FORMULATION AND RHEOLOGICAL CHARACTERIZATION OF CHITOSAN FILMS

by

HISHAMELDIN OMER AHMED

(Under the Direction of A. C. Capomacchia)

ABSTRACT

The study aims at developing a chitosan film bandage with physical attributes equivalent or better than the predicate Bandage and to further optimize the bandage quality by controlling rheological and viscometric properties of ingredients. Chitosan is a natural polysaccharide that has proven cost effective and the second most abundant natural polymer, it has favorable wound healing properties, biodegradable and biocompatible, the degradation of chitosan results in glucosamine which is endogenic and causes no adverse effects in the body. Chitosan from three grades of molecular weights, 150, 750 and 1190 were formulated using acetic and lactic acids, iodine iodide was added to the film, chitosan gels and films were tested for viscosity, water evaporation for up to three hours at 60 º C, water loss was investigated for periods of 15 minutes at 105 º C, film strength and resistance to breakage were also evaluated.

Chitosan can be formulated into gel formulas that can be optimized with understanding and controlling rheological and viscometric properties of ingredients. Chitosan can be cast into films that are stable at elevated temperature and are strong to resist breakage and absorb liquids while maintaining its physical form.

To evaluate chitsoan-metal ion complexation by using viscometric methodology.

Chitosan solutions from two grades (100 KDA and 500 KDA) were prepared in lactic acid. Complexes of chitosan with magnesium, zinc and ferrous salts (chloride and sulfate) were studied. The polymer to salts ratios were 4:1, 2:1, 1:1, and 1:2. Rheological properties of the mixtures were measured with a Brookfield rheometer, fitted with an SC-52 spindle, at 22 $\pm$ 2^{o} C.

Viscosity increased with the increase in the chitosan molecular weight, and with few exceptions, with the polymer metal ratio.Flow properties were studied by fitting the data to the rheological mathematical models of Bingham, Casson, NCA/Casson and the Power Law, the power law flow indices were less than 1 for the control samples as well as the six salts mixtures which indicates a pseudo plastic behavior for the chitosan complexes regardless of the complexing agent.

INDEX WORDS: Chitosan, Viscosity, Rheology, Complexation, Bingham, Casson, Power Law

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

The study aims to formulate a chitosan, a natural biopolymer, into a film suitable for use as a hemostatic dressing as well as a wound healing device. Viscometric and rheometric measurements will be utilized to control the specifications of the film to maintain physical characteristics necessary for reproducibility.

HISTORY AND USES OF CHITOSAN

Chitin is the second most abundant natural biopolymer, primary sources of chitin are seafood waste of shrimp, crab and lobster, while cell walls of fungi, algae and insects are used to produce smaller quantities of Chitin.

Chitin, a linear (unbranched) hetero-polysaccharide composed of 2-acetamido-2-deoxy-D-glucopyranose units linked by β-(1→4) glycosidic bonds, is very abundant in nature and comes next only to cellulose (Freier, T. et. Al., 2005; Kumar, M.N., 2000).

Chitosan is a linear hetero-polysaccharide made of two units of D-Glucosamine and N-Acetyl-D-Glucosamine, derived from partial N-de-acetylation of chitin, it is a copolymer composed of 2-amino-2-deoxy-D-glucopyranose2-acetamido-2-deoxy-D-glucopyranose units linked by β-(1→4) glycosidic bonds (2). Figure 1.1 chemical structure of chitosan.

Chitosan can also be termed de-acetyl-chitin or de-acetylated chitin. (Jones, D.S. and Mawhinney, H.J.,2003). The degree of de-acetylation is determined as the ratio

of D-Glucosamine and N-Acetyl-D-Glucosamine, thus a de-acetylation degree of 70% references a chitosan with 70% D-Glucosamine. The degree of de-acetylation corresponds directly to the number of amino groups along the backbone of the chitosan monomer.

Chitosan hydrochloride is listed in both the United States Pharmacopeia and European Pharmacopoeia.

METHOD OF MANUFACTURE FOR CHITOSAN

Chitin is produced by chemical treatment of shells of shrimp and crabs, the treatment is two-fold, an initial treatment with an alkali to remove proteins followed by an acid treatment for removal of calcium phosphate and calcium carbonates. (Jones, D.S. and Mawhinney, H.J., 2003). The de-proteinization is carried out with a 3-5% sodium hydroxide solution, while calcium is removed by treatment with a 3-5% hydrochloric acid solution. Chitin is then N-deacetylated to produce chitosan, the N-deacetylation treatment is conducted with a concentrated sodium hydroxide solution at elevated temperatures as high as 110 ^{o}C.

Early reports of chitosan manufacture have used alternative methods such as the one reported by Muzzarelli and group that Hoppe-Seyler in 1894 obtained chitosan by fusing potassium hydroxide to chitin at 180 ^{o} C. However, Recently sodium hydroxide is the alkaline of choice for the deacetylation of chitin into chitosan. (Muzzarelli, R.A., 1973).

Chitin and chitosan are similar in structure, since chitin is poly-N-acetyl-glucosamine, while chitosan is comprised of both acetyl-gucosamine and poly-N-acetyl-glucosamine, when minimal deacetylation occurs, chitin is the resultant, as deacetylation increases, chitosan content slowly increases as chitin content recedes.

The deacetylation process makes chitosan soluble in weak organic acids, such as formic acid, acetic acid and lactic acid; the solubilzation is attributed to the breakage of the hydrogen bond and the acquiring of positive charge due to the protonation of the amine groups.

Purification of chitosan is carried out by dissolving chitosan in excess acid, then filtration through a porous membrane, followed by pH adjustment with sodium hydoxide. (Muzzarelli et al., 1973).

CHITOSAN SAFETY

Chitosan is generally regarded as safe (GRAS) and as non-irritant and nontoxic. and is biocompatible with both healthy and infected skin.

Chitosan products were reported to be biocompatible with human tissues and cells and do not illicit any harmful reactions. Howling et al (2001) studied the impact of chitosan on human dermal fibroblasts and concluded that chitosan a with a high degree of deacetylation had proliferative effects on fibroblast cells. Weng et al (2008), studied the effects of chitosan hydrogels and their products on murine fibroblasts and reported that both chitosan hydrogels and their degradation products have no cytotoxic effects. Ma et al., 2007), tested a chitosan device for cytotoxic

effects on human fibroblasts, sensitization effects on guinea pigs, and irritation on rats and cell proliferation on dermal fibroblasts, the group reported that the chitosan device had no cytotoxic effects on fibroblasts, rather, it was reported to have a proliferative effects; as for sensitization and irritation effects such as edema or erythema, the device was reported to have no adverse reactions.

GENERAL USES OF CHITOSAN

Chitosan has been used in waste water treatment, due to its positive charge, chitosan sequester negatively charged metals such as copper and cadmium, the degree of sorption depends on the molecular weight of chitosan, the higher the molecular weight the greater the degree of sorption. (Muzzarelli et al (2005).

Amine groups on the backbone of Chitosan have made the polymer effective in chelation of metallic cations, chelation occurs at neutral pH at the Nitrogen sites on the polymer, however, protonation of the amine groups on the chitosan backbone, enables chitosan to chelate metal anions such as chloride due to electrostatic attractions. Muzzarelli et al 2005 have reported that chitosan has better immobilization of copper than chitin.

Chitosan have been used for ridding industrial refuse of heavy metals such as Copper and Chromium; these effective chelating abilities have presented chitosan as a viable and safe agent for waste water treatment plants (Muzzarelli et al. 1977, Guibal, E., et al., (1995) and (1998).

PHARMACEUTICAL USES OF CHITOSAN

Chitosan is a cationic biopolymer, the positive charge enables chitosan to possess muco-adhesive properties, and mucus layer has a negative charge from sialic acid; chitosan reactivity is also enhanced by the presence of amino groups and hydroxyl groups in the backbone. Chitosan is an attractive biopolymer due to its reactivity and biodegradability, the enzyme lysozyme naturally degrades chitosan into safe by-products that are absorbed by the body (Lehr et al., 1994 and Zikaki, J.P, 1984)

CHITOSAN USE IN WOUND HEALING PRODUCTS

Chitosan has excellent wound healing properties, those properties range from stimulation of wound healing and enhancement of angiogenesis and tissue granulation to sequestering negatively charged macrophages (Muzzarelli et al 2009). Due to the high porosity and hydro-gelling abilities, chitosan absorbs wound exudates to promote wound healing, while maintaining a moist wound environment to prevent dryness and scaling of the wound surface.

Chitosan bandage can be easily applied or pealed from the wound surface without application of force or causing trauma to the wound, due to its biodegradable and non-toxic nature, application to the wound does not pose any health risk.

Chitosan can also attract and bind to negatively charged platelets, thus contributing to fast clotting to protect wound from exposure to the environment and to seal the wound from opportunistic pathogens.

Chitosan is biocompatible with the extracellular matrix and promotes its production by the fibroblast (Ueno, H. et. al 2000 and 2001); poses no toxicity problems to the fibroblasts (Muzzarelli, R.A. et. al 2005, 1999, 1990; Berscht, P. et. al 1995). Has the capacity to release glucosamine and N-acetylgucosamine monomers and oligomers. Prevents excessive scar formations and promotes wound healing and closure (Muzzarelli, R.A. et. al 1999).

Chitosan has a high water absorption, high oxygen permeability, and very slow enzymatic degradation (Allan, G.G. et. al 1984); all these features allow for a less frequent wound dressing and lengthen the therapeutic window (Illum, L. et al 2001; Illum, L. et al 2000, Illum, L. et al., 1998, Illum, L., 1998). Chitosan has an antibacterial and antifungal effect (Hu, S.G. et. al 2004). Facilitates and enhances wound healing by promoting re-epithelialization which is the important granulation tissue formation process in the wound healing cascade of events (Muzzarelli, R.A. et. al 2005, and 1999; Ishihara, M., 2001and 2001).

It plays a very active role in the healing process and contributes to the acceleration and success of every step in the wound healing process, beginning from the promotion of anti-inflammatory factors through the remodeling and closure step. Using Chitosan as a delivery vehicle for the potent antiseptics is viewed as one of the best options for fast and complete healing.

