999). Its film characteristics vary from one report to another. This may be attributed to some factors such as chitin source, type of solvent used and method of film preparation (Samuels, 1981, Blair *et al*, 1987, Lim & Wan, 1995). Films of chitosan are clear, homogeneous and flexible with good oxygen barrier and mechanical properties (Hoagland & Parris, 1996, Kittur *et al*, 1998), but with relatively low water vapor barrier characteristics (Butler *et al*, 1996). In addition, films of chitosan are dense and do not possess pores (Muzzarelli, 1977, Hirano & okura, 1982). Since chitosan degrades before melting, it is necessary to dissolve it in an appropriate solvent prior to being casted into films.

Generally, the film properties of chitosan depend on its morphology, which is affected by its molecular weight (Butler *et al*, 1996, Chen & Hwa, 1996), degree of N-acetylation, solvent evaporation, and free amine regenerating mechanism. In addition, the solvent used has an influence on the properties of chitosan films as it has been mentioned earlier. For instance, chitosan films prepared from lactic acid are softer and more pliable and bioadhesive compared with those prepared from acetic acid (Khan *et al*, 2000).

There are several parameters to be considered in the selection of a chitosan membrane, among which are casting method (Samuels, 1981, Ogawa *et al*, 1992), molecular weight (Samuels, 1981), thickness (Nakatsuku & Andrady, 1992), swelling index (Mochizuki *et al*, 1989) and membrane preparing conditions (Kienzie-Sterzel *et al*, 1982).

1.5 Production of chitosan

Chitosan can be produced by the extraction of chitin from the shell of shrimps, lobsters, prawns and crabs, followed by the deacetylation with a strong base heated in special vessels under certain conditions of temperature, atmosphere and time. Several methods of preparing chitosan from chitin have been suggested in the literature (Muzzarelli, 1977). The most important among them are listed as follow:

1) The method of Horowitz, Roseman & Blumenthal and Horton & Lineback. In this method, chitosan is obtained by fusing chitin with a solid alkali hydroxide followed by stirring in a nitrogen atmosphere at 180^oC. The precipitate is washed with water and then purified.

2) The method of Rigby; Wolfram, Maher & Chaney and Wolfrom & Shen-Han. This method involves treating chitin with 40% aqueous solution of sodium hydroxide at 115^oC for some time under a nitrogen atmosphere. After that, the mixture obtained is left to cool, and then filtered and washed with water.

3) The method of Fujita. This method depends on mixing the same amount of chitin and sodium hydroxide together. Then, the mixture obtained is mixed with liquid paraffin. The final mixture is stirred at 120^oC, poured into cold water, filtered and washed with water.

1.6 Applications of chitosan

Chitosan shows a great promise for a wide variety of uses. As a matter of fact, many of chitosan's applications depend upon its unique properties such as biodegradability, cationic and non-toxic properties. Table 1.1 summarizes some applications of chitosan.

Table 1.1: Some application of chitosan

Application	**Examples**	**Reference**
Water treatment	Filtration Removal of metal iron Flocculant/Coagulant	(Li *et al*., 1992)
Medical	Bandages, Sponges	(Sathirakul *et al*, 1996),
	Cholesterol reducing agent	(Gordon *et al*, 1984),
	Membranes	(Li *et al*., 1992)
	Skin burns/Artificial skin	(Li *et al*., 1992)
	Contact lens	(Micheline *et al*, 1988, Olsen *et al*, 1989, Yuan & Wei, 2004)
	Wound healing agent	(lioyd *et al*, 1998, Cho *et al*, 1999, Ueno *et al*, 1999)
	Drug carriers	(Roseman, 1974, Pillai & Panchagnula, 2001, Hejazi & Amiji, 2003, Khor & Lim, 2003)
	Dressing burns	(Allan *et al*, 1984),
	Drug delivery system	(Sawayanagi *et al*, 1982, Park *et al*, 2001, Nunthanid *et al*, 2004),
	Producing artificial kidneys	(Hirano & Noishiki, 1985)
Cosmetics	Make-up powder	(Li *et al*., 1992)
	Nail lacquers	(Mark *et al*,1985)
	Moisturizer	(Li *et al*., 1992)
	Bath lotion and hair treatment	(Griesbach *et al*, 1999)
	Face, hand and body creams	(Gross *et al*, 1983, Chikamatsu *et al*, 1993)

	Toothpaste	(Li *et a*l., 1992)
paper industry	Surface treatment	(Li *et a*l., 1992)
	Photographic paper	(Li *et a*l., 1992)
	Carbonless copy paper	(Li *et a*l., 1992)
Biotechnology	Enzyme immobilization	(Li *et al*., 1992)
	Protein separation	(Li *et al*., 1992)
Agriculture	Seed coating	(Rawls, 1984)
	Making fertilizer	(Li *et al*., 1992)
	Controlled agrochemical release	(Li *et al*., 1992)
Food	Removal of dyes, solids, acids	(Li *et al*., 1992)
	Animal feed additives	(Li *et al*., 1992)
	Color stabilization	(Li *et al*., 1992)
Membranes	Solvent separation	(Li *et al*., 1992)
	Permeability control	(Li *et al*., 1992)

Objective of the present work

The overall objective of this work was to study some physical and chemical properties of chitosan dissolved in some weak acids solutions. Among the properties studied include the rheological properties, i.e., the dynamic viscosity and shear stress as a function of shear rate, thermal properties using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC), morphological properties employing scanning electron microscopy (SEM) and atomic force microscopy (AFM) and mechanical properties, i.e., tensile strength and elongation at break.

Chapter 2: Materials and methods

2.1 Materials

Shrimp source chitosan with a deacetylation percentage (DD) of 88.1% determined by UV method (Muzzarelli & Rochetti, 1985) was supplied by the chitin-chitosan laboratory at National University of Malaysia (UKM). This chitosan was acid soluble, white-colored and flaky. Formic acid (98-100% pure) and acetic acid (glacial 100%, pro analysi) were purchased from Merck KGaA (Darmstadt, Germany). Propionic acid (99.9% pure) was supplied by Riedel-de Haen (Seelze, Germany). Ultra pure water (Maxima Ultra Pure Water, Elga-Prima Corp, UK) with a resistivity greater than 18MΩ/cm was used to prepare all solutions. All chemicals were used without further purification and freshly prepared solutions were used in all experiments.

2.1.1 Preparation of the solutions

Chitosan was dried in the oven until constant weight was observed. Then, 5g of chitosan was dissolved in 500 mL (0.1 M) of the respective acids followed by mild stirring and heating at about 50^{o}C overnight to form a 10 g L^{-1} chitosan solution. The solution was then filtered to remove dust and other traces of impurities and was used as a stock solution. The stock solution was diluted with the respective acid to a range of concentrations between 2 to 10 g L^{-1}. Air bubbles were eliminated by keeping the solutions at room temperature for some time.

2.1.2 Film casting and drying

The prepared homogeneous solutions were cast onto polystyrene petri dishes for film formation, and then dried in the oven at 60^{o}C for 48 h. The obtained films were peeled off gently, and were further dried by keeping in a desiccator (with silica gel desiccant under vacuum) until use. All films obtained were transparent and free from air bubbles. The film thickness was about 0.012 mm measured with a digital micrometer (Mitutoyo, Japan)

2.2 Methods and equipments

2.2.1 Molecular weight measurements

The molecular weight of chitosan was about 5.5×10^5 g mol^{-1} determined by gel permeation chromatography (GPC) equipped with a Waters 1515 HPLC Pump and a Waters 2414 Refractive Index Detector. The column used was PL aquagel-OH 30 (8 μm, 300 × 7.5 mm) and the solvent used was 1% acetic acid. The GPC was calibrated using polystyrene standards.

2.2.2 Viscosity measurements

The dynamic viscosity of chitosan solutions was determined by using a Brookfield digital viscometer, model DV-II + Pro, with an attached UL adapter (Figure 4). Measurements were made at a constant shear rate (3.67 s^{-1}) as well as at varying shear rates. Viscosity values were reported in centipoises (cP). Each measurement was recorded as an average value of five readings.

Figure 2.1: Brookfield digital viscometer.

2.2.3 pH measurements

The pH measurements of the chitosan solutions were carried out using a microprocessor pH meter (pH 211, Hanna Instruments) as shown in Figure 5. Experiments were carried out in triplicate, and the average and standard deviation values were reported.

Figure 2.2: A microprocessor pH meter

2.2.4 Thermogravimetric measurements

It is possible to obtain information about the stability of polymers by investigating their thermal behavior as a function of weight loss with heat. Thermogravimetric analysis (TGA) has been used to study the thermal stability and decomposition of polymers.

In this work, TGA was used to evaluate the thermal stability and to determine the decomposition temperature of chitosan. Thermogravimetric measurements were realized using a Mettler-Toledo thermogravimetric analyzer model TGA/SDTA851e. The temperature range studied was 30–800^{o}C at a heating rate of 10^{o}C/min under a nitrogen atmosphere. The mass of the sample pan was continuously recorded as a function of temperature.

2.2.5 Differential scanning calorimetry measurements

DSC studies were performed using a DSC Mettler Toledo (model DSC822e). The samples were scanned under a nitrogen atmosphere at a constant rate of 10^{o}C/min. The experiment consisted of two runs. The first run was from 40^{o}C to 500^{o}C and the second was from 40^{o}C to 250^{o}C, after heating up to 200^{o}C and cooling to 40^{o}C.

2.2.6 Scanning electron microscopy

The surface morphology of the samples was investigated using SEM. A digital Leica S440 scanning electron microscope was used in order to study the surface smoothness and homogeneity.

2.2.7 Atomic force microscopy (AFM)

Atomic force microscopic observation was performed on a digital AFM nanoscope, Dimension 3000 (Digital instruments, Santa Barbara, CA). AFM studies were carried out using the tapping mode under air atmosphere at ambient temperature. The scan size, set point and scan rate are shown in the images. The tapping set-point was adjusted to minimize probe-sample interactions.

2.2.8 FTIR measurements

FTIR measurements of blend film were performed on a spectrometer (Perkin Elmer, model 2000). The spectra were obtained at a frequency range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and 8-times scanning.

2.2.9 Mechanical properties

The mechanical properties were measured using a universal mechanical tester (Instron, Model 5566, USA) according to the ASTM D 882-91 (ASTM, 1995). Dumbbell-shaped specimens of 50 mm long with a neck of 28 and 4 mm wide were used. The measurements were carried out at 23^{o}C and 50% relative humidity. The crosshead speed was fixed at 50 mm/min. A minimum of five specimens were tested for each sample and the average was recorded.

Chapter 3: Results and discussion

Rheology of chitosan solutions

3.1 The effects of temperature and concentration on pH of chitosan solutions

In general, chitosan in solution carries a positive charge along its backbone when the pH value is below 6.5. At pH values less than 4, most of the amino groups of chitosan are expected to be protonated. Figure 3.1 shows the effect of concentration on pH of chitosan solubilized in the three solvents at room temperature. The pH increases rapidly with increasing concentration (almost linear relationship), which is attributed to the consumption of H^+ ions in solution by the protonation of the free amine groups. The pH values of chitosan solutions span from 2.71 for 2 g L^{-1} solution to 3.16 for 10 g L^{-1} solution, from 3.52 for 2 g L^{-1} solution to 3.99 for 10 g L^{-1} solution and from 3.55 for 2 g L^{-1} solution to 3.97 for 10 g L^{-1} solution for chitosan dissolved in formic acid, acetic acid and propionic acid solutions respectively.

The variation of pH of chitosan solution (10 g L^{-1}) in the three solvents versus temperature is shown in Figure 3.2. In the beginning, the value of pH drops dramatically with increasing temperature as the solution becomes more acidic, and then, gradually becomes less sensitive to it.

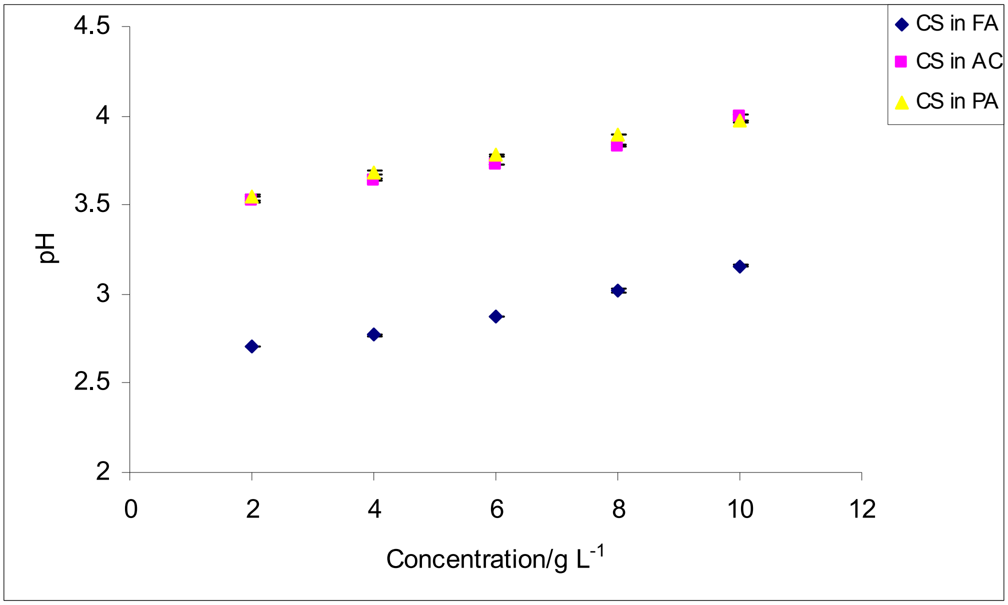


Figure 3.1: The influence of concentration on pH of chitosan solutions at 25°C (with very small standard deviation).

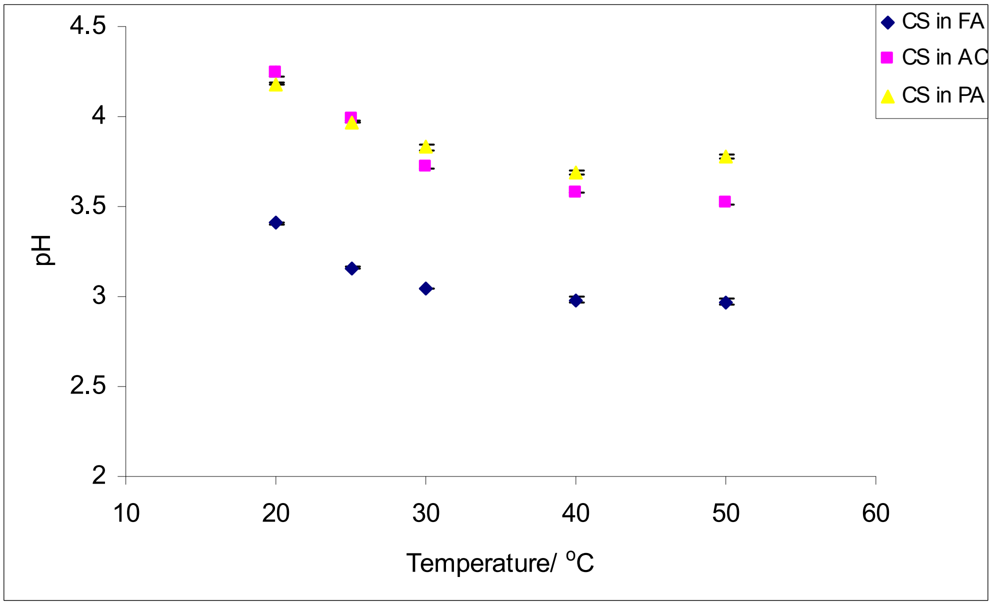


Figure 3.2: The influence of temperature on pH of chitosan solutions (with very small standard deviation).

3.2 The effects of temperature and concentration on the shear viscosity of chitosan solutions

In general, polymer solutions exhibit Newtonian behavior at low shear rates which means that the viscosity is independent upon shear rate. On the contrary, the viscosity is nearly dependent of shear rate at high shear rates (non-Newtonian behavior). In this study, the shear rate-dependent viscosity of chitosan solutions in the selected solvents as a function of shear rate at a range of temperature from 20°C to 50°C is presented in Figures 3.3, 3.4 and 3.5. For all solutions, at low temperature, non-Newtonian behavior is observed. However, increasing temperature leads to the appearance of a Newtonian behavior. In other words, decreases in the viscosities were more pronounced at lower temperature, i.e., lower shear thinning and higher viscosities are observed at lower temperature. The results obtained show good agreement with previous studies (Desbrie`res, 2002). In addition, these results indicate that the temperature has more effect on the solutions at lower shear rate values, i.e., the viscosity increase associated with temperature is less important at high shear rates. This behavior was also reported in the literature (Delben *et al*, 1990).

The relationship between shear viscosity (determined at a constant shear rate) and temperature for a chitosan concentration of 10 g L^{-1} in the three selected solvents is demonstrated in Figures 3.6, 3.7 and 3.8. These graphs show a nearly linear relationship whereby the viscosity of chitosan in the three acids solutions decreases significantly with increasing temperature, i.e., it is a strong function of temperature. The viscosity varies from 19.52 cP for 20°C to 10.56 cP for 50°C, from 9.3 cP for 20°C to 4.2 cP for 50°C and from 7.28 cP for 20°C to 3.60 cP for 50°C for chitosan in formic, acetic and propionic acid solutions respectively.

The viscosity values obtained at a constant shear rate can be correlated with temperature according to the Arrhenius equation:

$$\eta = A \, . \, e^{-Ea/RT} \qquad \text{EQ. 1}$$

where A is a constant related to molecular motion, E_a is the activation energy for viscous flow at constant shear rate, R is the gas constant and T is the absolute temperature in K. Figures 3.9, 3.10 and 3.11 present Arrhenius plots for chitosan solutions in the three solvents with concentration of 10 g L^{-1}. These graphs also show linear relationships with apparent activation energies of 16.19 kJ mol^{-1}, 20.86 kJ mol^{-1} and 17.62 kJ mol^{-1} for chitosan solutions in formic, acetic and propionic acids respectively. The determination of activation energy is important in deducing the sensitivity of a process towards temperature. It is also useful for signifying the minimum amount of energy required for the reactants to start a reaction.

The obtained values of the activation energy in this work are in accordance with the reported values using acetic acid. For example, Wang and Xu (1994) revealed that the activation energy varies from 25 kJ mol^{-1} when DD is 91% to 15 kJ mol^{-1} when DD is 75% for a chitosan concentration of 20 g L^{-1} (in 0.2 M AcOH/0.1 M AcONa). Desbrieres (2002) reported values of activation energy at zero shear rate vary from about 15 kJ mol^{-1} to 37 kJ mol^{-1} for a chitosan concentration ranges between 0 g L^{-1} and 40 g L^{-1} respectively (solvent, 0.3 M AcOH/0.05 M AcONa). This shows that the type of acid does not have significant influence on the E_a.

Figures 3.12, 3.13 and 3.14 show the effect of temperature on the shear stress of chitosan solutions as a function of shear rate. Shear stress increased with increasing shear rate. At the same shear rate, shear stresses were higher at lower temperature. In addition, increases in shear stress with increasing shear rate were more remarkable at lower temperature. From the graphs it is clear that all solutions exhibit pseudoplastic non-Newtonian behavior.

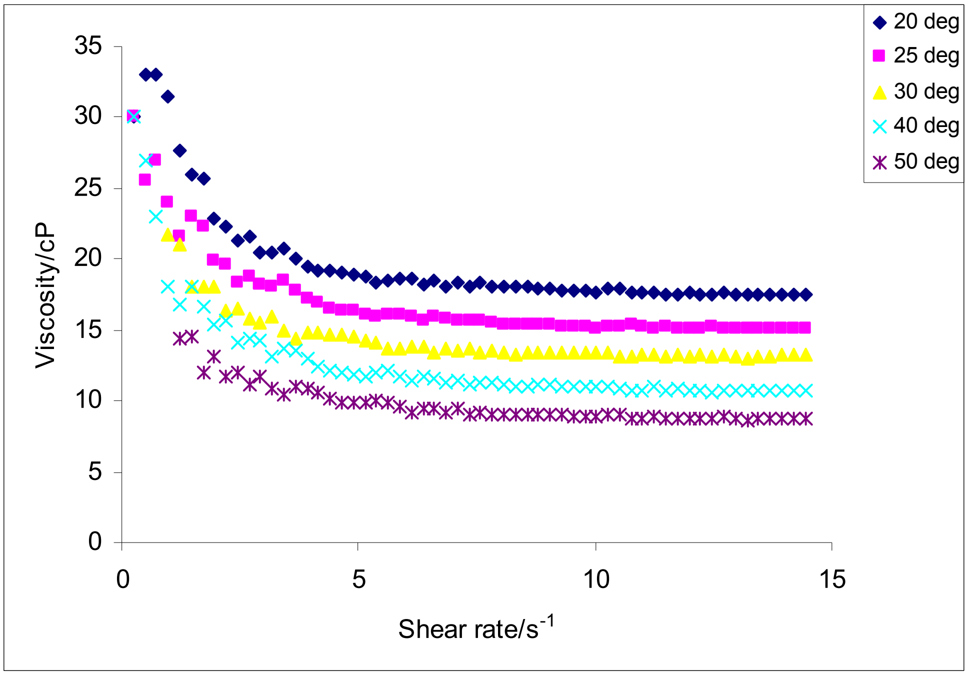


Figure 3.3: The influence of the shear rate and temperature on the rheological curves of chitosan in formic acid solutions.

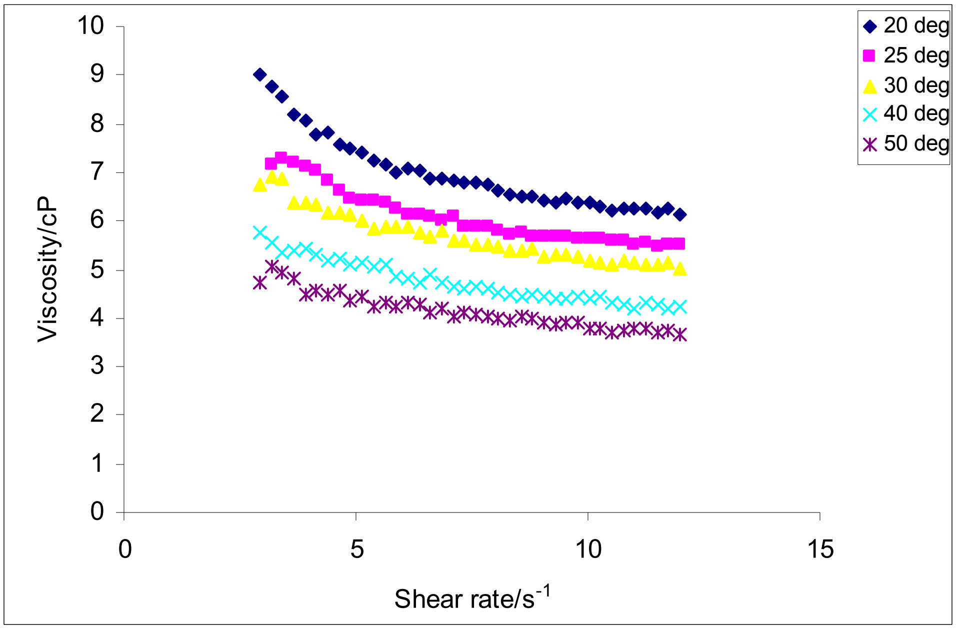


Figure 3.4: The influence of the shear rate and temperature on the rheological curves of chitosan in acetic acid solutions.

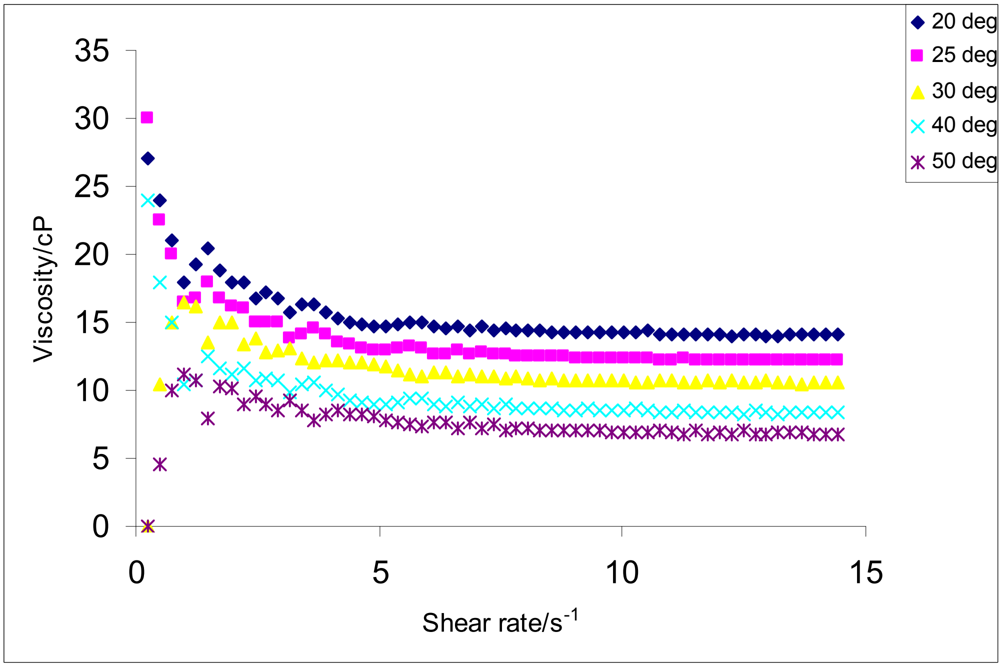


Figure 3.5: The influence of the shear rate and temperature on the rheological curves of chitosan in propionic acid solutions.

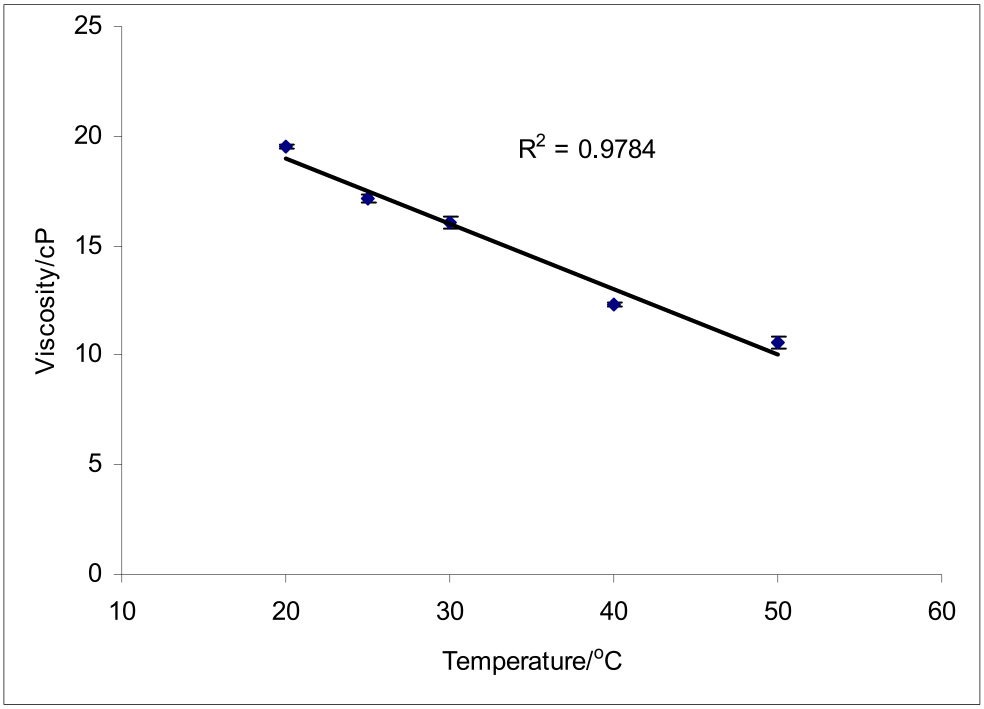


Figure 3.6: The influence of temperature on viscosity of chitosan in formic acid solutions at a constant shear rate.

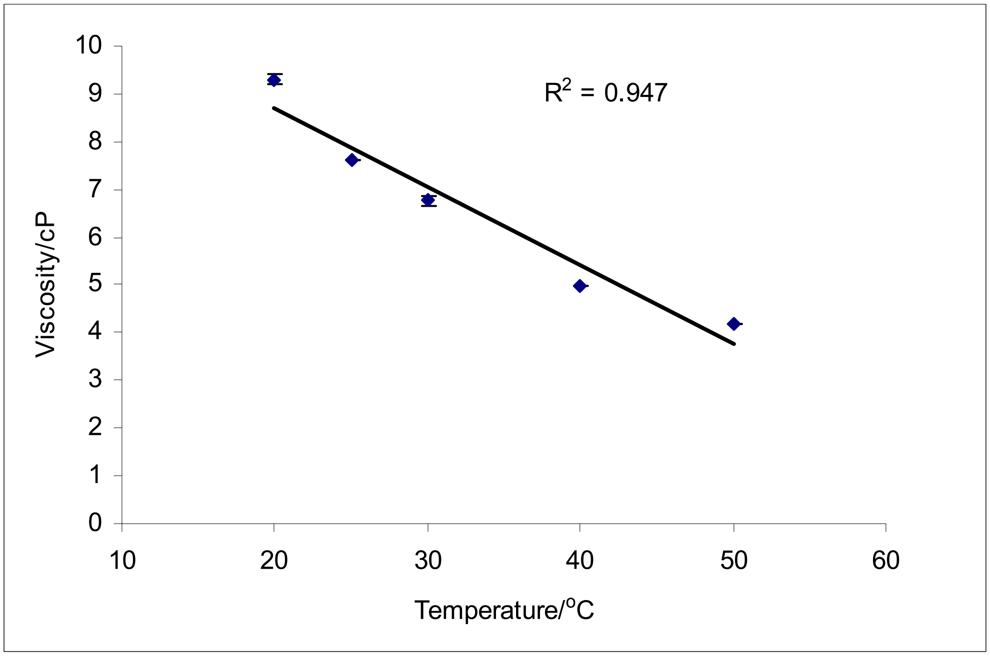


Figure 3.7: The influence of temperature on viscosity of chitosan in acetic acid solutions at a constant shear rate.