Chitosan was reported to promote wound healing by absorbing wound exudates and promoting granulation and fibroblast formation. Several chitosan based wound

healing products have been available in the markets; these products are effective in wound healing as well as hemostasis.

Chitosan effectiveness in hemostasis and wound healing stems from its biodegradability and non-toxicity, (Muzzarelli et al., 2009) reported that chitosan of high molecular weight possessed hemostatic capabilities. Other reports have credited chitosan for being effective in hemostasis and clotting on its own, while some reports have attributed chitosan clotting and hemostasis to its positive charge which enables it to bind to negatively charged platelets.

Several researchers have developed a wound dressing based on chitosan and silver nanoparticles that proved to be effective as both a hemostatic and antimicrobial product, silver had expanded the antimicrobial activity of chitosan in terms of species as well as wound acidity.

VISCOMETRIC AND RHEOMETRIC METHODS

Materials flow and deformation properties differ due to their unique intermolecular makeup, when a material is subjected to force or stress, the resultant deformation or strain defines the material's rheological behavior, while the resistance to flow is measured or represented by viscosity, the deformation is represented and measured by rheology.

While gases and fluids flow as a force is applied, solids will deform by a fixed amount and may regain original shape when force is removed. To optimally design

chitosan for wound healing products, gels or devices, it is paramount to understand the flow and deformation behaviors (Smithrod and Haug, 1971).

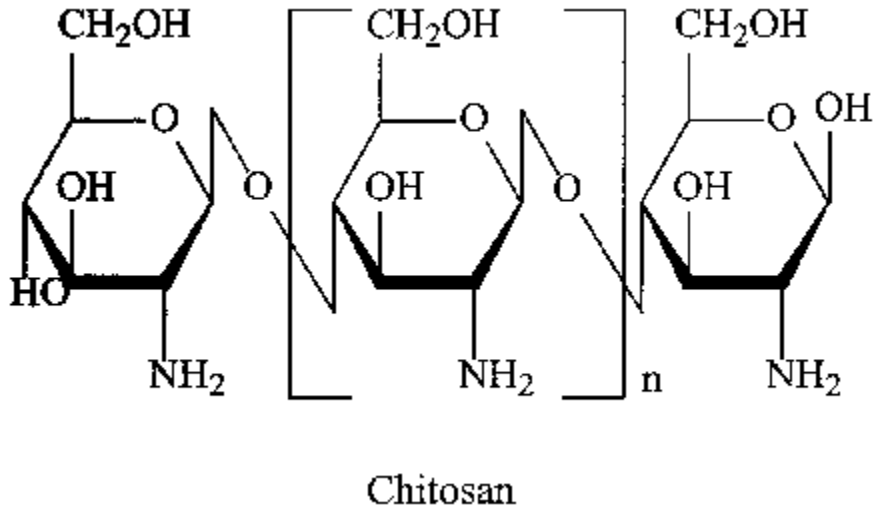
Chitosan, a natural biopolymer with a potential for use in the biomedical field can only be fully utilized after elucidation of flow and deformation properties (Hwang et al., 1997).

Understanding viscosity and rheology provides valuable information about material textures and flow properties, thus allows for better handling and design of products. Viscosity is reported in units of centipoise (cps) or milli Pascal-seconds (mPa.s). Basic components of viscosity and rheology of materials are stress, which is the force divided by the area over which it is applied, strain is the relative deformation, measured as deformation per unit area.

Viscosity range is inversely proportional to the size of the spindle and to the rotational speed, when measuring high viscosity, a small spindle or slow speed need to be used.

When measuring viscosity and rheology with a viscometer or a rheometer, the basic principle of operation is to drive a spindle by immersing it in the measured or tested material, the spindle is connected to a spring. The viscous drag of the tested material against the spindle is captured by the spring deflection which is measured by a rotary transducer. The values measured depend on the rotational speed, the spindle size and shape and torque of the spring.

Yield stress is the measure of applied force that forces the material to begin to flow, the deformation endured by the material prior to beginning to flow is termed yield strain.



Chitosan

Figure 1.1: Chemical structure of chitosan

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CHAPTER 2

FORMULATION OF CHITOSAN FILMS[1]

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Abstract

The study aims at developing a chitosan film bandage that physical attributes equivalent or better than the predicate Bandage and to further optimize the bandage quality by controlling rheological and viscometric properties of ingredients. Chitosan is a natural polysaccharide that has proven cost effective and the second most abundant natural polymer, it has favorable wound healing properties, biodegradable and biocompatible, the degradation of chitosan results in glucosamine which is endogenic and causes no adverse effects in the body. Chitosan from three grades of molecular weights, 150, 750 and 1190 were formulated using acetic and lactic acids, iodine iodide was added to the film, chitosan gels and films were tested for viscosity, water evaporation for up to three hours at 60 º C, water loss was investigated for periods of 15 minutes at 105 º C, film strength and resistance to break were also evaluated.

Chitosan can be formulated into gel formulas that can optimized with understanding and controlling rheological and viscometric properties of ingredients. Chitosan can be cast into films that are stable at elevated temperature and are strong to resist breakage and absorb liquids while maintaining its physical form.

Introduction

Chitosan, a natural based polysaccharide comprised of copolymers of glucosamine and N-acetyl-glucosamine, is manufactured by the de-N-acetylation of chitin, Chitosan is very abundant in nature and comes next only to cellulose (Freier, T. et. al 2005; Kumar, M.N. 2000). It has many attributes that are conducive to burn and wound healing, it is biocompatible with the extracellular matrix and promotes its production by the fibroblast (Ueno, H. et. al 2000 and 2001); possesses no toxicity problems to the fibroblasts (Muzzarelli, R.A. et. al 2005, 1999, 1990; Berscht, P. et. al 1995). Has the capacity to release glucosamine and N-acetylgucosamine monomers and oligomers. Prevents excessive scar formations and promotes wound healing and closure (Muzzarelli, R.A. et. al 1999).

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It plays a very active role in the healing process and contributes to the acceleration and success of every step in the wound healing process, beginning from the promotion of anti-inflammatory factors through remodeling and closure step.

Using Chitosan as a delivery vehicle for the potent antiseptics is viewed as one of the best options for fast, complete healing.

Chitosan is obtained from natural polymer that is comprised of poly-N-acetyl-glucosamine, according to Muzzarelli (1973), Hoppe-Seyler in 1894 obtained chitosan by fusing potassium hydroxide to chitin at 180 ^{o} C. Recently sodium hydroxide is alkaline of choice for the deacetylation of chitin into chitosan. (Muzzarelli, R.A 1973 Natural chelating polymers).

Chitin and chtiosan are similar in structure, since chitin is poly-N-acetyl-glucosamine, while chitosan is comprised of both acetyl-gucosamine and poly-N-acetyl-glucosamine, when minimal deacetylation occurs, chitin is the resultant, as deacetylation increases, chitosan slowly takes over and chitin recedes.

The deacetylation process makes chitosan soluble in weak organic acids, such as formic acid, acetic acid and lactic acid; the solubilzation is attributed to the breakage of the hydrogen bond and the acquiring of positive charge due to the protonation of the amine groups.

Purification of chitosan is carried out by dissolving chitosan in excees acid, then filtration through a porous membrane, followed by pH adjustment with NaOH or NH_4OH, the latter alkaline cause solidification of chitosan since it is not soluble in alkaline solutions with pH values near neutral.

Summary of beneficial wound healing effects for Chitosan

ChitosanReferences

Antibacterial, antifungal	Hu, S.G et al 2004
Enhancement of re-eptheliazation	Muzzarelli, R.A. et al 1999, 2005
Widen therapeutic window	Illum, L. et al 1998, 2000
Water absorption, high O_2 Permeability	Allan, G.G. et al 1984
Prevention of scar formation	Muzzarelli, R.A. et al 1999
Biocompatible with extracellular matrix and promotes it production	Ueno, H. et al 2000, and 2001
Poses no toxicity problems to fibroblast	Muzzarelli, R.A. et al 2005, 1999; Berscht, P. et al 1995

In the following study, chitosan was the sole film forming component in the formula to generate gels and films. Acetic acid, lactic acid and water were the solvents, while glycerin was used as a plasticizer to enhance elasticity and flexibility.

Important physical attributes for chitosan gels that were cast into films were: The viscosity of gel solutions which impact suitability for casting films, chitosan molecular weight variations which impact viscosity, and possible reactions between formula ingredients. Loss on drying (LOD) was examined to assess film ability to contain/absorb liquids while maintaining the physical integrity of the film. Film and iodine stability was assessed at elevated temperature, while mechanical strength of the film was tested to assess resistance to breakage.

Materials and Methods

Materials:

Three grades of chitosan, 150, 750 and 1190 cps were used in the study, iodine solutions were prepared from iodine (I) and potassium iodide (KI) in cascading strength in the range 0.1 – 1%, ratio of I: KI was varied between 1:1 to 1:2, glycerin mixed with purified water in the ratio of 1:10 was used as a plasticizer.

Solution preparation:

Several chitosan solutions with polymer concentrations in the range of 1% to 6%, and acetic or lactic acid solutions at in concentrations ranging from 1% to 5%. the procedure to manufacture chitosan solutions begins with slowly adding chitosan to the solubilizing acid, acetic acid or lactic acid, while mixing on an IKA mixer at high speeds of 200 -500 RPM, speed depends on the amount of acid used, large acid amounts need higher speed to create a vortex, when a homogeneous solution is obtained, the solution is de-aerated by securing an air tight lid on the container then,

a vacuum pump is fitted to the lid to remove air bubbles, iodine iodide solution is added slowly to the chitosan solution while mixing at 400 – 700 RPM.

Viscosity studies

Chitosan gel solutions were tested on Rheometer, model DV-Ultra III, a batch file was created to upload the description of spindle properties as well as the speed and duration of the test, sample size was 0.5 g (± 0.05 g), the test ran for 10 minutes, a speed ramp was included in the test where the velocity of the rheometer was increased steadily, all testing was done at room temperature.

Loss on drying studies (LOD)

An Ohaus moisture analyzer model MB23 was used for testing LOD. Triplicate samples weighing 2 g of were placed in an aluminum pan with temperature at 105 ^{o} C examined water loss over a 15 minute period. The study indicated the stability and water retention capacity of the films under elevated temperature.

Film casting

a casting knife model can be adjusted to cast film in variable thicknesses.
Use the knobs on the side of the casting knife to adjust film thickness to 4 mm. Thickness was chosen to match dimensions for predicate film and to minimize fragility upon drying, a liability that hampers thinner films, albeit thicker films were found to take too long to dry. Once dry, the physical attributes of resistance to break and desirable moisture content outweigh process development challenges.

To cast a chitosan film, place a plastic sheet on a flat surface, place the casting knife on the edge of the plastic sheet with backside of the knife resting on the edge of the sheet and the inside facing the balance of stretched out the plastic sheet. Pour the gel solution on the plastic sheet on the inside part of the casting knife, slowly move the casting knife to spread out the gel solution on the plastic bag. Leave the cast film on the plastic sheet to dry at room temperature.

Water Evaporation:

Water content allows for malleability and elasticity of dried films, too little water content results in films that are too rigid to use over countered surfaces, and too dry to maintain a moist wound environment.

This study tested the stability of water, how much water remains in the films that were exposed to elevated temperature for 1, 2, and 3 hours.

An equal amount of fresh gels from the three grades of chitosan were placed in an oven, with temperature preset to 60º C, triplicate samples were collected after 1, 2, and 3 hours and immediately tested for water content.

Tensile Strength:

Tensile strength is the amount of force a material can endure without breaking. As it is subjected to a force lower than its tensile strength it stretches without breaking. But, as soon as the applied force reaches the tensile strength of the tested material, no further stretching occurs, the material breaks, and a value is assigned to the force at breaking point as well as to the distance stretched. The TA Plus Texture Analyzer

was used for determining the tensile strength of the films prepared as described above.

Statistical analyses:

Analysis of variance (ANOVA) was performed in a randomized complete bock design (Steel and Torrie, 1980) with triplicate treatments using PROC GLM of SAS (Statistical Analysis System, SAS Institute 2001) on all data collected as described above.

Results and Discussion

Effects of chitosan grade (150, 750, and 1190) and acid type (acetic acid or lactic acid), and chitosan concentration were tested on parameters such as viscosity, evaporation over extended period of times, one, two and three hours at 60 ^{o}C, evaporation tests stability during extended stress, while loss on drying (LOD) tests water stability during short lived stress under very elevated temperatures 105 ^{o}C .

Viscosity Results: Tables 1.1 and 1.2, indicate that chitosan grade, concentration and acid type are important for viscosity readings, all variables were reported to have a Pr>F values of <0.0001; in order or design a film with a target viscosity, chitosan grade and concentration need to be controlled as well as acid type, results based on figures 1.1, and 1.2, reported that chitosan grade of 150, concentration of 3% and acid choice of acetic would render viscosity reading that are higher than their alternative variables of lactic acid, chitosan grades of 750 or 1190 or polymer concentration of 1.5%, 2.5%, or 5%.

High viscosity reading allow for development of films that are both strong, and have desirable water retention to allow for elasticity for ease of application and handling and to sustain a moist wound environment conducive to healing.

Evaporation Results: Table 1.3 indicates, evaporation is strongly impacted by chitosan grade and acid type, figures1.4 – 1.9 reported evaporation results for one hour, two hours and three hours in a controlled temperature oven of 60 ^{o} C.
Films made with acetic acid were reported more resistant to extended stress conditions, all tested variables were lost water initially during the first hour, then maintained the level of water content during the ensuing two and three hours, acetic acid had 63% water evaporation during the first hour, 60% after two hours in the oven and 60% after three hours. Lactic acid tapered from an initial evaporation of 53% to 50% after three hours, difference between acid is not significant to favor one over the other. Chitosan grade evaporation profile had exhibited similar trend as the acid effects, grade of 1190 had the least evaporative loss of 45% after one hour, then steadied at 40% after two and three hours, results for 1190 grade were better that those for 750 grade which had 55% evaporation for the three tested tine frames. 150 grade had the most loss of 72% after one hour, two and three hours values were similarly high at 70% and 69%.
Values for evaporation losses were high, which suggests that films are not designed to maintain integrity or functionality under extended heat stress, if films are needed to be used in areas where temperatures may reach as high as 60 ^{o} C, then storage conditions need to be in controlled temperature to avoid high levels of evaporation.

grade 1190 chitosan proved to be most suitable for films that are to be shipped to high temperature regions.

Loss on drying Results (LOD): Loss on drying results are presented in Table 1.4 and Figures 1.10, 1.11 and 1.12, results indicate chitosan grade had the most contribution towards desirable LOD results, desirable LOD range for films is 16% to 25% with a target LOD of 20% as optimal. Too high loss on drying will render the film dry and fragile, leading to easy breakage or excessive depletion of moisture from the wound environment. Chitosan grade 150 had an LOD of 24%, grade 750 had 17%, both are acceptable and within limits, however grade 1190 had an LOD value of 9%, pointing to too strong molecular bonding that prevented moisture loss and therefore had a high moisture content that adversely impact the functionality of the film, too soft and moist, presenting shape deformation challenges and due to high moisture content may not be effective as moisture absorbent from the wound environment.

Tensile Strength Results: Tensile testing results were presented in Table 1.5, two films made with acetic acid and lactic acid were tested for tensile strength, how much force is needed for a measured distance before it fails or ruptures.
Films made with acetic acid were reported to stretch for 9.899 mm and endure 3.667 kg before rupture, lactic acid films were stretched to a distance of 8.899 mm and endured a force of 2.284. reading for both films are comparable.

Conclusions:

Chitosan can be formulated into gel formulas that can optimized with understanding and controlling rheological and viscometric properties of ingredients.

Chitosan can be cast into films that are stable at elevated temperature and are strong to resist breakage and absorb liquids while maintaining its physical form.

Chitosan films can be developed to meet wound care needs.

Films quality may be compromised at extended exposure to temperature that exceed 60 ^{o} C, film can stored at controlled temperature conditions.

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Table 2.1: Mean squares, F values and probabilities of the effect of Chitosan concentration on pH, viscosity, evaporation (EV) and moisture content (LOD).

	pH	Viscosity	Ev_1h	Ev_2h	Ev_3h	LOD
df	4	4	4	4	4	4
MS	0.3535	269619149	650.73	877.0506	893.131	36.786
F	46.64	80.52	17.62	71.51	60.86	4.04
Pr>F	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	0.033

Table 2.2: Mean squares and variance components for Chitosan grades, Acid types and Chitosan grade x Acid type for Viscosity

Source	df	MS	F	Pr>F
Chitosan grade	2	3304106	1012.13	<0.0001
Acid type	1	311786.7	95.51	<0.0001
Chitosan grade x Acid type	2	123916.7	37.95	<0.0001
error	12	3264.5		

Table 2.3: Mean squares and variance components for Chitosan grades, Acid types and Chitosan grade x Acid type for Evaportation-3hr

Source	df	MS	F	Pr>F
Chitosan grade	2	1083.6	124.67	<0.0001
Acid type	1	464.1	53.39	<0.0001
Chitosan grade x Acid type	2	68	7.83	0.0067
error	12	8.7		

Table 2.4: Mean squares and variance components for Chitosan grades, Acid types and Chitosan grade x Acid type for Lose on drying (LOD)

Source	df	MS	F	Pr>F
Chitosan grade	2	389.7	64.51	<0.0001
Acid type	1	36.3	6	0.0306
Chitosan grade x Acid type	2	9.9	1.65	0.2327
error	12	6.04		

Table 2.5: Tensile strength results for chitosan films made with lactic and acetic acids

Texture Analysis Test for Chitosan Films			
Lactic acid film		**Acetic acid film**	
Force (kg)	Distance (mm)	Force (kg)	Distance (mm)
2.284	**8.664**	**3.667**	**9.899**