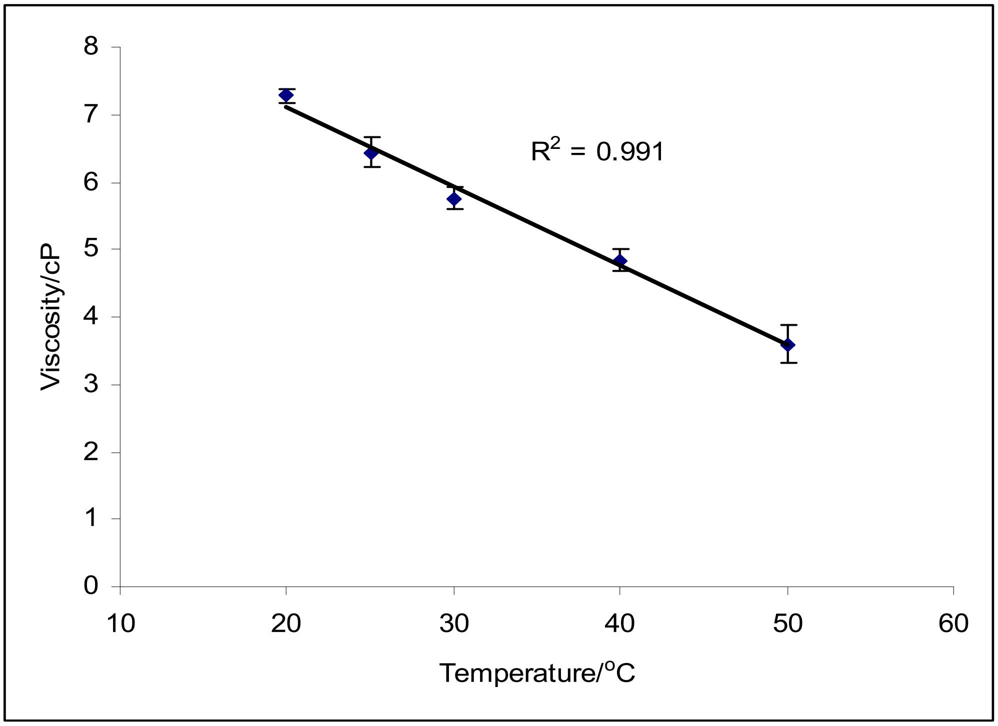


Figure 3.8: The influence of temperature on viscosity of chitosan in propionic acid solutions at a constant shear rate.

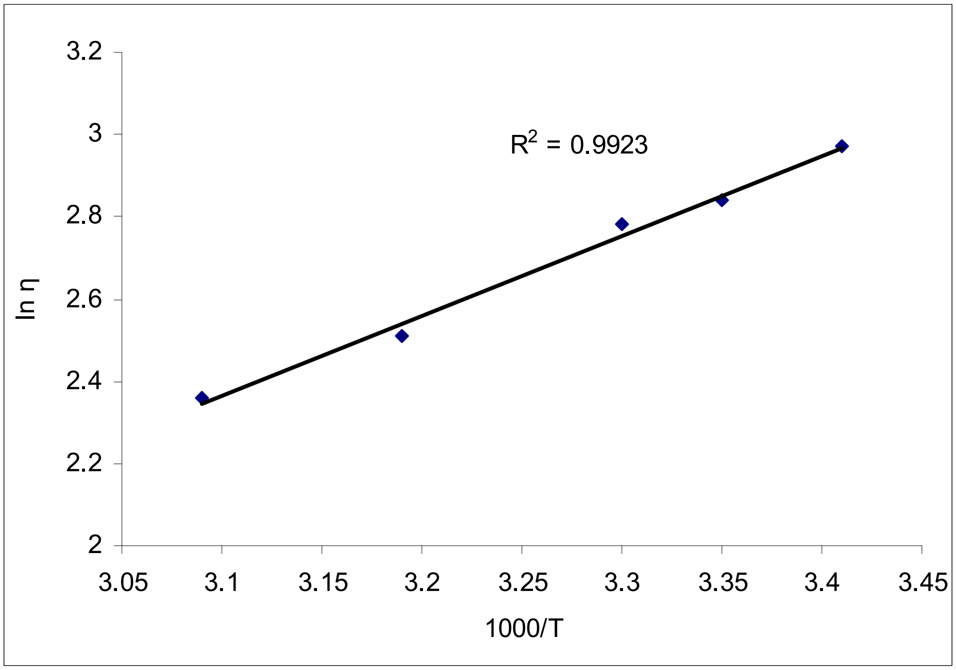


Figure 3.9: The Arrhenius plot of ln η versus 1/T for chitosan in formic acid solutions.

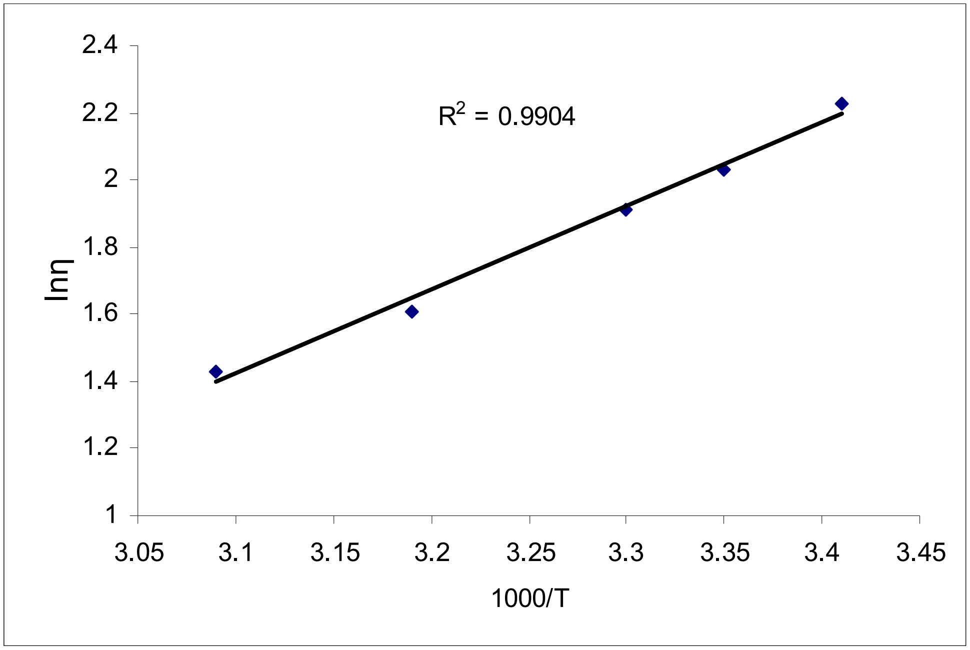


Figure 3.10: The Arrhenius plot of ln η versus 1/T for chitosan in acetic acid solutions.

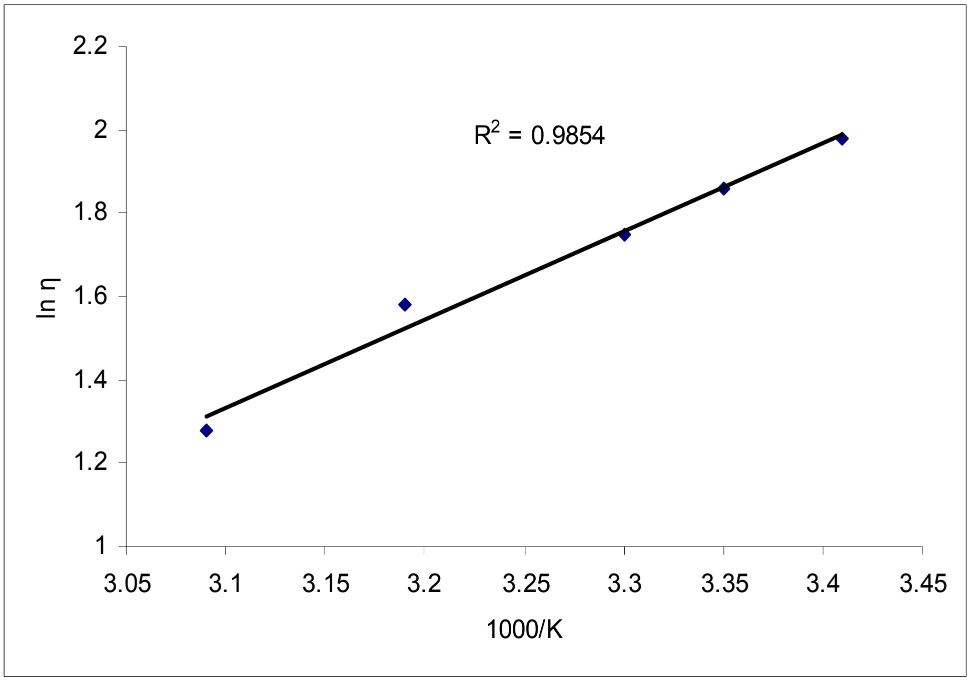


Figure 3.11: The Arrhenius plot of ln η versus 1/T for chitosan in propionic acid solutions.

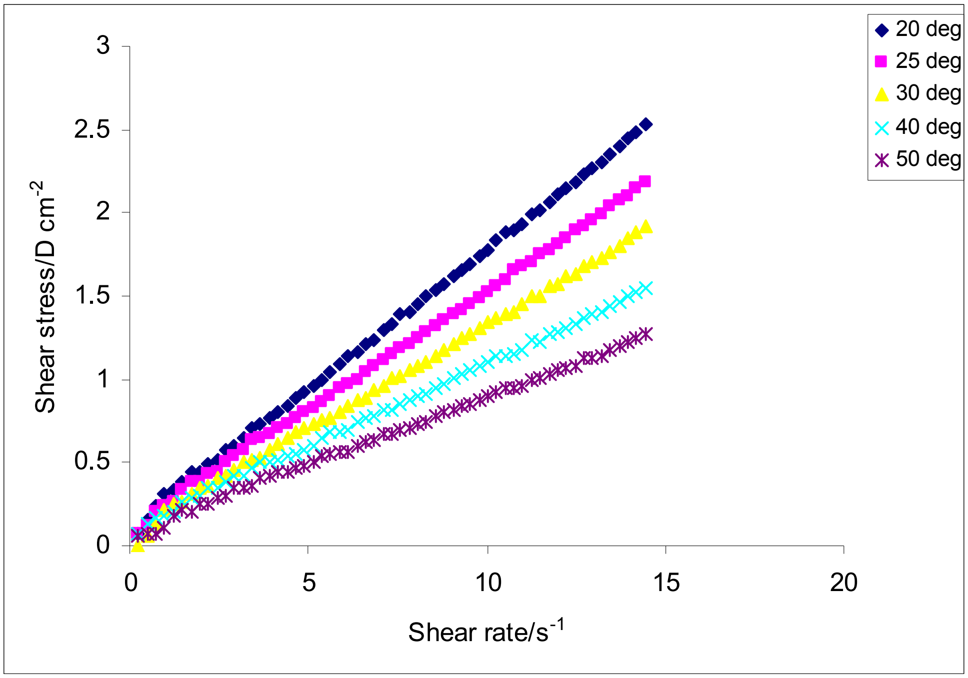


Figure 3.12: The shear stress of chitosan in formic acid solutions at different range of temperature versus the shear rate.

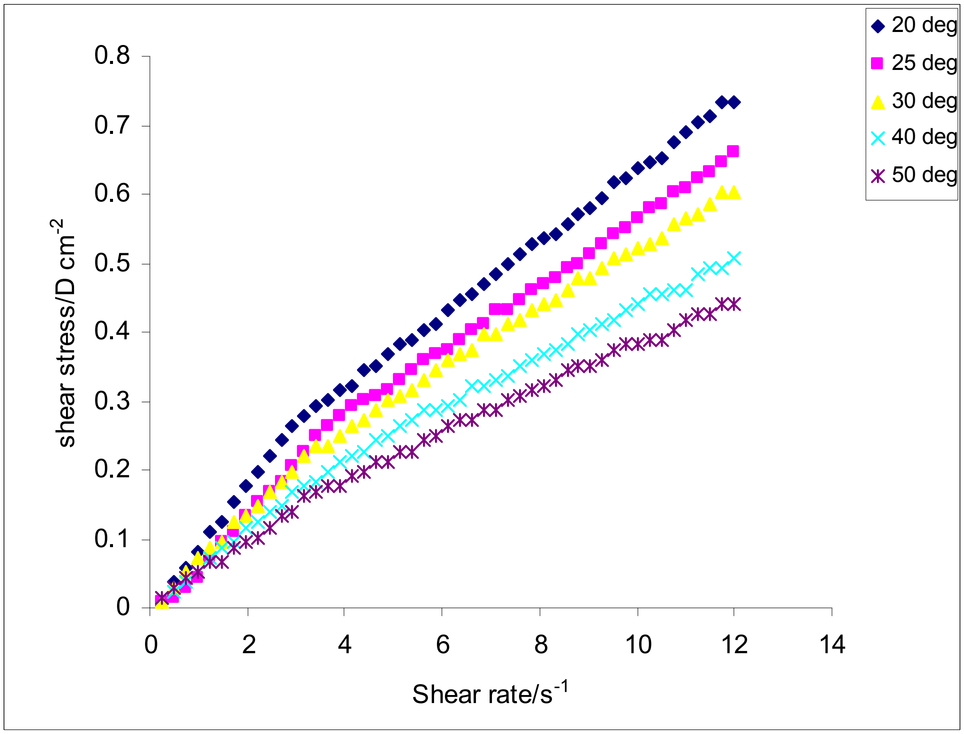


Figure 3.13: The shear stress of chitosan in acetic acid solutions at different range of temperature versus the shear rate.

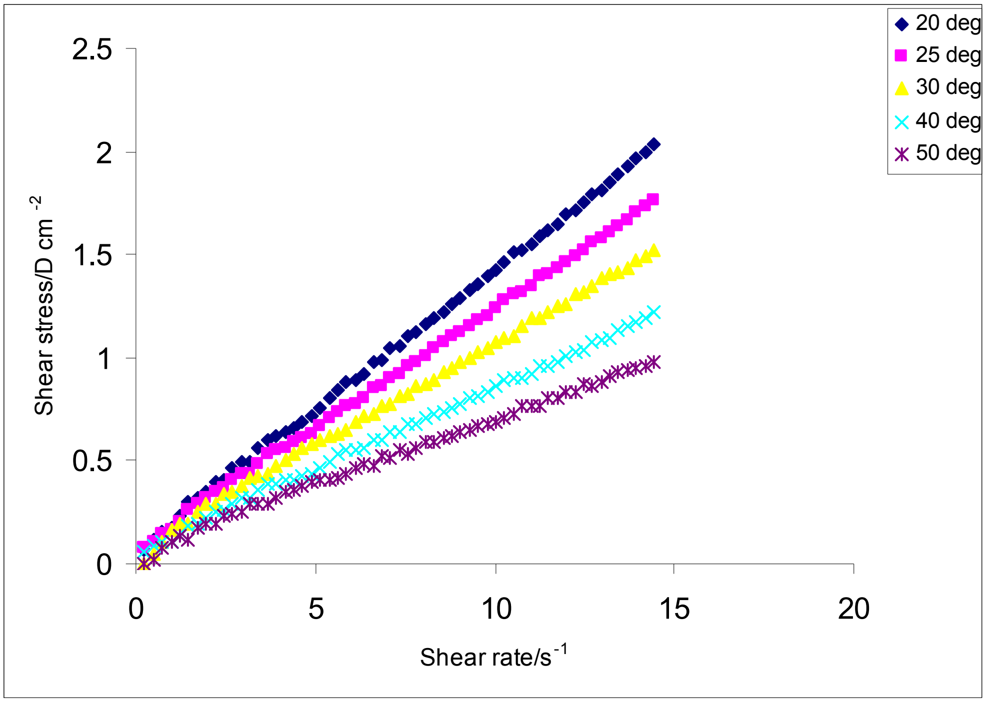


Figure 3.14: The shear stress of chitosan in propionic acid solutions at different range of temperature versus the shear rate.