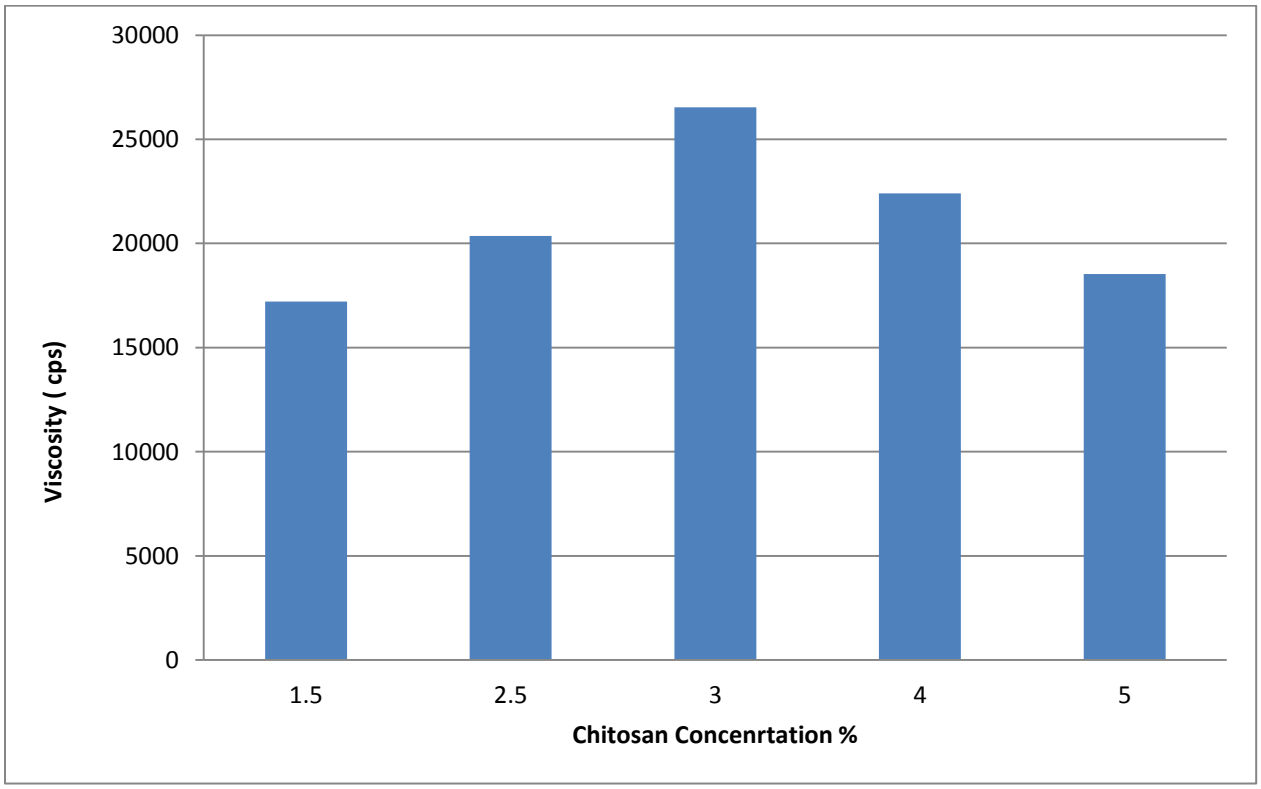

Figure 2.1: Effect of chitosan 150 grade concentrations on Viscosity

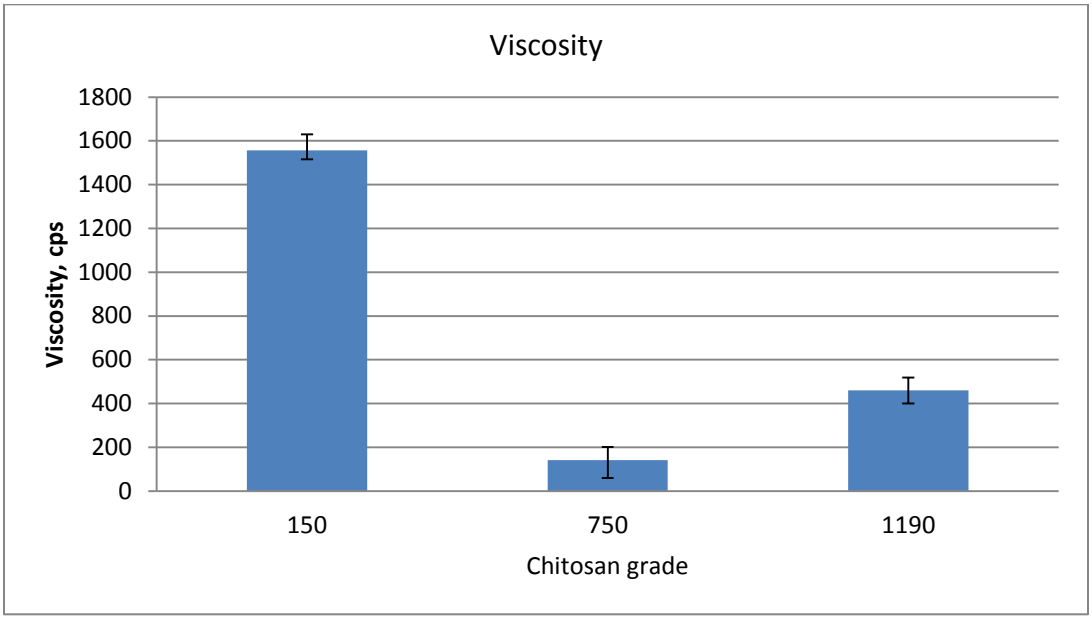

Figure 2.2: Effect of chitosan grade on viscosity

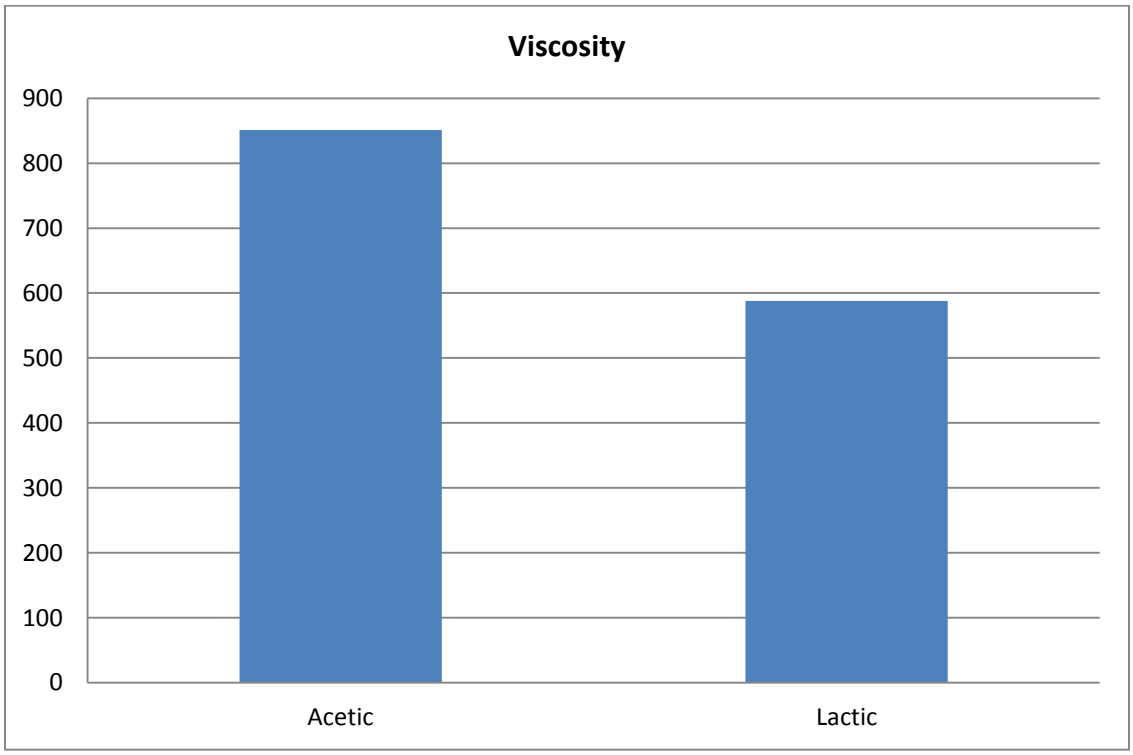

Figure 2.3: Effect of acid type on viscosity

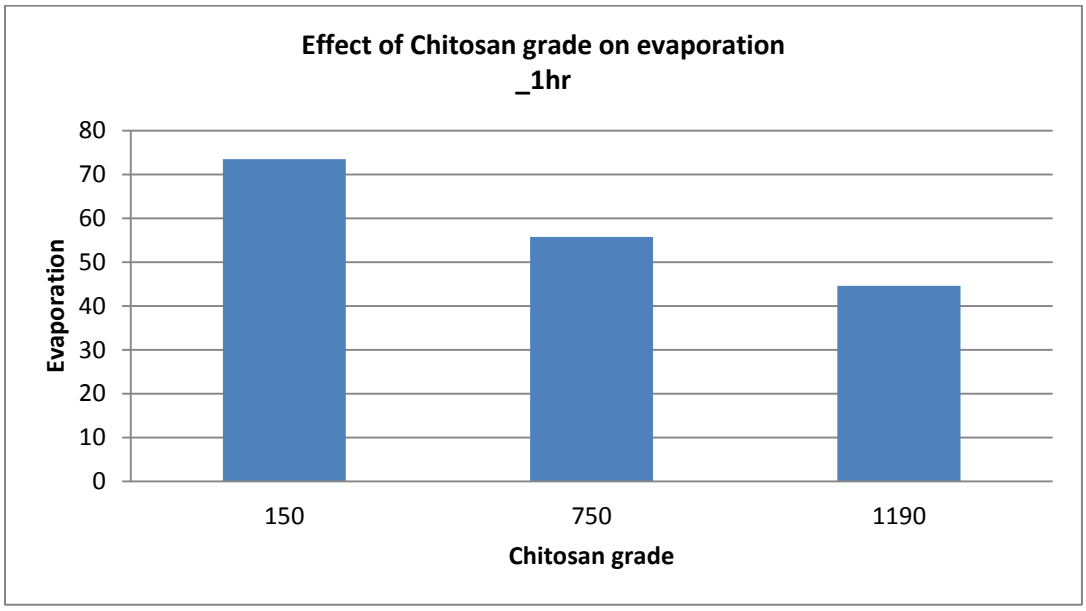

Figure 2.4: Effect of chitosan grade on evaporation for 1 hour

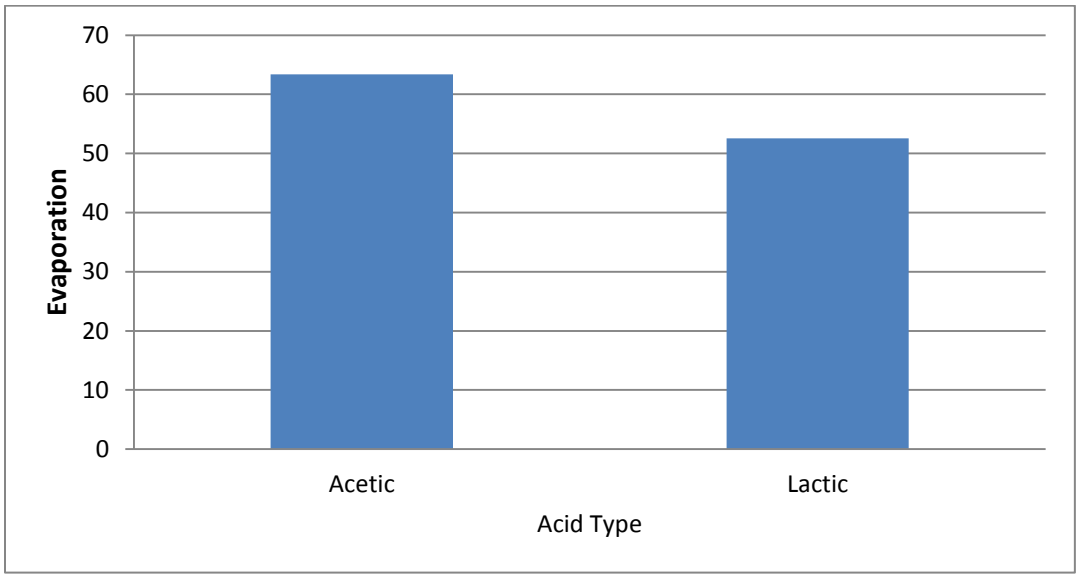

Figure 2.5: Effect of chitosan grade on evaporation for 1 hour

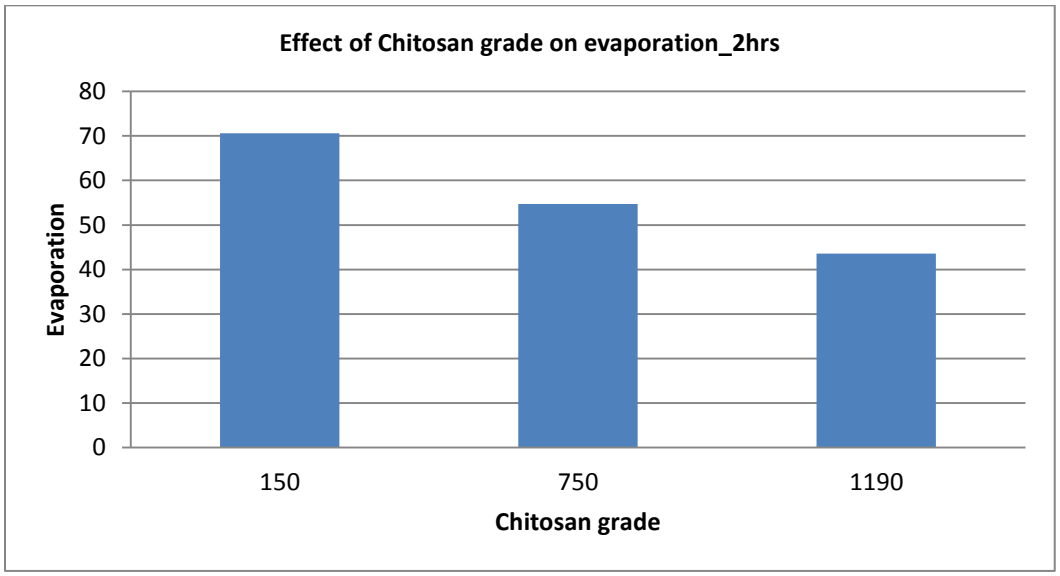

Figure 2.6: Effect of chitosan grade on evaporation for 2 hours

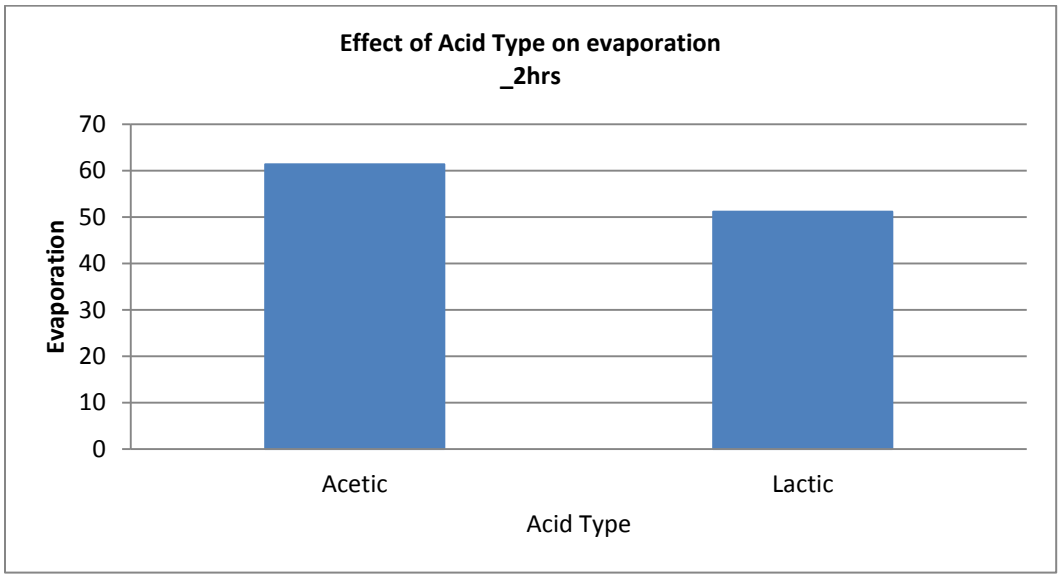

Figure 2.7: Effect of acid type on evaporation for 2 hours

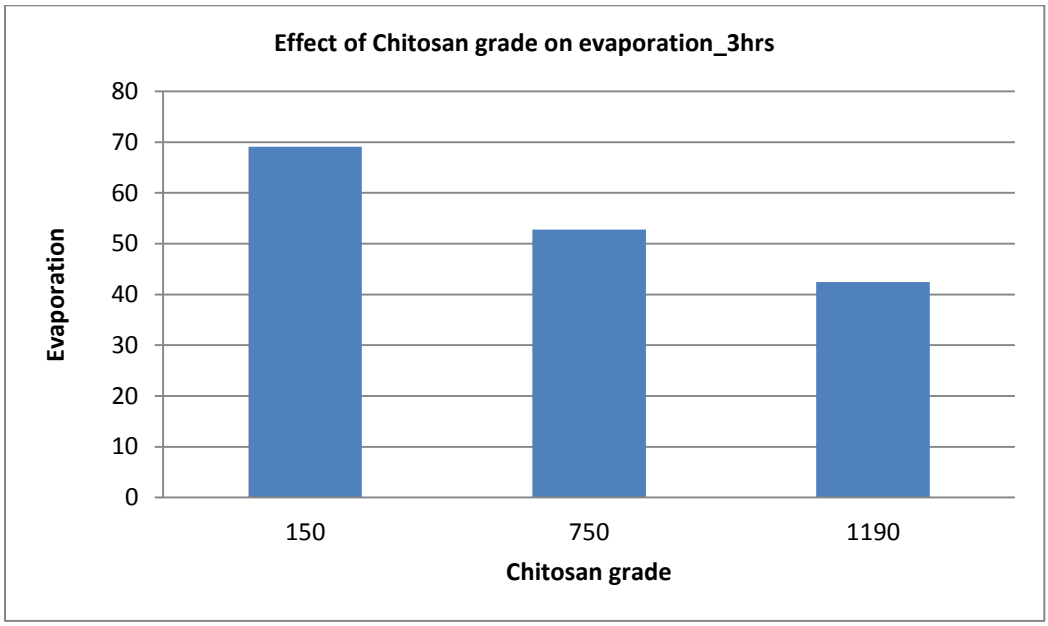

Figure 2.8: Effect of chitosan grade on evaporation for 3 hours

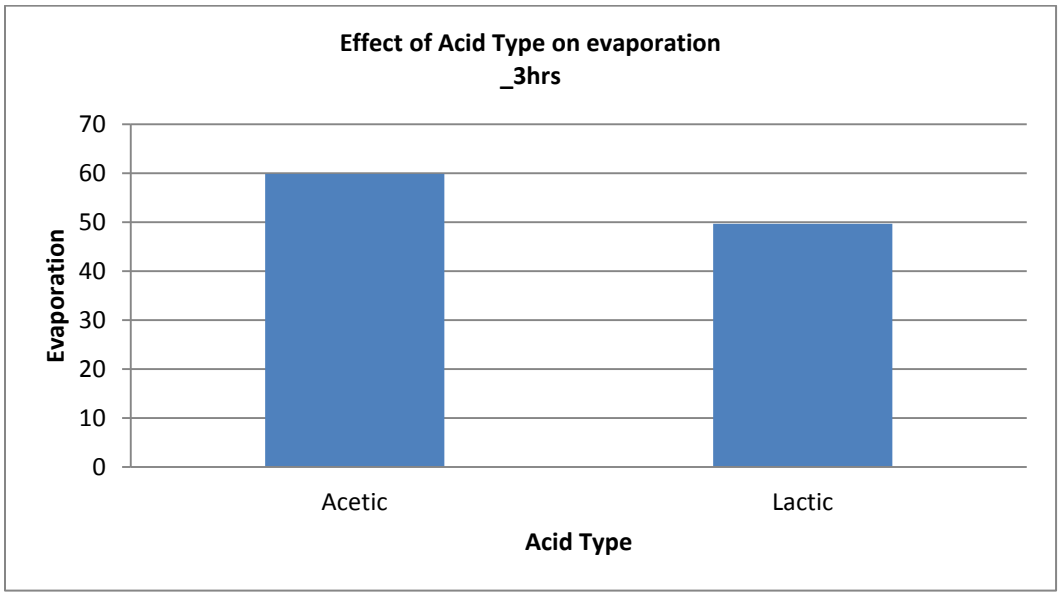

Figure 2.9: Effect of chitosan grade on evaporation for 3 hours

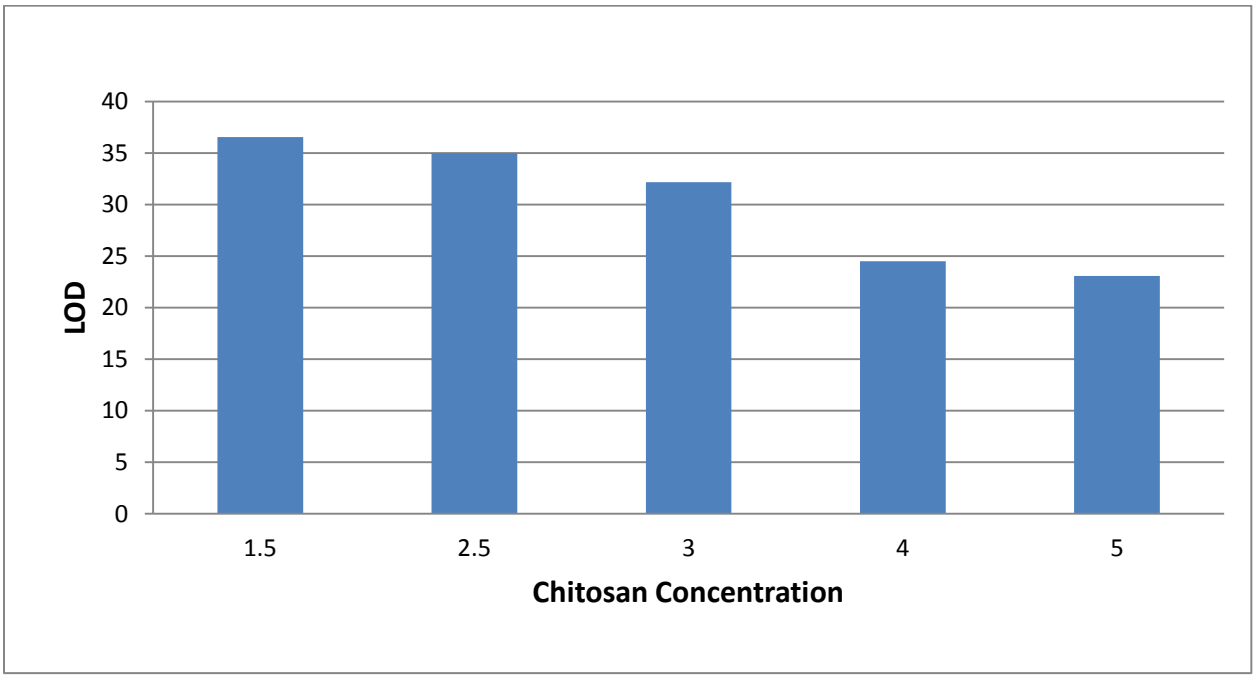

Figure 2.10: Effect of chitosan concentrations on loss on drying (LOD).