From Figures 3.15, 3.16 and 3.17, it is apparent that these types of fluid display increasing shear rate with decreasing viscosity suggesting a pseudoplastic behavior. Generally, more shear thinning is observed with increasing chitosan concentration. Mucha (1997), Delben *et a*l. (1990) and Kjønisken *et al*. (1997) also reported a stronger shear thinning at higher concentration for different systems. This is probably attributed to the increasing in intermolecular entanglement as chitosan concentration increases, which in turn leads to more restriction in the freedom of movement of the individual chains (Graessley, 1974).

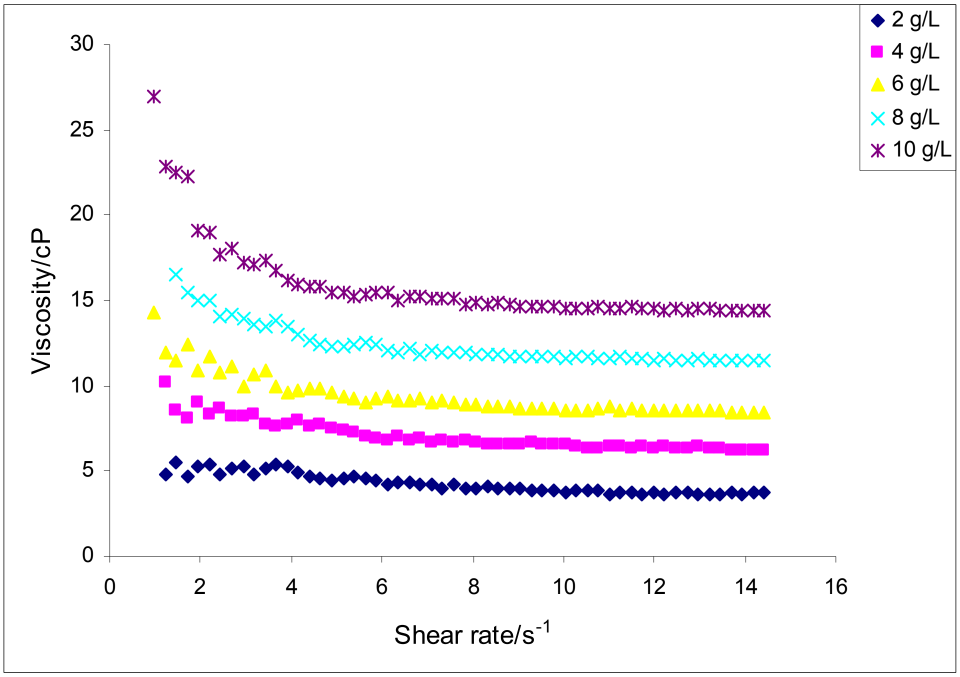


Figure 3.15: The influence of the shear rate and concentration on the rheological curves of chitosan in formic acid solutions at 25°C.

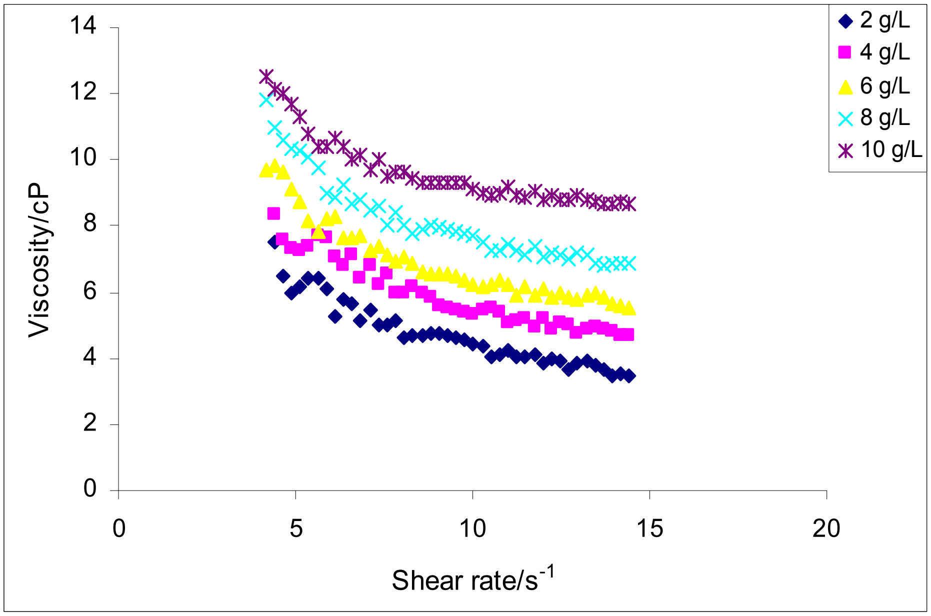


Figure 3.16: The influence of the shear rate and concentration on the rheological curves of chitosan in acetic acid solutions at 25°C.

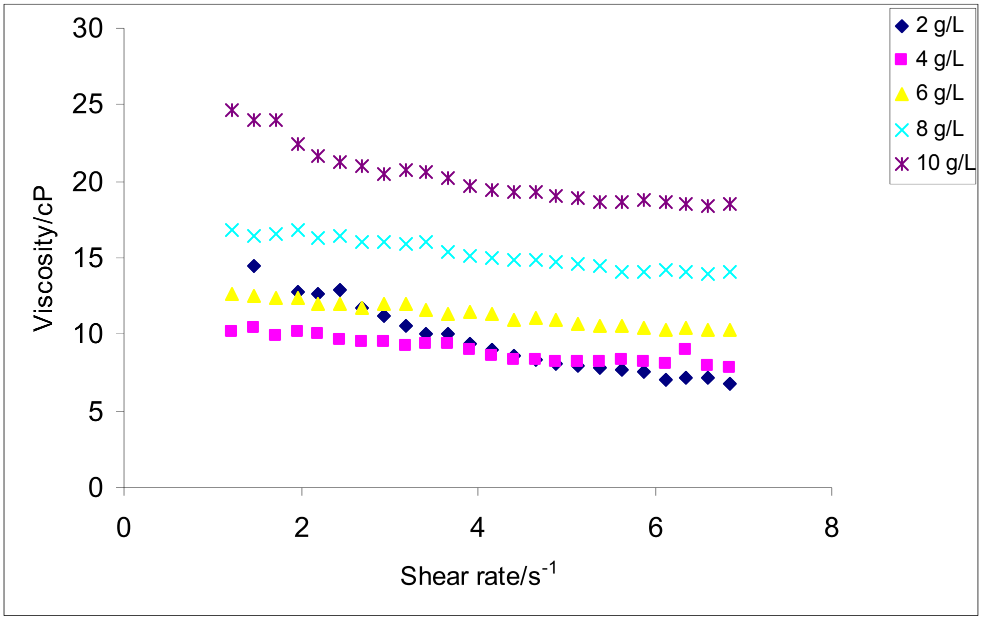


Figure 3.17: The influence of the shear rate and concentration on the rheological curves of chitosan in propionic acid solutions at 25°C.

3.3 The effects of shearing and storage time on the apparent viscosity of chitosan solutions.

Viscosity measurements were performed as a function of shear rate for 15, 30, 45, 60 and 75 seconds at 25°C to study the shearing time effect (Figures 3.18, 3.19 and 3.20). At all shearing times, all chitosan solutions exhibit similar behavior; the viscosity of chitosan solutions is shear rate dependent at low shear rates, but remains nearly constant at higher shear rates. Also, the shear stress as a function of shear rate curves show similar response for all shearing times evaluated in this study (Figures 3.21, 3.22 and 3.23). In addition, more shear thinning behavior and lower values of viscosity of chitosan solutions in formic acid were observed when the period of storage was extended to four months (Figure 3.24). However, the opposite behavior was observed for chitosan in acetic and propionic acids solutions (Figures 3.25 and 3.26). Since the viscosity of chitosan solutions in formic acid decreased with time while it is subjected to a constant shear rate, this may suggest a thixotropic behavior of chitosan in formic acid solutions (Table 3.1). However, a general increase in the viscosity of chitosan in acetic and propionic acids solutions with time was observed suggesting a rheopexy behavior (Tables 3.2 and 3.3). As for pH values, no significant changes were recorded as a function of time.

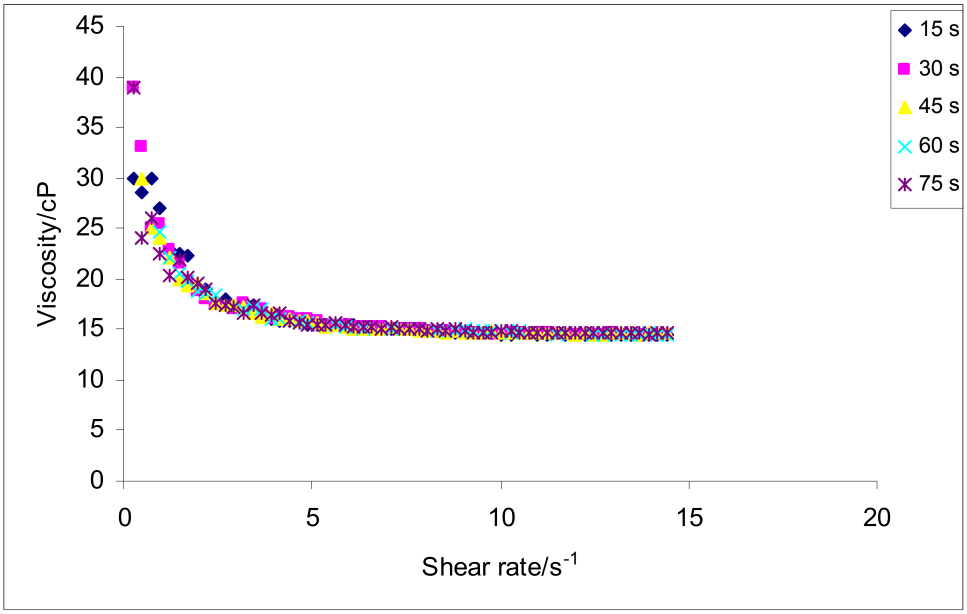


Figure 3.18: The influence of the shear rate and shearing time on the rheological curves of chitosan in formic acid solutions at 25^{o}C.

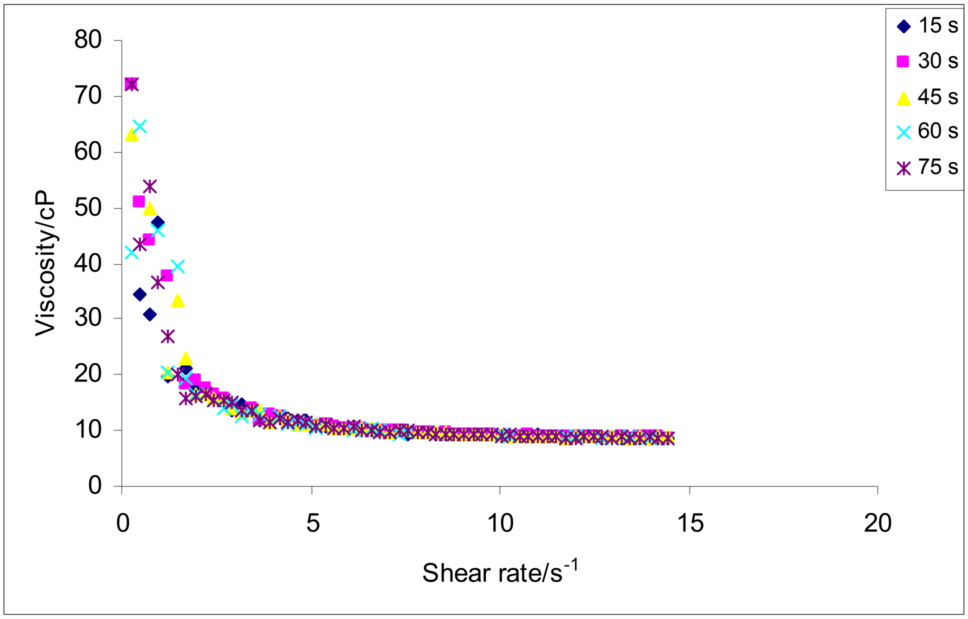


Figure 3.19: The influence of the shear rate and shearing time on the rheological curves of chitosan in acetic acid solutions at 25°C.

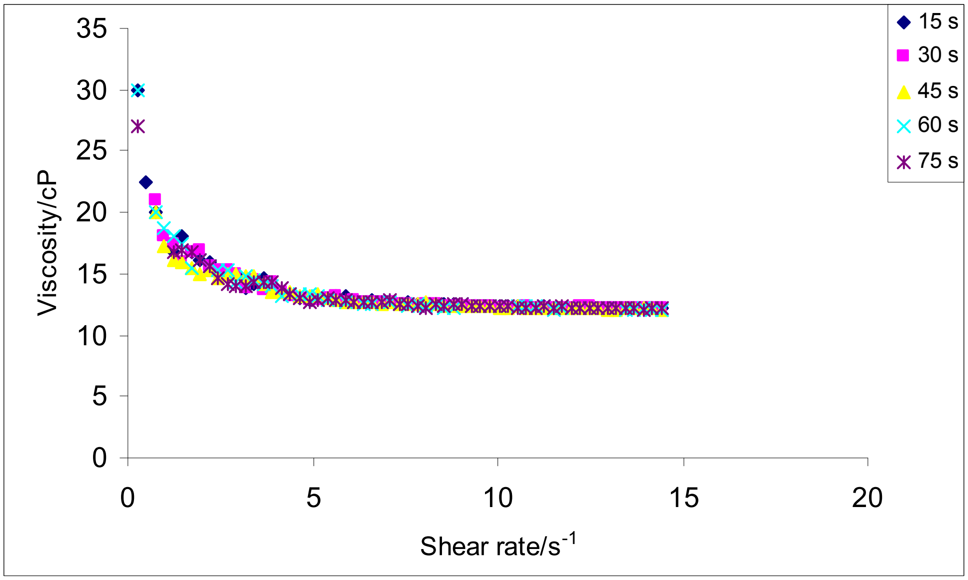


Figure 3.20: The influence of the shear rate and shearing time on the rheological curves of chitosan in propionic acid solutions at 25°C.

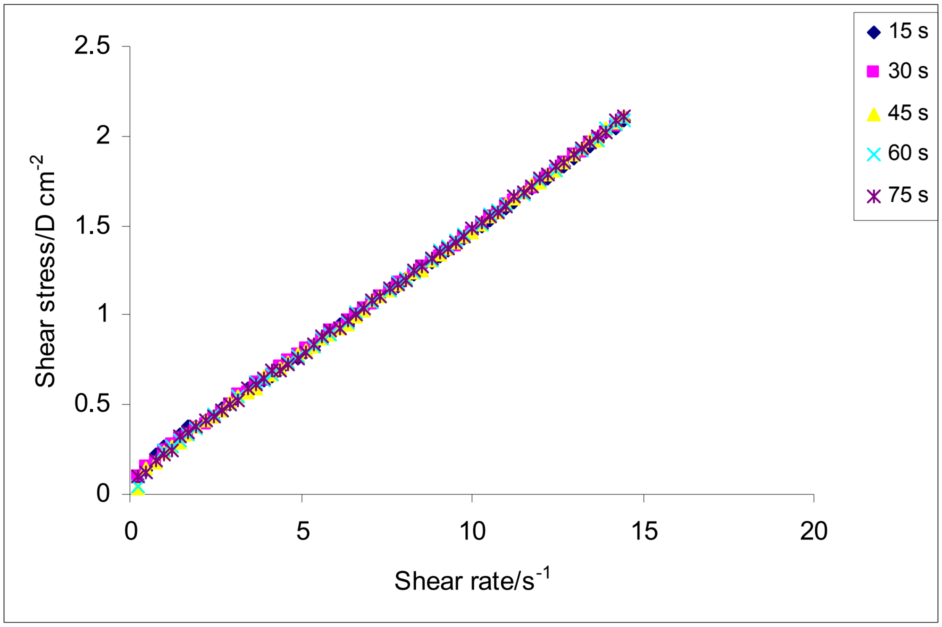


Figure 3.21: The influence of the shear rate and shearing time on the shear stress curves of chitosan in formic acid solutions at 25°C.

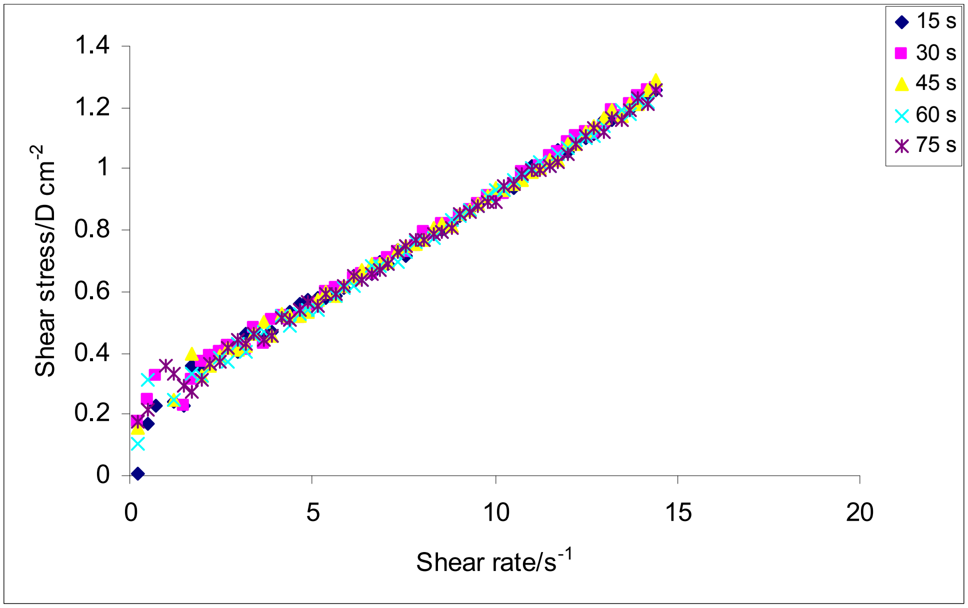


Figure 3.22: The influence of the shear rate and shearing time on the shear stress curves of chitosan in acetic acid solutions at 25°C.

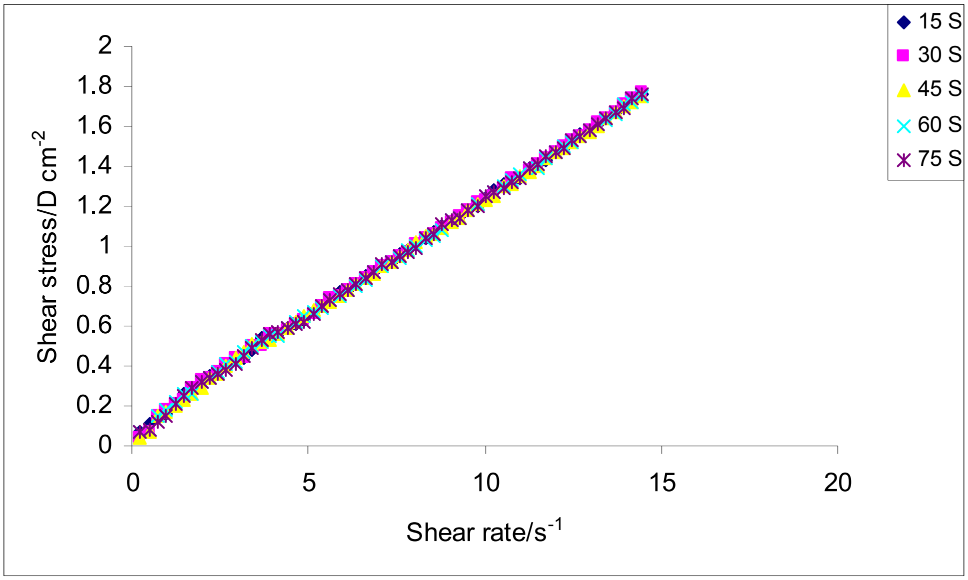


Figure 3.23: The influence of the shear rate and shearing time on the shear stress curves of chitosan in propionic acid solutions at 25^{o}C.

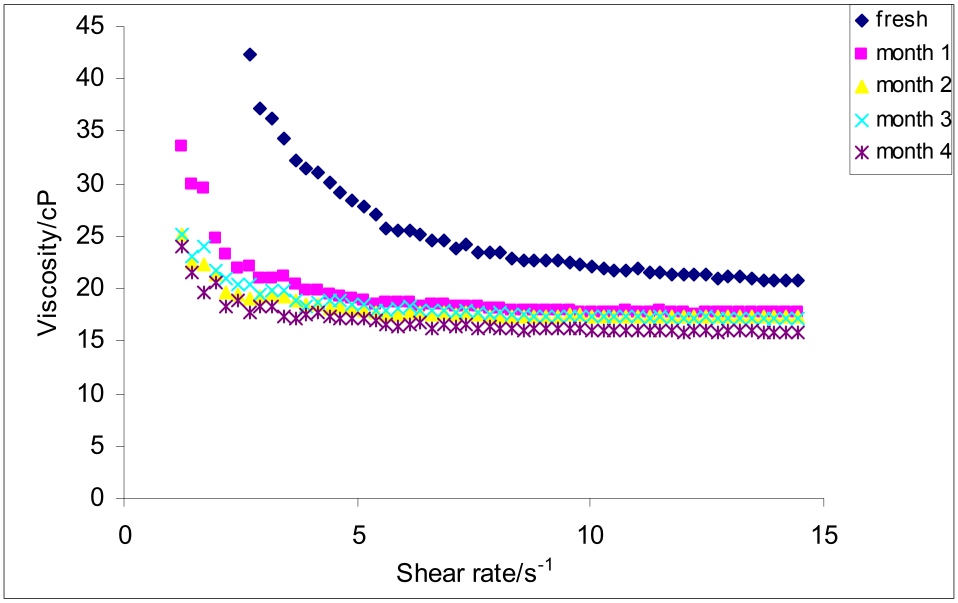


Figure 3.24: The influence of the shear rate and storage time on the rheological curves of chitosan in formic acid solutions at 25^{o}C.

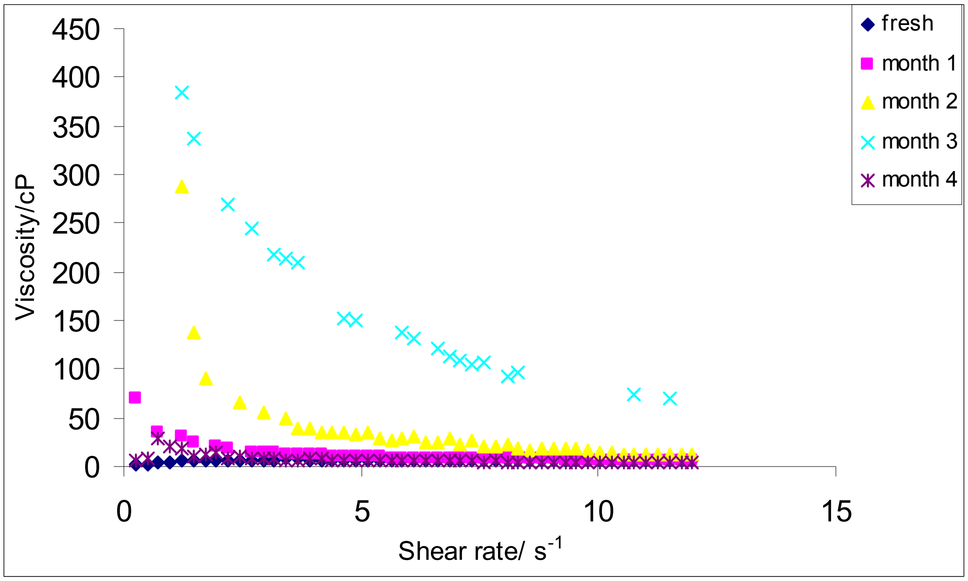


Figure 3.25: The influence of the shear rate and storage time on the rheological curves of chitosan in acetic acid solutions at 25°C.

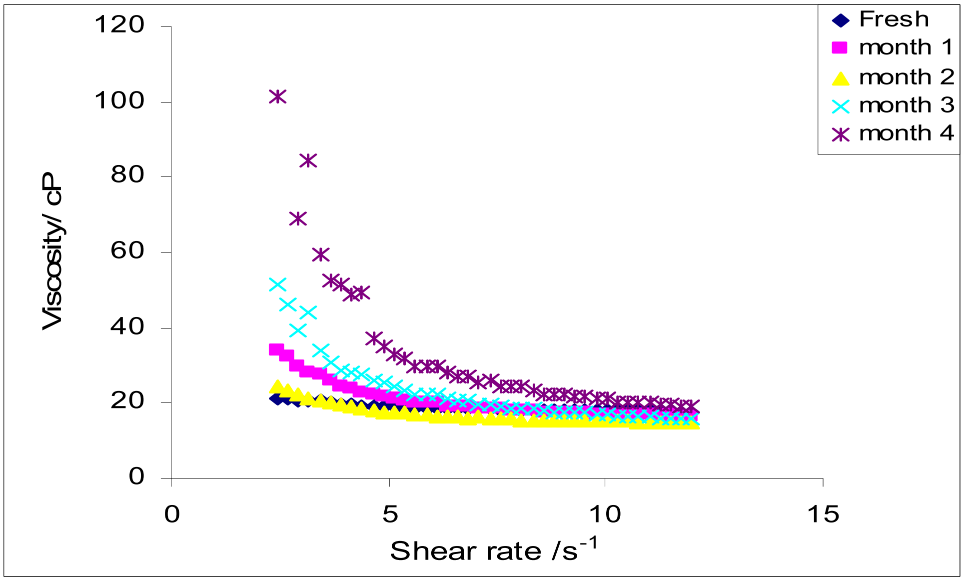


Figure 3.26: The influence of the shear rate and storage time on the rheological curves of chitosan in propionic acid solutions at 25°C.

Table 3.1: The effect of storage time on dynamic viscosity, shear stress (determined at a constant shear rate) and pH of chitosan in formic acid solutions (mean ± SD).

Time (month)	Viscosity (cP)	Shear stress (D/cm^2)	pH
0	36.36± 0.10	1.34± 0.03	3.16± 0.01
1	21.36± 0.41	0.78± 0.01	3.21± 0.01
2	19.84± 0.17	0.73± 0.00	3.26± 0.01
3	18.92± 0.23	0.69± 0.01	3.22± 0.00
4	17.56± 0.17	0.65± 0.00	3.24± 0.00

Table 3.2: The effect of storage time on dynamic viscosity, shear stress (determined at a constant shear rate) and pH of chitosan in acetic acid solutions (mean ± SD).

Time (month)	Viscosity (cP)	Shear stress (D/cm^2)	pH
0	7.60 ± 0.00	0.28 ± 0.00	3.99 ± 0.02
1	11.20 ± 0.14	0.41 ± 0.01	4.12 ± 0.00
2	41.11 ± 1.42	1.51 ± 0.05	4.33 ± 0.00
3	140.45 ± 9.23	5.51 ± 0.34	4.38 ± 0.00
4	7.68 ± 0.36	0.28 ± 0.01	4.32 ± 0.00

Table 3.3: The effect of storage time on dynamic viscosity, shear stress (determined at a constant shear rate) and pH of chitosan in propionic acid solutions (mean ± SD).

Time (month)	Viscosity (cP)	Shear stress (D/cm^2)	pH
0	20.16 ± 0.10	0.74 ± 0.00	3.97 ± 0.01
1	25.95 ± 0.26	0.95 ± 0.01	4.02 ± 0.01
2	19.56 ± 0.66	0.71 ± 0.02	4.09 ± 0.01
3	30.35 ± 0.23	1.12 ± 0.01	4.31 ± 0.01
4	54.27 ± 3.55	1.99 ± 0.13	4.23 ± 0.00

Chapter 4: Results and discussion

Thermal and morphological properties of chitosan films

4.1 Thermogravimetric analysis (TGA)

Figures 4.1-4.3 show the results of thermogravimetric analysis of chitosan films made with the three solvents. The chitosan film made with formic acid showed two stages of thermal degradation. The first stage is a weight loss of about 8% with a maximum rate occurring at about 75°C, which is due to the evaporation of water present in the sample. The second thermal event starts at about 220°C with the amount of loss at about 54% and is attributed to the depolymerisation of chitosan chains.

There are two weight losses observed in the TGA curve of chitosan film prepared from acetic acid solution. The first stage ranges between 30ºC and 145ºC and shows about 5% loss in weight. This is thought to be due to moisture vaporization. The second weight loss begins at about 150°C and the corresponding weight loss is about 53% which is attributed to the decomposition of chitosan.

The thermal degradation curve of chitosan film with propionic acid solvent showed two stages of weight loss. The first weight loss occurs at 30°C with an amount of loss of 3%. This weight loss is due to the removal of water. The second stage is at about 130°C showed a weight loss of about 57%, which is due to the polymer decomposition.

The total weight loss of all samples at about 800°C are around 62%, 58% and 60% for chitosan films with formic, acetic and propionic acid solvents respectively as shown in

Table 4.1. These results indicate that the chitosan film with acetic acid solvent has more thermal stability at a higher temperature than the other films.

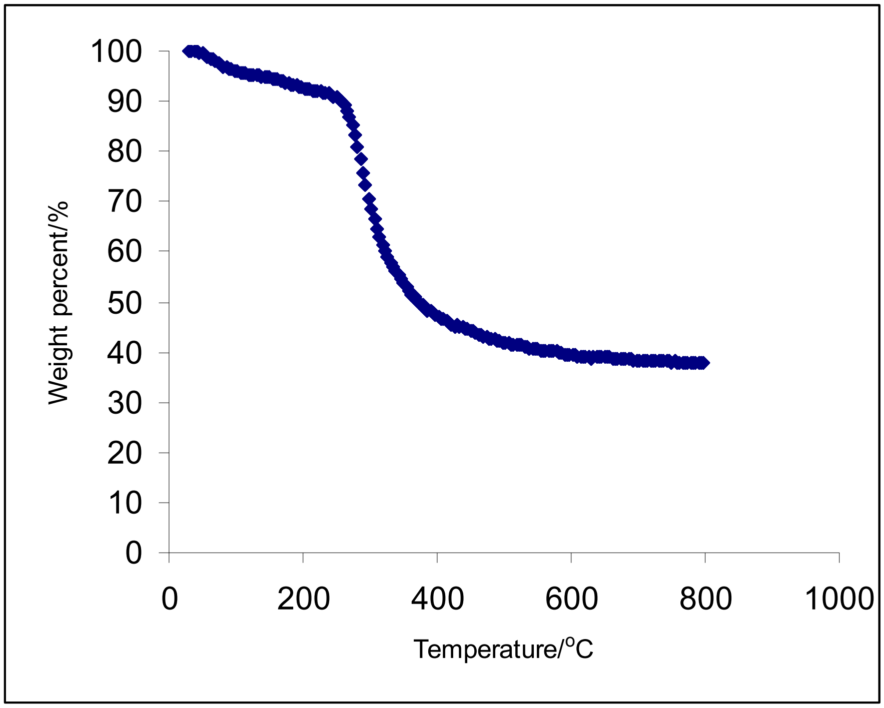


Figure 4.1: The TGA thermogram for chitosan film with formic acid.