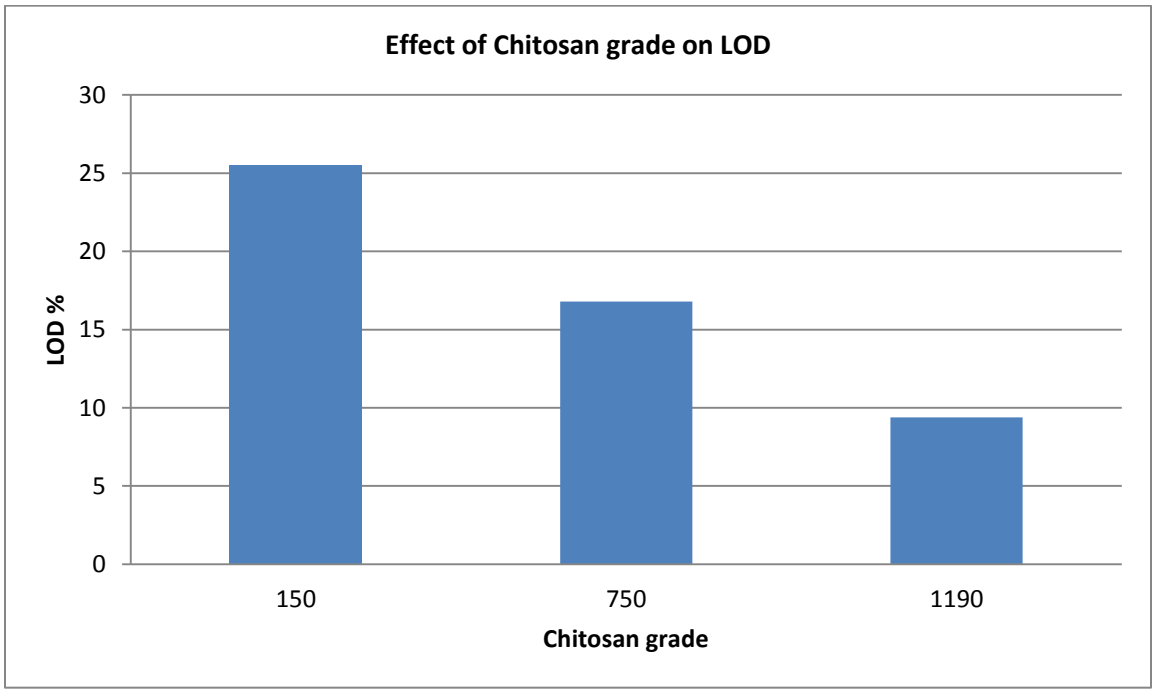

Figure 2.11: Effect of chitosan grade on loss on drying (LOD).

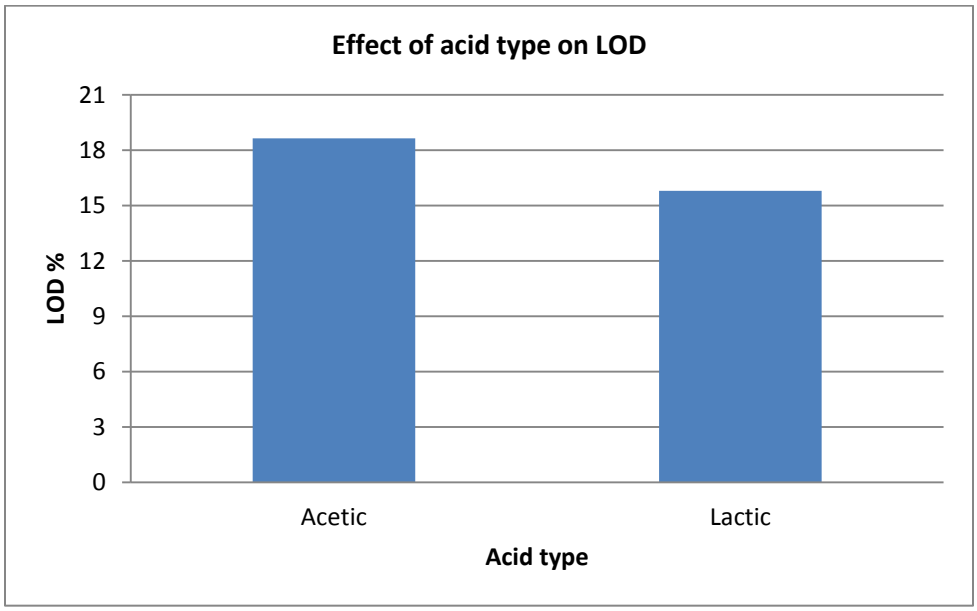

Figure 2.12: Effect of acid type on loss on drying (LOD).

CHAPTER 3

RHEOMETRIC CHARACTERIZATION OF CHITOSAN COMPLEXES[1]

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[1] To be submitted to the *Int. J. of Pharmaceutics*

Abstract:

To evaluate chitsoan-metal ion complexation by using viscometric methodology.

Chitosan solutions from two grades (100 KDA and 500 KDA) were prepared in lactic acid. Complexes of chitosan with magnesium, zinc and ferrous salts (chloride and sulfate) were studied. The polymer to salts ratios were 4:1, 2:1, 1:1, and 1:2. Rheological properties of the mixtures were measured with a Brookfield rheometer, fitted with an SC-52 spindle, at 22 $\pm$ 2^{o} C.

For all experiments, the viscosity measurement increased with the increase in the chitosan molecular weight, and with few exceptions, there was a similar increase in viscosity with the polymer metal ratio, thus it was higher for the 500 KDA formulations. The highest increase in viscosity values was recorded for the polymer to metal ratio of 4 to 1. The flow properties were studied by fitting the data to the rheological mathematical models of Bingham, Casson, NCA/Casson and the Power Law, the power law flow indices were less than 1 for the control samples as well as the six salts mixtures which indicates a pseudo plastic behavior for the chitosan complexes regardless of the complexing agent.

Introduction

Chitosan, a natural based polysaccharide comprised of copolymers of glucosamine and N-acetylglucosamine, is manufactured by the de-N-acetylation of chitin;chitin and Chitosan are very abundant in nature and come next only to cellulose (Freier, T. et. al 2005; Kumar, M.N. 2000). The process of N-dacetylation enables chitosan to possess more amino groups than chitin, and thus is more suited to adsorb metal ions, adsorption capacity not only exceeds that of chitin but also of activated carbon and cellulose (Piron and Domard (1997 and 1998); Helder et al (2008). Prominent among the factors that control chitosan chelating ability are the degree of deacytelation (DA), solution pH, metal ion type and concentration. Juang, RS et al (2002) and Rhazi, M et al (2002). Viscometric and rheological methods measure a polymer is complexed, adsorbed or bond to another polymer, metal or salt. Data generated from the reaction of chitosan with selected salts to understand differences among chitosan grades as well differences that stem from type of metal or salt used.

Mathematical models will be used to understand rheometric data that represent the extent of chitosan metal chelation and how the chelation or complexation affects flow properties of chitosan ion complexes. Rheometer measures the flow behavior of materials; hence it determines the flow and deformation of materials better known as rheology of materials.

Newtonian materials viscosity values are the same at all shear rates, few materials, such as water and very thin syrups are classified as Newtonian. Most pharmaceutical semi-solids and liquids are non-Newtonian. Polymeric complexes are known to produce non-Newtonian viscosity values due to the complex structures and presence of

molecules of different sizes whose alignment within the complex introduces the variability in viscosity readings. Chitosan salt solutions exhibit shear thinning, due to the difference in complexing molecules shapes and sizes, viscosity decreases when the shear rate is increased. Mucha, M. (1997 and 1998).

Metal Sorption and Chelation by Chitosan

Deacetylation of chitosan frees amine groups to interact with and sequester metals; the deacetylation process modifies chitin, which lacks free amine groups due to the presence of the acetyl groups, thus chitin is neither active nor effective in metal sequestration as chitosan (Guibal et al 1995; and Oyrton et al 1999).

Chitosan Metal Sorption:

Presence of free amine groups empowers chitosan to sorb metals, sorption prowess is further enhanced by the protonation which results from chitosan solubility in weak organic acids, protonation of chitosan unleashes the amine groups and adds more sorption sites to the chitosan.

Besides degree of protonation, metal sorption is influenced by metal type, concentration and media pH, and the sorption method employed by the amine groups (Jansson-Charrier et al 1996; Guibal et al 1998 and 2000; Piron et al 1997; and Rhazi et al (2002), methods of sorption are chelation through chemical interactions, electrostatic reactions by ion exchange (Muzzarelli et al 1982, and 2005), or by forming ion pairs with sorbed metals (Inue et al 1993).

Metal Chelation Models:

Chelation methods are based on chemical interactions between sorbed metals and chitosan, two chelation models compete to explain the metal uptake mechanism (Muzzarelli et al 1980; and Domard 1987), a “bridge model” proposes metal ions encircled by amine groups from one or several chains, and a “pendant model” where an amine group is bound to a metal ion as a pendant.

Material Methods:

Methods: Chitosan solutions from two grades (100 KDA and 500 KDA) were prepared in in lactic acid. Complexes of chitosan with magnesium, zinc and ferrous salts (chloride and sulfate) were studied. The polymer to salts ratios were 4:1, 2:1, 1:1, and 1:2. A control was prepared by mixing the chitosan solution with purified water in the same ratios as the prepared salts. Samples were measured in triplicate. Rheological properties of the mixtures were measured with a Brookfield rheometer (Model DV-III Ultra) fitted with an SC-52 spindle, at 22 ± 2^{o} C.

Two different grades of chitosan were used in the study; both were obtained from Protan Inc. (Raymond, WA), a low molecular weight grade with a viscosity of 31 cps at 1% solution, and a medium molecular weight grade with a viscosity of 746 cps in 1% solution. Chitosan complexes were prepared from solution of 4% of chitosan in 2% ascetic acid.

The viscosity of different molecular weight chitosan solutions were measured in presence of various molar concentrations of complex-forming heavy metals using a Brookfield rheometer (Model DV-III Ultra)(Stoughton, MA). The tested metals were calcium chloride, magnesium sulfate, and ferrous sulfate. Solutions with different

concentrations of themetals were prepared; in 0.8, 0.6, 0.4, and 0.2 mmole. Viscosities in centipoises were compared to equivalent polymer solutions at different ionic strengths of noncomplex forming electrolytes, such as sodium chloride.

Rheological (Mathematical) Models:

Brookfield rheometer (Model DV-III Ultra) is loaded with a software program Rheocalc32 which facilitates the use of the rheometer in data collection, analysis, and generation of mathematical models that are utilized to fit the data and describe the behavior. Polymeric solutions are pseudo-plastic materials that behave as non-Newtonian media, where the relationship between the shear stress and shear rate are non-linear, increasing the shear rate may not necessarily lead to an increase in the shear stress. In Newtonian media, the relationship between shear stress and shear rate is linear; the shearing stress is proportional to the shear rate. Newtonian flow assumes plasticity, as in the case of water, if stress is applied water surface may be disturbed, however, the water surface return to its original form as soon as the stress is removed.Eugene Bingham described the flow behavior for non-Newtonian materials by formulating an equation with a yield stress parameter, yield stress describes a material that deforms under stress and will retain the deformation until more stress is applied.

Power Law:

A model used to describe the relationship between shear stress, the force per unit area used to move a material, to shear rate, the velocity gradient in a moving material and consistency index, which indicates flow behavior at low shear rates. Shear stress estimates the product of shear rate and consistency index as indicated at the specified flow index. The model is useful when no viscosity reading is available.

$\tau = \kappa\gamma^{n}$ (1)

Casson's Model:

The model estimates shear stress in the absence of flow index, utilizing consistency indices shear rate and yield stress, which is the amount of force required to initiate the flow of a material.

The model can alternatively be calculated using yield stress, and viscosity.

$\tau^{1/2} = \tau_0^{1/2} + \kappa\gamma^{1/2}$ (2)

$\tau^{1/2} = \tau_0^{1/2} + \eta D^{1/2}$ (3)

Bingham's Model:

One of the most popular models, simplifies estimation of the shear stress by using yield stress, and viscosity

$\tau = \tau_0 + \eta \mathrm{D}$ (4)

NCA/CMA Casson Model:

Model combines useful estimation of shear stress by utilizing yield stress, an aspect ratio and the product of viscosity and shear rate.

$(1+\alpha)\tau^{1/2} = 2\tau_0^{1/2} + (1+\alpha)\,\eta\gamma^{1/2}$ (5)

Symbols

τ =Shear stress

γ = Shear rate

η = viscosity

κ = consistency index

n = flow index

α = aspect ratio

Shear thinning and shear thickening:

Shear thinning is and shear thickening are viscosity anomalies that are observed in the flow behavior of non-Newtonian materials, shear thinning is a property of some materials whose viscosity decreases as the shear stress is increased, thus shear thinning materials tend to dissipate away from the mixing shaft which produces the shearing stress. Shear thickening is the propensity of some materials to exhibit an increase in the viscosity as the shear stress is increased, thus materials that exhibit shear thickening, when mixed at high speed, tend to climb the mixer shaft rather than dissipate or move away.

Results and discussion:

Parameters studied were viscosity, yield stress, consistency index and shear sensitivity. All parameters were proportional to the chitosan grade, low for chitosan grade of 100 and high for grade of 500. Figures 3.1, 3.5 - 3.7, 3.11, 3.15 - 3.17, 3.21, 3.25 - 3.27.

For all experiments, the viscosity measurement increased with the increase in the chitosan molecular weight, and with few exceptions, there was a similar increase in viscosity with the polymer metal ratio, thus it was higher for the 500 KDA formulations. The highest increase in viscosity values was recorded for the polymer to metal ratio of 4 to 1. High viscosity readings attest to strong bonding or cohesion between the complexed ingredients, while highly viscous formulations can bind better and longer to target surfaces, however, if an ingredient is deigned to be released from the formulation, release will be inversely commensurate to the viscosity of the formulation, the higher the viscosity the slower the release. Viscosity information can impact the rate and duration of release from a formulation. Viscosity and rheological behavior affects the

absorption of drugs at site of delivery, e.g. skin, the higher the viscosity the lower will be the absorption, however, the longer the drug delivery time.

Formulation flow properties are crucial for dispensing and affect the ease of application or spreading. The flow properties were studied by fitting the data to the rheological mathematical models of Bingham, Casson, NCA/Casson and the Power Law, the power law flow indices were less than 1 for the control samples as well as the six salts mixtures which indicates a pseudo plastic behavior for the chitosan complexes regardless of the complexing agent, (Mucha, M., 1998) have reported similar shear thinning behavior for chitosan complexes with acetic acid.

Viscosity data from low molecular weight chitosan complexes were not different from that of the polymer alone. However, the medium molecular weight chitosan complex data showed a steady decrease in viscosity with increasing the salt concentration. For example, the viscosity was 5.13 x 10^3cps upon mixing 0.8 mmole of ferrous sulfate with 8 g 4% (w/w) chitosan solution, whereas when mixed with 0.2 mmole of the same salt the viscosity was 9.61 x 10^3cps. Similar profiles were observed for calcium chloride and magnesium chloride, the decrease in viscosity indicates chitosan chain de-entanglement (Hwang et al., 2000).

Ogawa and group (Ogawa et al., 1984) and (Nieto et al., 1992) have argued that zinc chloride is complexed with chitosan through a pendant-like arrangement, where a molecule of zinc chloride is attached to a single amino molecule from chitosan in a 1:1 pendant model.

Sodium and calcium complexes behave differently due to the difference in charge strength, calcium having stronger charge will support larger particles whereas the

smaller charge of sodium will result in attracting smaller particle sizes. (Gungor, A.A. et al., 2005). Ferrous complexation results were reported in figures 3.1 to 3.10, while magnesium complexation results were reported in figures 3.11 to 3.20 and zinc complexation results were reported in figures 3.21 to 3.30.

Consistency index is inversely proportional to the amount of salt, the higher the amount of salt added, the lower the consistency index; consistency index defines the viscous nature of the complex, when the added salt has a lower molecular weight than the polymer, the viscosity will be lowered due to the decrease in the overall molecular weight t of the complex.

Shear thinning is exhibited due to higher alignment of molecules at high shear rates, and mis-alignment at lower rates.

Bingham and Casson models fit the data better than the rest of the models due to the yield stress expression.

One way ANOVA analysis is presented in Table 3.1 to 3.13; chitosan grade, chitosan concentration and salt type, sulfate or chloride.

Viscosity Results: Tables 3.1, 3.5, and 3.8 shows statistical significance, PR>f was 0.0001for chitosan grade and concentration as well as salt type for tested salts. Refer to figures 3.1, 3.5 - 3.7, 3.11, 3.15 - 3.17, 3.21, 3.25 - 3.27.

Yield Stress Results: Tables 3.2, 3.6, and 3.10 shows yield stress for the ferrous, magnesium and zinc salts, when complexed with magnesium salts of sulfate and chloride as well as zinc sulfate and chloride, chitosan grade, concentration were reported to be statistically significant, however, yield stress results for ferrous sulfate and chloride were significant for chitosan grade and concentration, but were not

significant for salt type with reported Pr>F value of 0.1694. Refer to figures 3.1, 3.2, 3.6, and 3.10.

Consistency Index Results: Tables 3.3, 3.7, and 3.11, magnesium and zinc complexes with chitosan were significant Pr>F was <0.0001 for chitosan grade, concentration as well as salt type, while ferrous salt complexes only chitosan grade and concentration were significant, salt type was insignificant at Pr>F value of 0.0181.

Shear Sensitivity Results: Tables 3.4, 3.8, and 3.12, zinc salts complexation with chitosan had proved significant for chitosan grade, concentration and salt type, all have a reported Pr>F of <0.0001, Refer to figures 3.21 to 3.30; for magnesium complexes only chitosan concentration and salt type were significant at Pr>F values of <0.0001, while the chitosan grade was reported insignificant at 0.021, shear sensitivity results for ferrous salts with chitosan were reported to have only the chitosan concentration as significant at Pr>F value of <0.0001.while chitosan grade and salt type were reported insignificant with Pr>F values of 0.6672 and 0.2701 respectively.

Conclusions:

Chitosan can form complexes with metal salts.

Quality of complexes depend on molecular interaction between chitosan.

Rheological models allow for controlling the complex formation between chitosan and other compounds.

Viscosity, yield stress, consistency indices, and shear sensitivities can be defined by use of rheological models of Bingham, Casson, and Power Law.

Bingham and Power Law models are suitable for use with chitosan due to the use of yield stress.