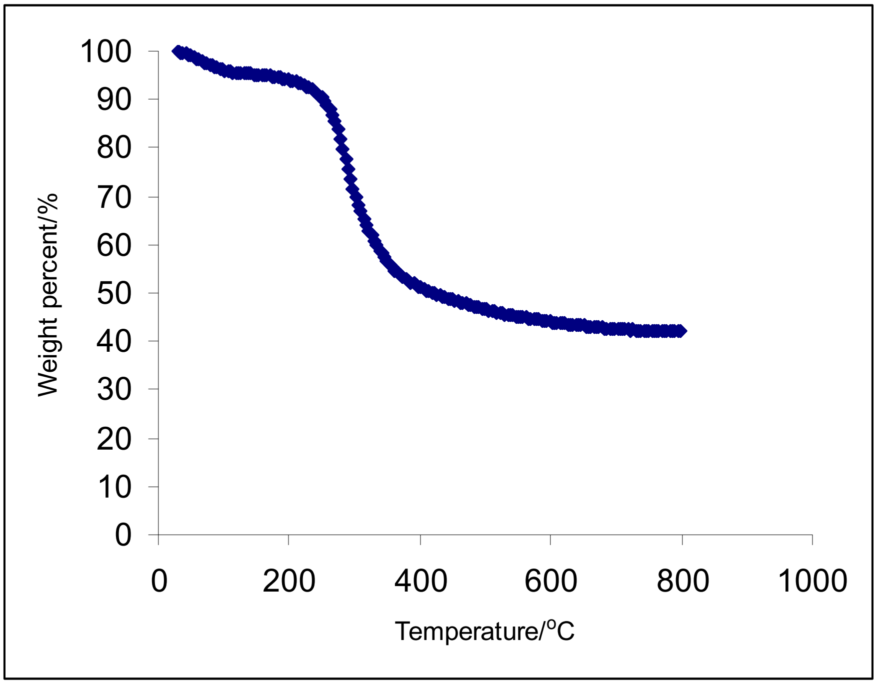


Figure 4.2: The TGA thermogram for chitosan film with acetic acid.

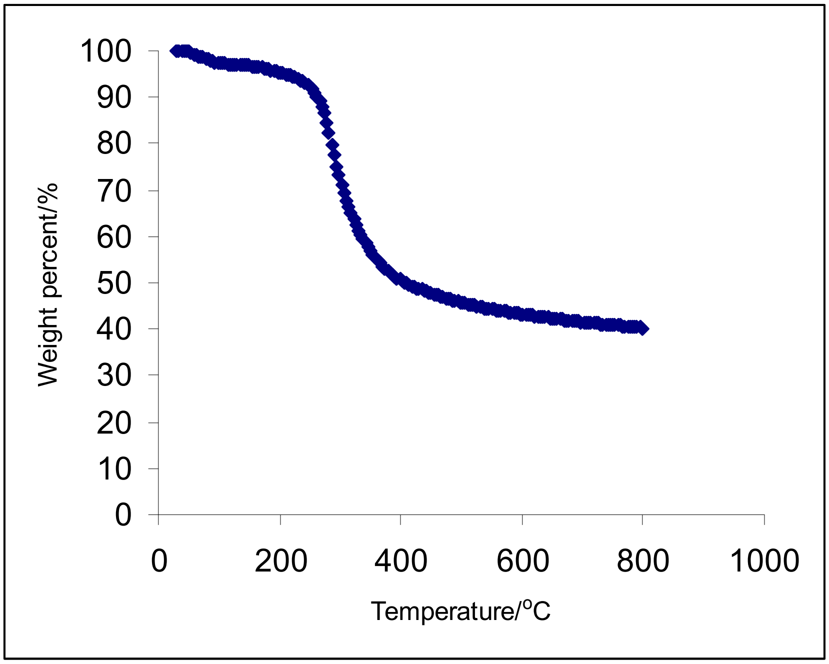


Figure 4.3: The TGA thermogram for chitosan film with propionic acid.

Table 4.1: The weight loss stages of chitosan films with the three selected solvents.

Stage No.(weight loss %)	**CS film with FA solvent**	**CS film with AC solvent**	**CS film with PA solvent**
Stage I	7.94	4.78	3.01
Stage II	54.35	53.23	56.74
Total weight loss (%)	62.29	58.01	59.75

4.2 Differential scanning calorimetry (DSC)

Figure 4.4 shows the DSC thermograms of chitosan films. After drying, all samples were kept in a desiccator before analysis. All film membranes exhibited a broad endothermic peak centered at around 80°C, 79°C and 75°C for chitosan films with formic, acetic and propionic acids respectively. A closer examination of Figure 4.4 reveals that there are differences in the endothermic peaks area for the three systems, i.e., there are differences in their water-holding capacity, showing that chitosan film with acetic acid solvent has the lowest denaturation energy. The endothermic peaks are attributed to the loss of water associated with the hydrophilic groups of polymers (Cheung *et al*, 2002, Gonzalez *et al*, 2000). This suggests that the samples were not completely anhydrous and that some bound water was still not removed when dried in the desiccator, which was confirmed by results obtained from TG analysis. The exothermic peaks, which appear in the temperature range between about 250°C up to 350°C correspond to the degradation of the chitosan chains. Sakurai *et al*. (2000) and Zeng *et al*. (2004) also reported that the thermal degradation of chitosan begins at about 250°C.

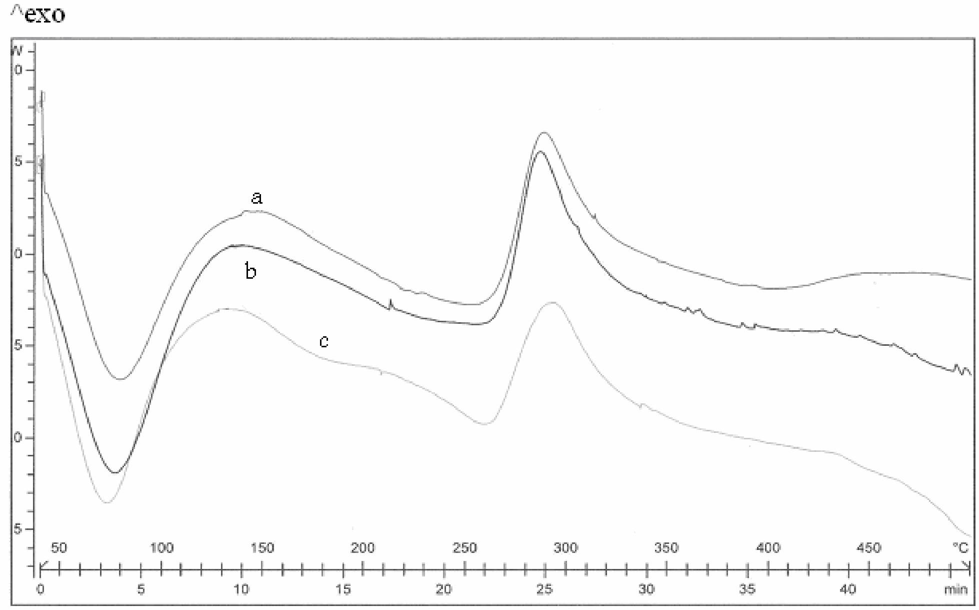


Figure 4.4: DSC first run curves of the chitosan films with (a) AC, (b) PA and (c) FA solvents.

Since chitosan is apt to absorb moisture, a second heating run of the DSC, after heating up to 200°C and cooling to 40°C, was employed to eliminate the effect of moisture (Figures 4.5-4.7). The main feature in the three curves is that there is a small endothermic peak at 186.20°C, 179.41°C and 171.43°C for chitosan films with formic, acetic and propionic acid solvents respectively. This shows that the endothermic peak position shifts toward lower temperature as the number of carbons in the molecule of the solubilizing agent increased according to the following sequence: formic acid >acetic acid >propionic acid as shown in Figure 4.8. Zeng *et al*. (2004) and Chuang *et al*. (1999) also reported an endothermic peak of chitosan (using acetic acid as a solvent) at around 180°C, which is very close to our value (179.41°C). This peak may be due to the dissociation process of interchain hydrogen bonding of chitosan (Chuang *et al*, 1999) which strongly formed among the $–NH_2$ and $–OH$ functional groups.

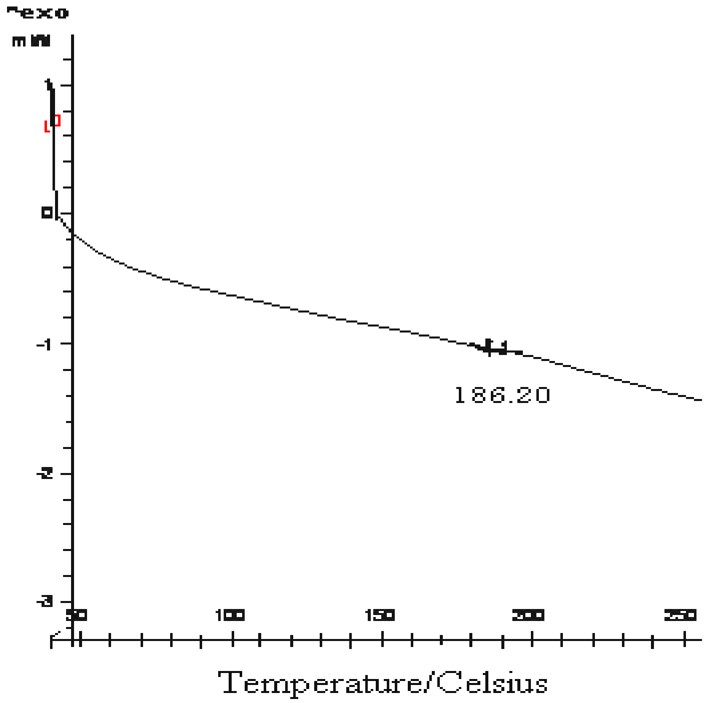


Figure 4.5: The DSC second run curve of chitosan film with formic acid solvent.

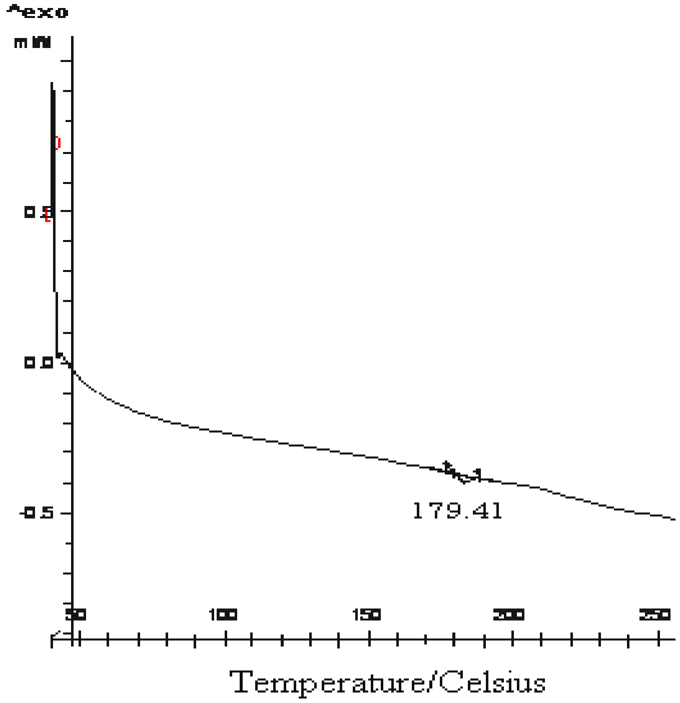


Figure 4.6: The DSC second run curve of chitosan film with acetic acid solvent.

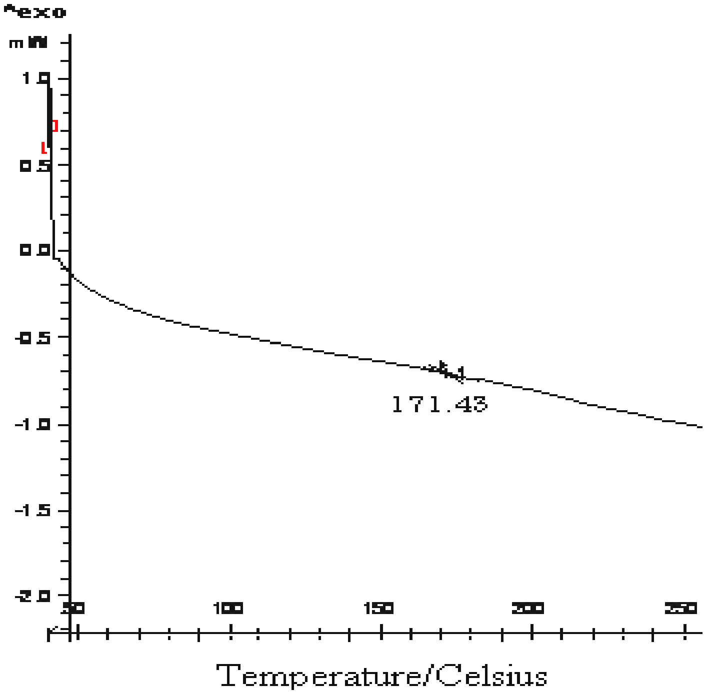


Figure 4.7: The DSC second run curve of chitosan film with propionic acid solvent.

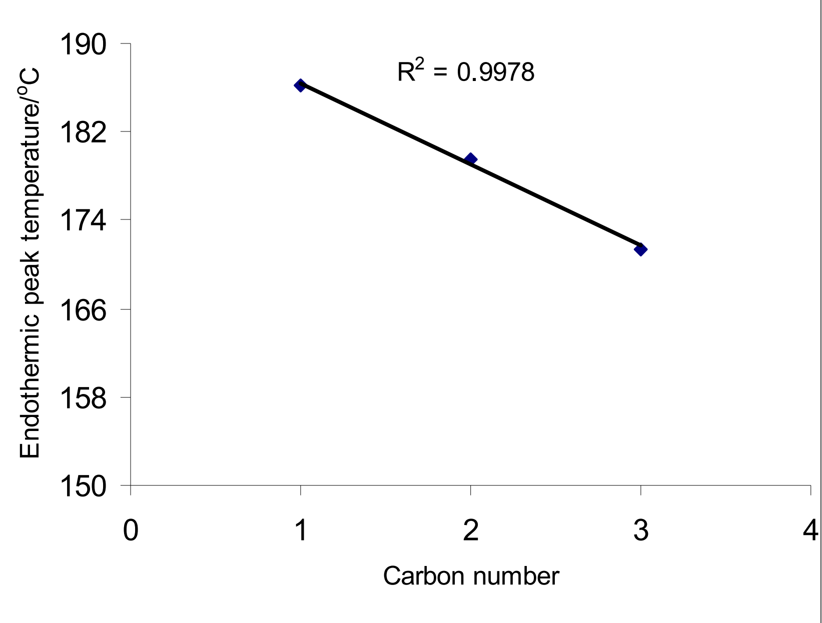


Figure 4.8: The effect of carbon number in the three solvents on the endothermic peak position after the second run of DSC.

4.3 Scanning electron microscopy

The scanning electron microscopy was used to quantify the surface smoothness and homogeneity of the films. The SEM images of chitosan films are shown in Figure 4.9. It is obvious that all films displayed clear, smooth and homogeneous surfaces and seemed not to be influenced by the type of solvent used.

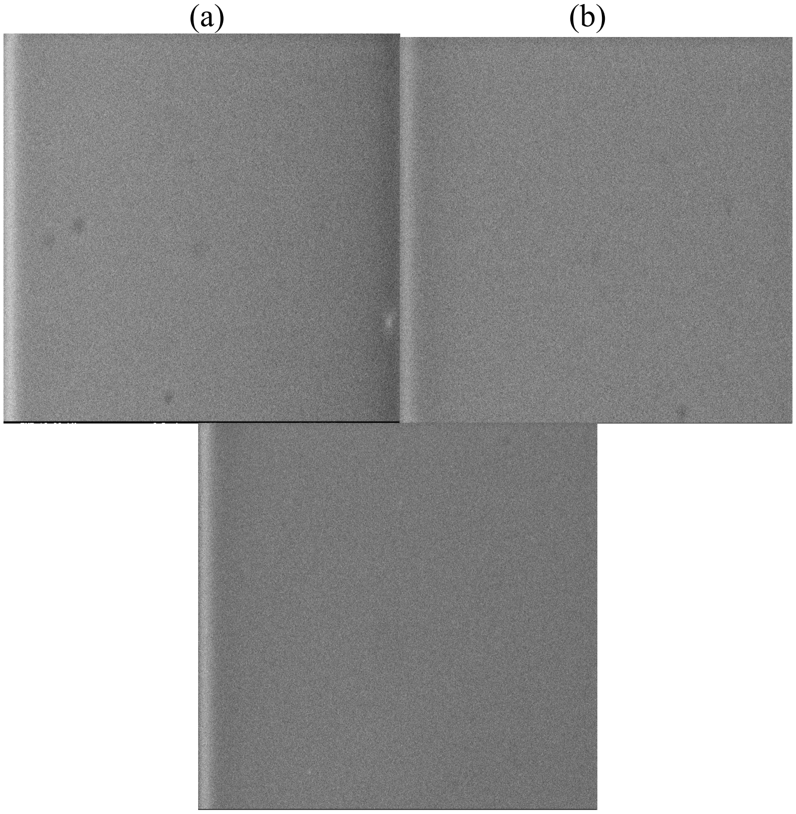


Figure 4.9: The scanning electron micrographs of the chitosan films with (a) formic acid, (b) acetic acid and (c) propionic acid solvents ((magnification 150X).

4.4 Atomic force microscopy

In order to further study the surface of chitosan films cast from the three solvents, atomic force microscopy was employed to evaluate the morphology of the films. As it can be seen in Figures 4.10-4.12, all films give a relatively smooth and homogeneous surface without any pores or cracks. However, among the three types of films, chitosan film cast from acetic acid gives the smoothest surface. The values of surface roughness (Ra) are shown in

Table 4.2. These observations show agreement with previous studies (Nosal *et al*, 2005, Lauto *et al*, 2007, Takahashi *et al*, 2009).

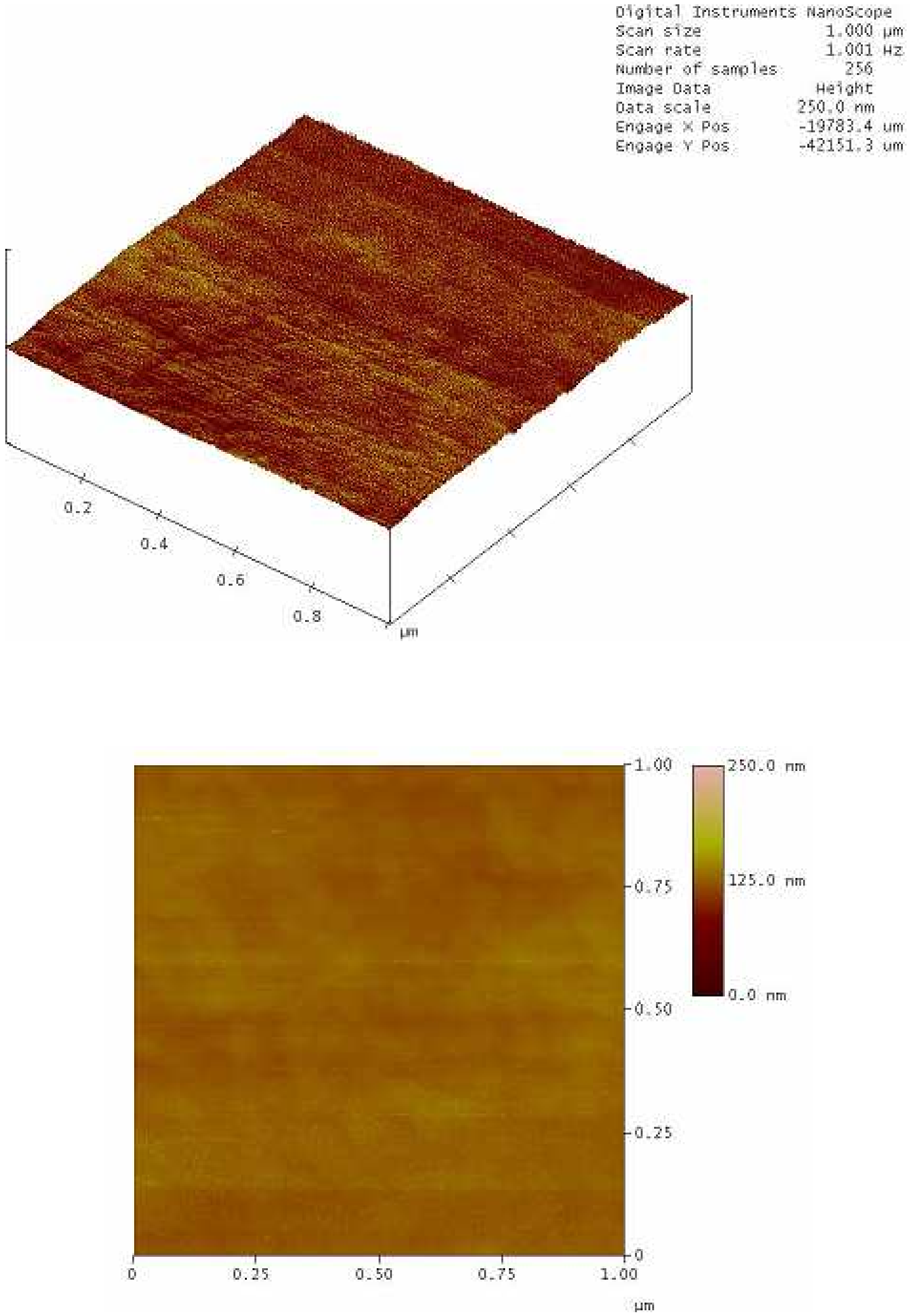


Figure 4.10: AFM image (1 μm x 1 μm) of chitosan film cast from formic acid.

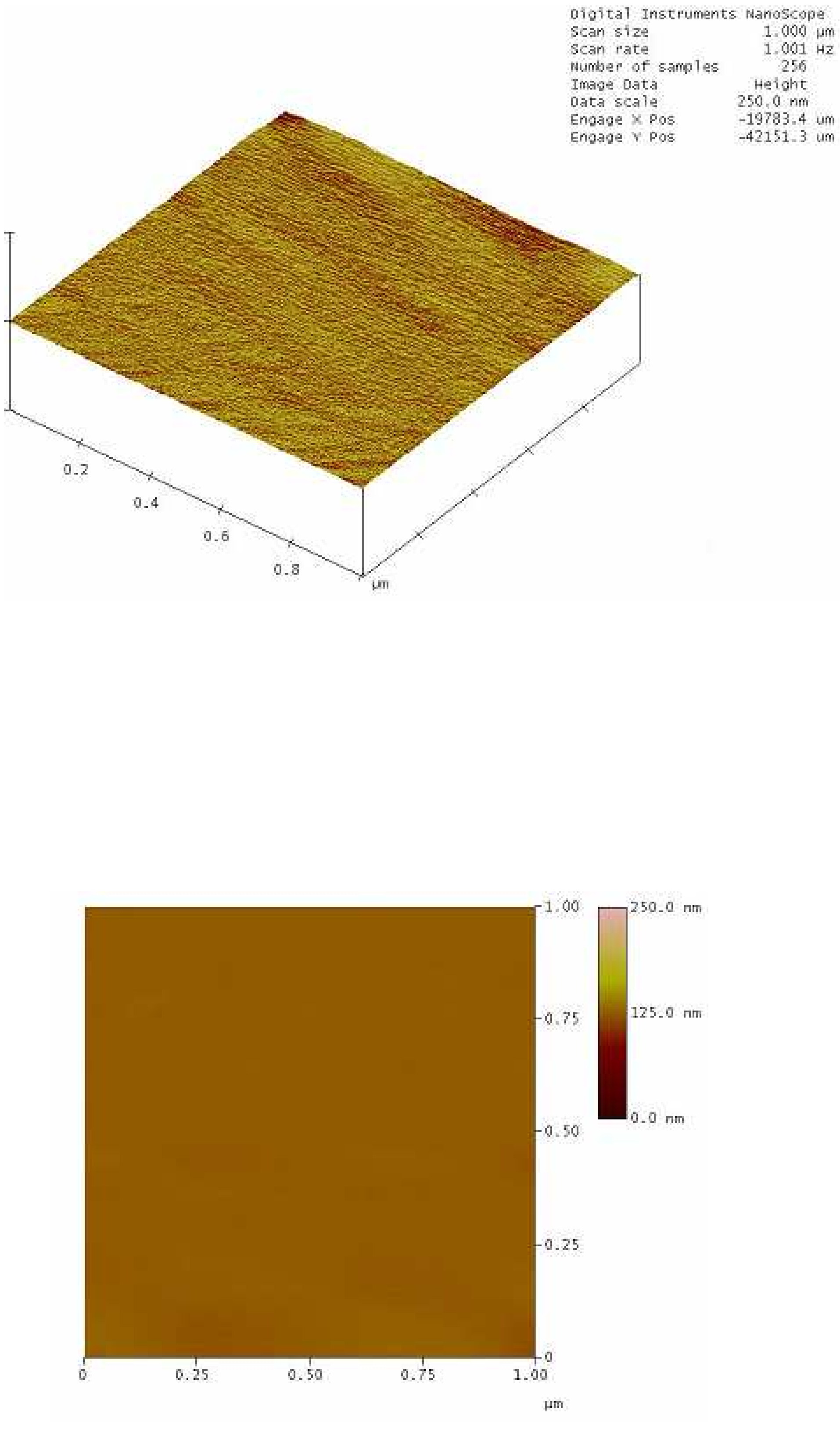
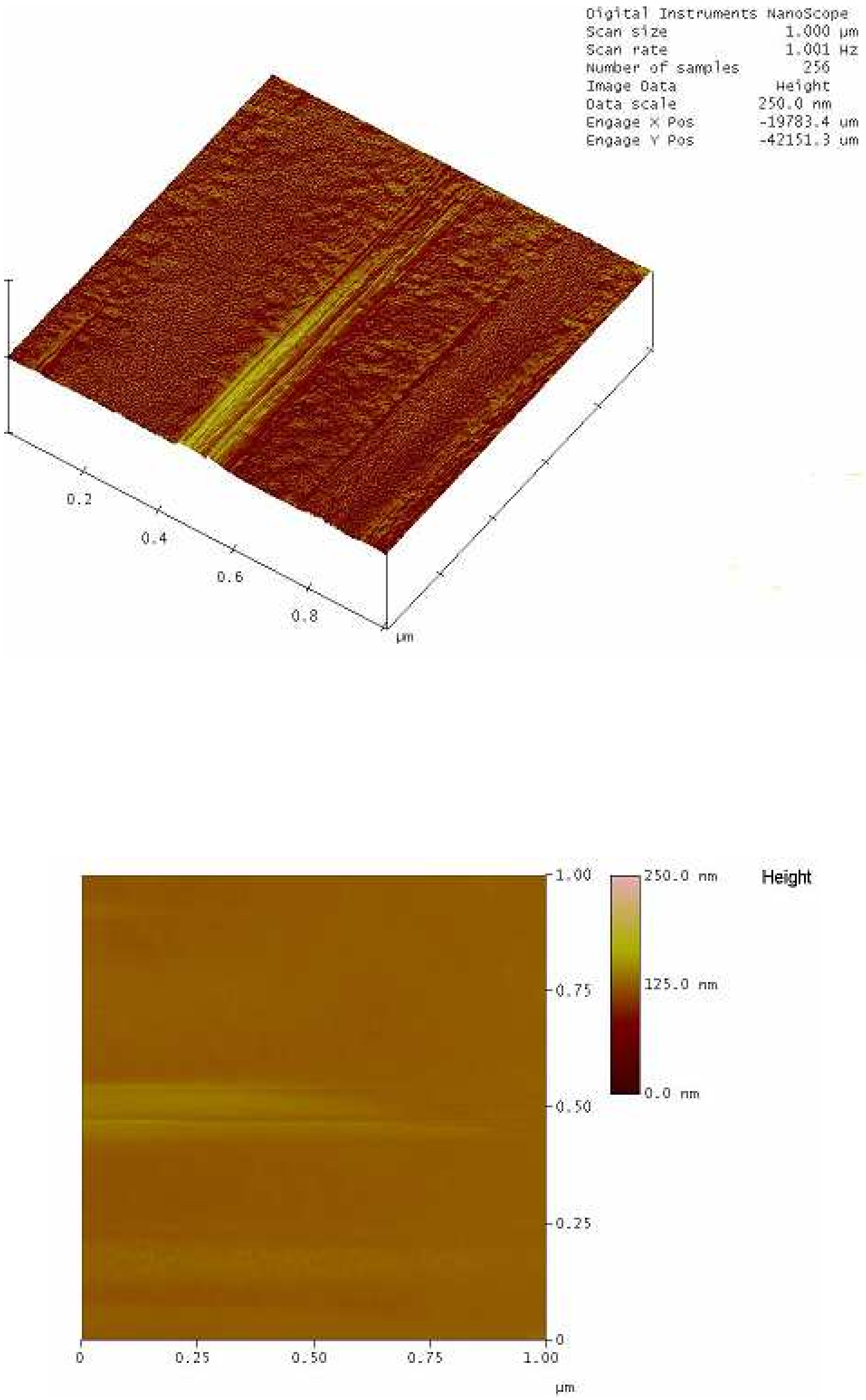


Figure 4.11: AFM image (1 μ m x 1 μ m) of chitosan film cast from acetic acid.



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Digital Instruments NanoScope
Scan size             1.000 µm
Scan rate             1.001 Hz
Number of samples       256
Image Data            Height
Data scale          250.0 nm
Engage X Pos       -19783.4 um
Engage Y Pos       -42151.3 um
```

Figure 4.12: AFM image (1 μ m x 1 μ m) of chitosan film cast from propionic acid.

Table 4.2: The surface roughness (Ra) of the prepared samples

Sample	Surface roughness Ra (nm)
CS in FA	3.894
CS in AC	0.755
CS in PA	2.041

4.5 Infrared spectroscopy

Figures 4.13-4.15 show the FTIR spectra of chitosan film cast from different solvents. All types of films showed similar spectra, in that the characteristic bands of chitosan films have almost similar wave numbers as shown in Table 4.2. However, some peaks seem to be slightly shifted to lower or higher wave numbers with increase the number of carbon in the solvent such as the peaks assigned to C=O stretching (amide I). The broad band at around 3367 cm^{-1} is due to the OH stretching. The band at 1560 cm^{-1} is assigned for the NH bending (amide II) (NH_2) while the small peak at 1647 cm^{-1} is attributed to the C=O stretching (amide I) O=C-NHR. These two bands could be due to the incomplete deacetylation. The characteristic absorption bands at 2927, 2884, 1411, 1321 and 1260 cm^{-1} are assigned to CH_2 bending due to pyranose ring (Pawlak & Mucha, 2003). The band at 1380 cm^{-1} is due to CH_3 wagging. The characteristic features of chitosan spectra in this study are similar to previous reports (Nunthanid *et al*, 2001, Ritthidej *et al*, 2002, Xu *et al*, 2005).

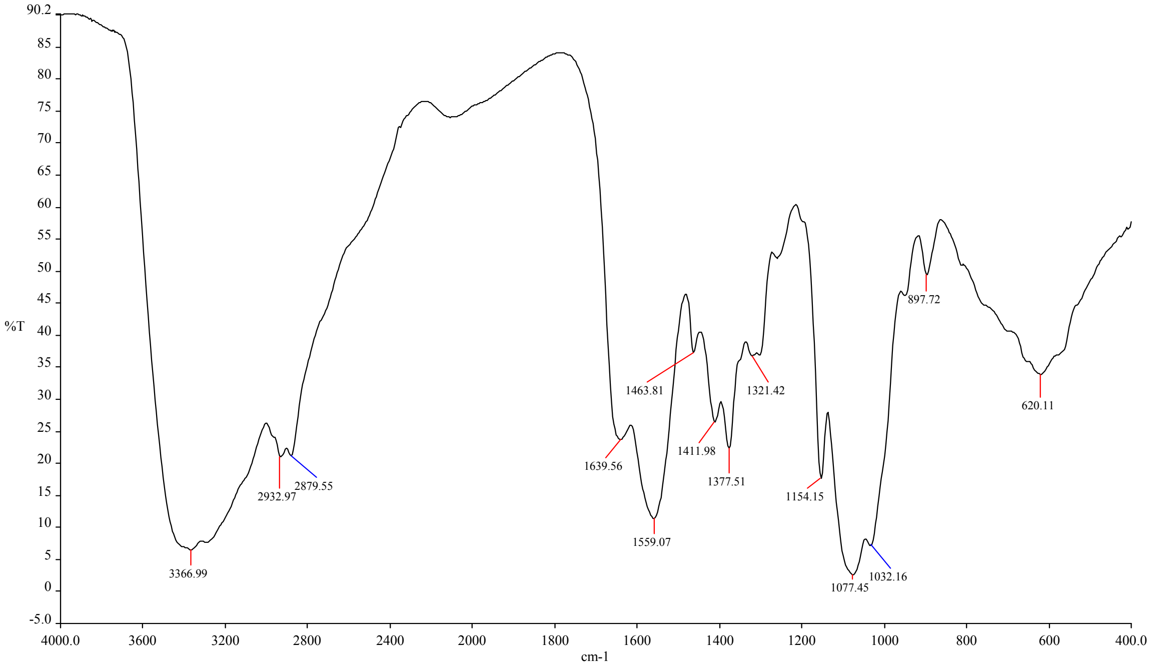

Figure 4.13: FTIR spectrum of chitosan cast from formic acid.

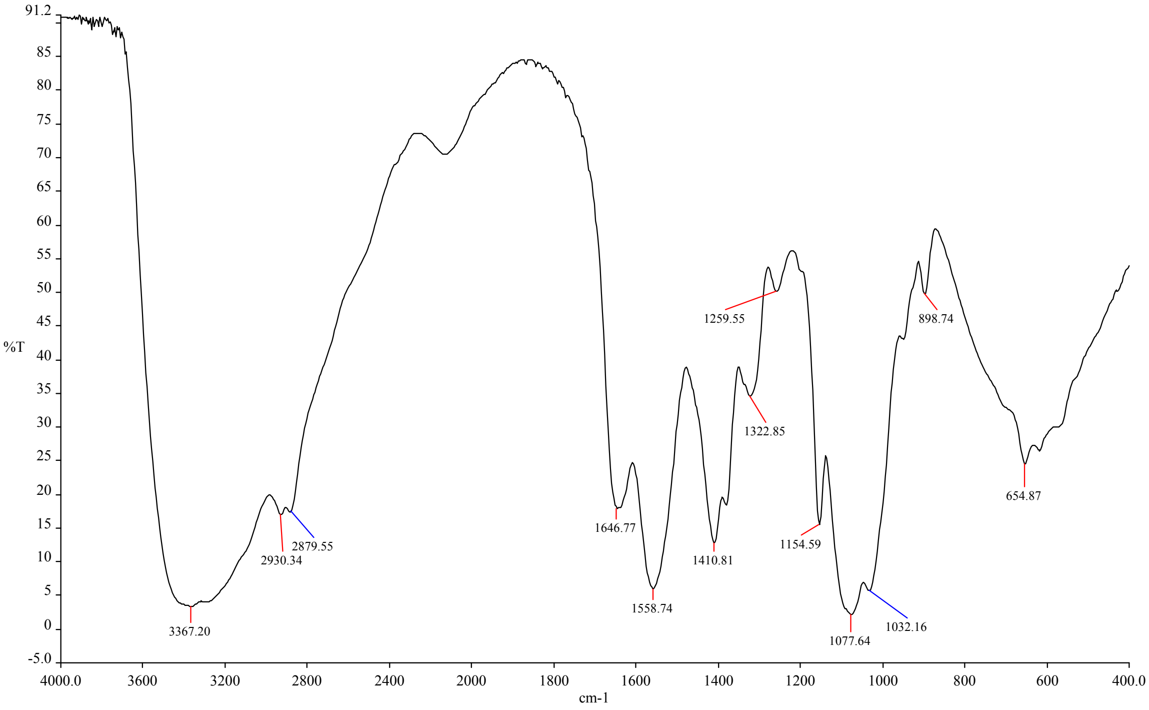

Figure 4.14: FTIR spectrum of chitosan cast from acetic acid.

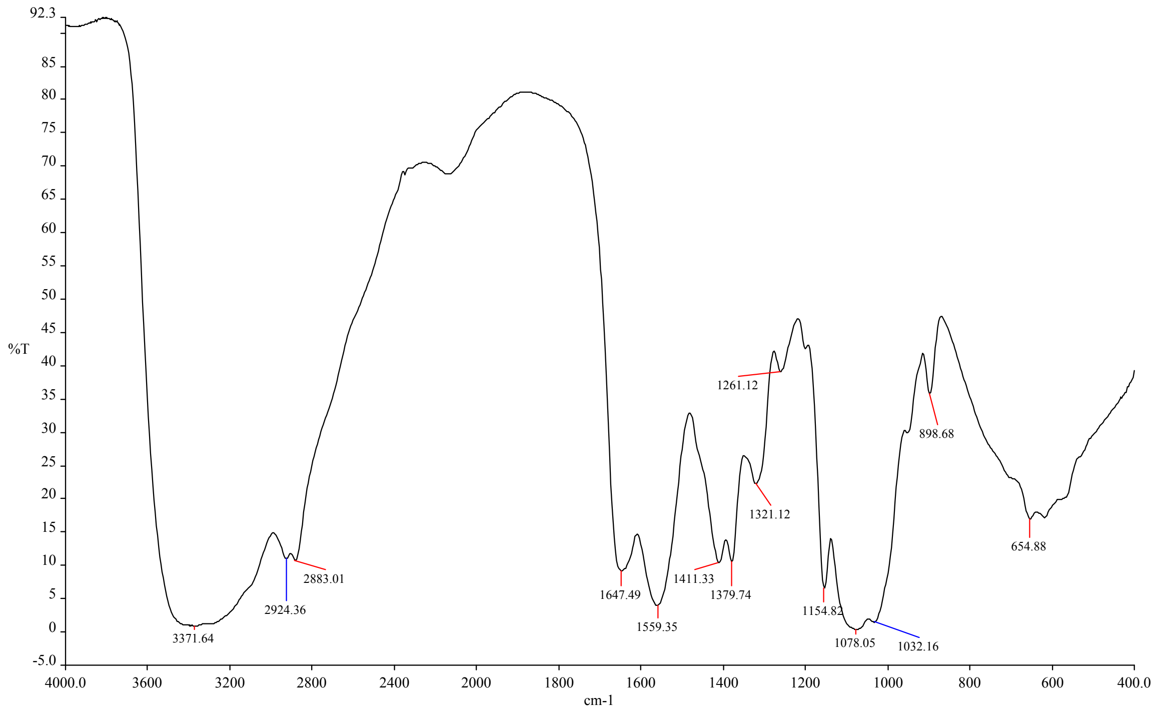

Figure 4.15: FTIR spectrum of chitosan cast from propionic acid.

Table 4.3: A summary of FTIR bands of chitosan films cast from different solvents.

	OH	C=O (amide I)	NH (amide II)	CH (amide II)	C-C	C-O
CS in FA	3367	1640	1559	1412	1378	1035
CS in AC	3367	1647	1559	1411	1380	1032
CS in PA	3363	1647	1559	1412	1380	1032

4.6 Tensile strength and percent elongation at break

The tensile strength and the percent elongation at break of chitosan films cast from the three solvents are shown in figure 4.16. It was found that the chitosan films cast from formic and propionic acids recorded higher tensile strength but lower elongation at break than the film cast from acetic acid. Kim *et al*. (2006) reported the tensile strength and elongation of chitosan film prepared from dissolving chitosan in a number of solvents, *i.e.*, formic, acetic, propionic and lactic acids. They found that chitosan films cast from acetic and propionic acid solvents had the highest TS, while films cast from lactic acid solvent had the highest elongation %. It was also demonstrated that the tensile strengths at break of chitosan films decreased and percent elongations increased for chitosan films cast from lactic acid instead of films cast from acetic acid (Niamsa and Baimark, 2009).

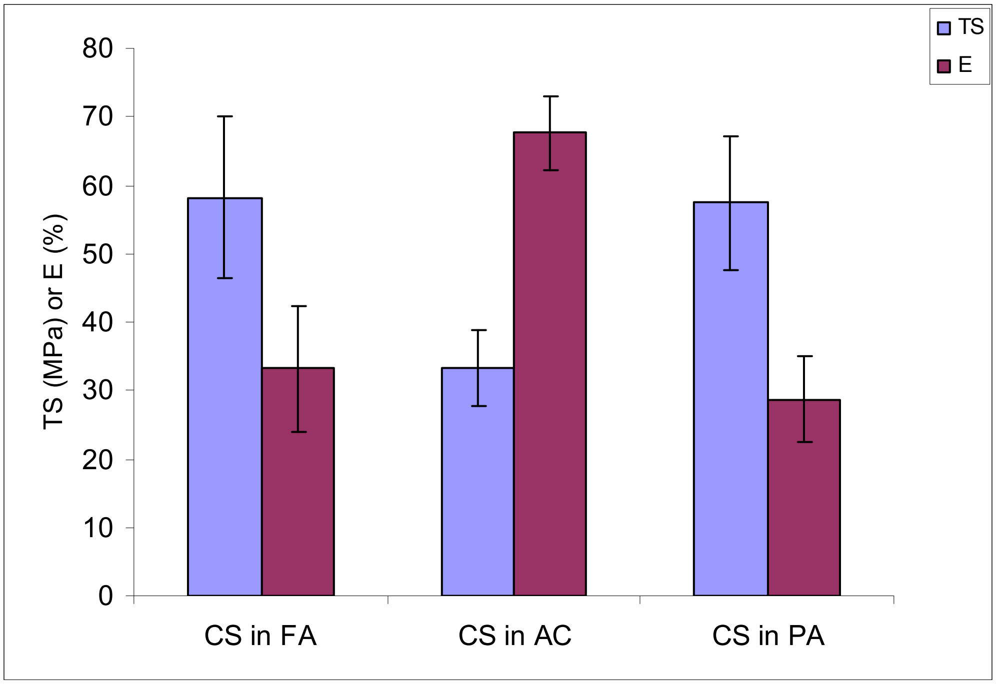

Figure 4.16: Tensile strength and percent elongation chitosan film cast from different solvents.

Chapter 5: Conclusions

This study has shown that the pH of chitosan in formic, acetic and propionic acids solutions are significantly affected by factors, such as temperature and concentration, whereas a decrease in pH with increasing temperature and an increase with increasing concentration were observed in all solutions. This is due to the increased availability of polymer units with increasing concentration which allows more protonation to occur, hence increase pH.

This study has also shown that the dynamic rheological measurements of chitosan solutions in the three selected solvents suggested a pseudoplastic non-Newtonian behavior. The shear thinning behavior was remarkable at temperatures between 20^{o}C and 50^{o}C. In addition, all chitosan solutions were found to exhibit less shear thinning and an increase in viscosity with increasing concentration. The E_a was found to be almost independent of the number of carbons in the acid. Furthermore, more shear thinning behavior for chitosan in formic acid solutions, but less shear thinning for chitosan in acetic and propionic acids solutions were observed with extending the period of storage to four months. A general decrease in viscosity with time for chitosan in formic acid solutions was observed while an increase in viscosity as a function of time were recorded for chitosan in acetic and propionic acids solutions at a constant shear rate, which may suggest a thixotropic and rheopexy behaviors respectively. The viscosity decrease observed over time might be due to partial degradation of chitosan by the acid solutions as well as due to the promotion of acidic hydrolysis.

The TGA curves of chitosan films showed that the chitosan film with acetic acid solvent had the best thermostability among the three types of film studied. As far as the DSC measurements are concerned, all samples showed endothermic and exothermic peaks in the first run. However, the smallest endothermic peak area was recorded for the chitosan film with acetic acid solvent. After the second run of the DSC, all samples showed an endothermic peak at a different position. These observations indicate that acetic acid may be the best solubilizing acid for dissolving chitosan. SEM images revealed that all types of films had a relatively smooth and clear morphology, which was confirmed by AFM. The FTIR spectra of chitosan films cast from different solvents showed similar spectra with a slight shift of some bands towards lower or higher wave numbers. Studying the mechanical properties of the films showed that chitosan film cast from acetic acid recorded the highest elongation at break, but the lowest tensile strength among the three types of films.

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Publications

1- Esam A. El-hefian, **Elham. S. Elgannoudi**, A. Mainal and A. H. Yahaya. *Characterization of chitosan in Acetic acid: Rheological and thermal studies*. Turkish Journal of Chemistry (ISI Journal). Accepted for publication.

2- An article submitted to the Malaysian Journal of Science titled "*Characteristics of chitosan films based on organic solvent type*".

3- An article submitted to the Malaysian Journal of Science titled "*Rheological study of chitosan in propionic acid solutions*".

Conferences

1- **Elham. S. Elgannoudi**, Esam A. El-hefian , Azizah Mainal and A. H. Yahaya: *Rheology of chitosan solutions in acetic acid*. International Graduate Congress 2007: 3rd Mathematics & Physical Science Graduate Congress (MPSGC).Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia. (12-14 December 2007).

2- Esam A. El-hefian, **Elham. S. Elgannoudi**, R Yahya, A Mainal and A. H. Yahaya: *Study of the effects of pH and dynamic viscosity on the chitosan solutions in formic acid*. 2nd Penang International Conference For Young Chemists (ICYC). University Sains Malaysia, Penang, Malaysia. (18-20 June 2008).

3- Esam A. El-hefian, **Elham. S. Elgannoudi**, A. H. Yahaya, R. Yahya and A. Mainal. *Rheological study of chitosan in propionic acid solutions*. Simposium Kimia Analisis Malaysia Ke-21 (SKAM-21). University Malaysia Sabah, Kota kinabalu, Malaysia. (24-27 November,2008).

4- **Elham. S. Elgannoudi**, Esam A. El-hefian, A. Mainal and A. H. Yahaya. *Study of the effect of the solvent on the thermal and morphological properties of chitosan films.* Simposium Kimia Analisis Malaysia Ke-21 (SKAM-21). University Malaysia Sabah, Kota kinabalu, Malaysia. (24-27 November, 2008).