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Table 3.1: Mean squares and variance components for viscosities for chitosan salts with Fe chloride and Fe sulfate

Source	*DF*	*MS*	*F*	*Pr>F*
Chitosan grd	*1*	*17124113.17*	*210.44*	*<.0001*
Chitosan conc	*2*	*202677.42*	*2.49*	*0.0887*
Salt type	*2*	*6269838.66*	*77.05*	*<.0001*
Salt conc	*1*	*61047.07*	*0.75*	*0.3888*
Chitosangrd x Chitosanconc	*2*	*150474.48*	*1.85*	*0.1634*
Chitosangrd x salttype	*2*	*4369506.28*	*53.7*	*<.0001*
Chitosangrd x saltlevel	*1*	*18604.69*	*0.23*	*0.6337*
Chitosanconc x salttype	*4*	*111743.84*	*1.37*	*0.2497*
Chitosanconc x saltlevel	*2*	*539937.99*	*6.64*	*0.0021*
Salttype x saltlevel	*2*	*156872.47*	*1.93*	*0.1516*
Error	*88*	*7160721.55*		

Table 3.2: Mean squares and variance components for yield stress results for chitosan salts with Fe chloride and Fe sulfate

Source	DF	MS	F	Pr>F
Chitosangrd	1	40318.13541	26.63	<.0001
Chitosanconc	2	20421.74318	13.49	<.0001
Salttype	2	2743.15502	1.81	0.1694
Saltlevel	1	13167.85001	8.7	0.0041
Chitosangrd x chitosanconc	2	9177.98151	6.06	0.0034
Chitosangrd x salttype	2	2860.37626	1.89	0.1572
Chitosangrd x saltlevel	1	353.05901	0.23	0.6303
Chitosanconc x salttype	4	6810.08774	4.5	0.0024
Chitosanco x saltlevel	2	3387.85436	2.24	0.1127
Salttype x saltlevel	2	1460.01591	0.96	0.3852
Error	88	133219.5592		

Table 3.3: Mean squares and variance components for consistency indices for chitosan salts with Fe chloride and Fe sulfate

Source	DF	MS	F	Pr>F
Chitosangrd	1	419032008.6	25.46	<.0001
Chitosanconc	2	203375902.3	12.36	<.0001
Salttype	2	69091379.9	4.2	0.0181
Saltlevel	1	129525580.8	7.87	0.0062
Chitosang x chitosanco	2	80798292.7	4.91	0.0095
Chitosangra x salttype	2	67102396.9	4.08	0.0203
Chitosangr x saltlevel	1	2704935.6	0.16	0.6862
Chitosancon x salttype	4	73823837.3	4.49	0.0024
Chitosanco x saltlevel	2	28242264.4	1.72	0.1857
Salttype x saltlevel	2	20833967.7	1.27	0.2871
Error	88	1448257061		

Table 3.4: Mean squares and variance components for shear sensitivities for chitosan salts with Fe chloride and Fe sulfate

Source	DF	MS	F	Pr>F
chitosangrd	1	0.01020833	0.19	0.6672
chitosanconc	2	0.54035833	9.85	0.0001
Salttype	2	0.07285833	1.33	0.2701
Saltlevel	1	0.858675	15.66	0.0002
Chitosangrd x chitosanconc	2	0.04840833	0.88	0.4172
Chitosangrd x salttype	2	0.49725833	9.07	0.0003
Chitosangrd x saltlevel	1	0.00140833	0.03	0.873
Chitosanconc x salttype	4	0.12115833	2.21	0.0744
Chitosanconc x saltlevel	2	0.206725	3.77	0.0269
Salttype x saltlevel	2	0.025675	0.47	0.6277
Error	88	4.8254		

Table 3.5: Mean squares and variance components for viscosity results for chitosan salts with Mg chloride and Mg sulfate

Source	DF	MS	Pr>F	Pr>F
Chitosangrd	1	1.95E+08	248.95	<.0001
Chitosanconc	2	36763693	46.92	<.0001
Salttype	2	45258921	57.76	<.0001
Saltlevel	1	34359783	43.85	<.0001
Chitosangrd x chitosanconc	2	35948731	45.88	<.0001
Chitosangrd x salttype	2	32197081	41.09	<.0001
Chitosangrd x saltlevel	1	23780558	30.35	<.0001
Chitosanconc x salttype	4	7798077	9.95	<.0001
Chitosanconc x saltlevel	2	5301047	6.77	0.0018
Salttype x saltlevel	2	6201880	7.92	0.0007
Error	88	783507.6		

Table 3.6: Mean squares and variance components for yield stress results for chitosan salts with Mg chloride and Mg sulfate

Source	DF	MS	F	Pr>F
Chitosangrd	1	447258.2	64.81	<.0001
Chitosanconc	2	36512.09	5.29	0.0068
Salttype	2	320562.6	46.45	<.0001
Saltlevel	1	45596.34	6.61	0.0118
Chitosangrd x chitosanconc	2	64657.62	9.37	0.0002
Chitosangrd x salttype	2	79646.36	11.54	<.0001
Chitosangrd x saltlevel	1	45621	6.61	0.0118
Chitosanconc x salttype	4	58174.2	8.43	<.0001
Chitosanco x saltlevel	2	91773.74	13.3	<.0001
Salttype x saltlevel	2	31284.64	4.53	0.0134
Error	88	607330.7		

Table 3.7: Mean squares and variance components fo consistency indices for chitosan salts with Mg chlorid and Mg sulfate

Source	DF	MS	F	Pr>F
Chitosangrd	1	2.18E+09	16.66	<.0001
Chitosanconc	2	7.39E+08	5.64	0.005
Salttype	2	3.26E+09	24.83	<.0001
Saltlevel	1	2.23E+08	1.7	0.1952
Chitosangrd x chitosanconc	2	3.54E+08	2.7	0.0729
Chitosangrd x salttype	2	6.81E+08	5.19	0.0074
Chitosangrd x saltlevel	1	2.35E+08	1.79	0.184
Chitosanconc x salttype	4	1.89E+09	14.44	<.0001
Chitosanconc x saltlevel	2	6.65E+08	5.07	0.0082
Salttype x saltlevel	2	1.77E+09	13.48	<.0001
Error	88	1.15E+10		

Table 3.8: Mean squares and variance components for shear sensitivities for chitosan salts with Mg chloride and Mg sulfate

Source	DF	MS	F	Pr>F
Chitosangrd	1	541.229	6.18	0.0148
Chitosanconc	2	460.4866	5.26	0.007
Salttype	2	467.736	5.34	0.0065
Saltlevel	1	499.6171	5.7	0.0191
Chitosangrd x chitosanconc	2	480.2021	5.48	0.0057
Chitosangrd x salttype	2	496.2738	5.67	0.0048
Chitosangrd x saltlevel	1	479.6881	5.48	0.0215
Chitosanconc x salttype	4	487.4397	5.56	0.0005
Chitosanconc x saltlevel	2	465.801	5.32	0.0066
Salttype x saltlevel	2	477.2085	5.45	0.0059
Error	88	7708.413		

Table 3.9: Mean squares and variance components for viscosity results for chitosan salts with Zn chloride and Zn sulfate

Source	DF	MS	F	Pr>F
Chitosangrd	1	3.05E+08	1880.49	<.0001
Chitosanconc	2	57944857	356.77	<.0001
Salttype	2	2243520	13.81	<.0001
Saltlevel	1	48413420	298.08	<.0001
Chitosangrd x chitosanconc	2	59084944	363.78	<.0001
Chitosangrd x salttype	2	912007.1	5.62	0.0051
Chitosangrd x saltlevel	1	47359220	291.59	<.0001
Chitosanconc x salttype	4	176964.4	1.09	0.3667
Chitosanconc x saltlevel	2	5257089	32.37	<.0001
Salttype x saltlevel	2	172954.2	1.06	0.3492
Error	88	162417.3		

Table 3.10: Mean squares and variance components fo yield stress results for chitosan salts with Zn chloride and Zn sulfate

Source	DF	MS	F	Pr>F
Chitosangrd	1	557847.8	347.44	<.0001
Chitosanconc	2	122776.8	76.47	<.0001
Salttype	2	24140.85	15.04	<.0001
Saltlevel	1	130523.1	81.29	<.0001
Chitosangrd x chitosanconc	2	164219.9	102.28	<.0001
Chitosangrd x salttype	2	2435.586	1.52	0.2251
Chitosangrd x saltlevel	1	154607.4	96.29	<.0001
Chitosanconc x salttype	4	530.6585	0.33	0.8568
Chitosanconc x saltlevel	2	46967.7	29.25	<.0001
Salttype x saltlevel	2	1332.632	0.83	0.4394
Error	88	1605.616		

Table 3.11: Mean squares and variance components fo consistency indices for chitosan salts with Zn chloride and Zn sulfate

Source	DF	MS	F	Pr>F
Chitosangrd	1	2.54E+09	418.14	<.0001
Chitosanconc	2	5.38E+08	88.61	<.0001
Salttype	2	1.02E+08	16.85	<.0001
Saltlevel	1	5.58E+08	91.87	<.0001
Chitosangrd x chitosanconc	2	7.74E+08	127.5	<.0001
Chitosangrd x salttype	2	2372032	0.39	0.6777
Chitosangrd x saltlevel	1	6.75E+08	111.19	<.0001
Chitosanconc x salttype	4	1306963	0.22	0.9293
Chitosanconc x saltlevel	2	1.69E+08	27.87	<.0001
Salttype x saltlevel	2	3246249	0.53	0.5877
Error	88	6070894		

Table 3.12:Mean squares and variance components fo shear sensitivities for chitosan salts with Zn chloride and Zn sulfate

Source	DF	MS	F	Pr>F
Chitosangrd	1	0.421875	21.92	<.0001
Chitosanconc	2	0.284558	14.78	<.0001
Salttype	2	0.218608	11.36	<.0001
Saltlevel	1	0.165675	8.61	0.0043
Chitosangrd x chitosanconc	2	0.191425	9.95	0.0001
Chitosangrd x salttype	2	0.099925	5.19	0.0074
Chitosangrd x saltlevel	1	0.185008	9.61	0.0026
Chitosanconc x salttype	4	0.013433	0.7	0.5954
Chitosanconc x saltlevel	2	0.028825	1.5	0.2293
Salttype x saltlevel	2	0.013075	0.68	0.5096
Error	88	0.019247		

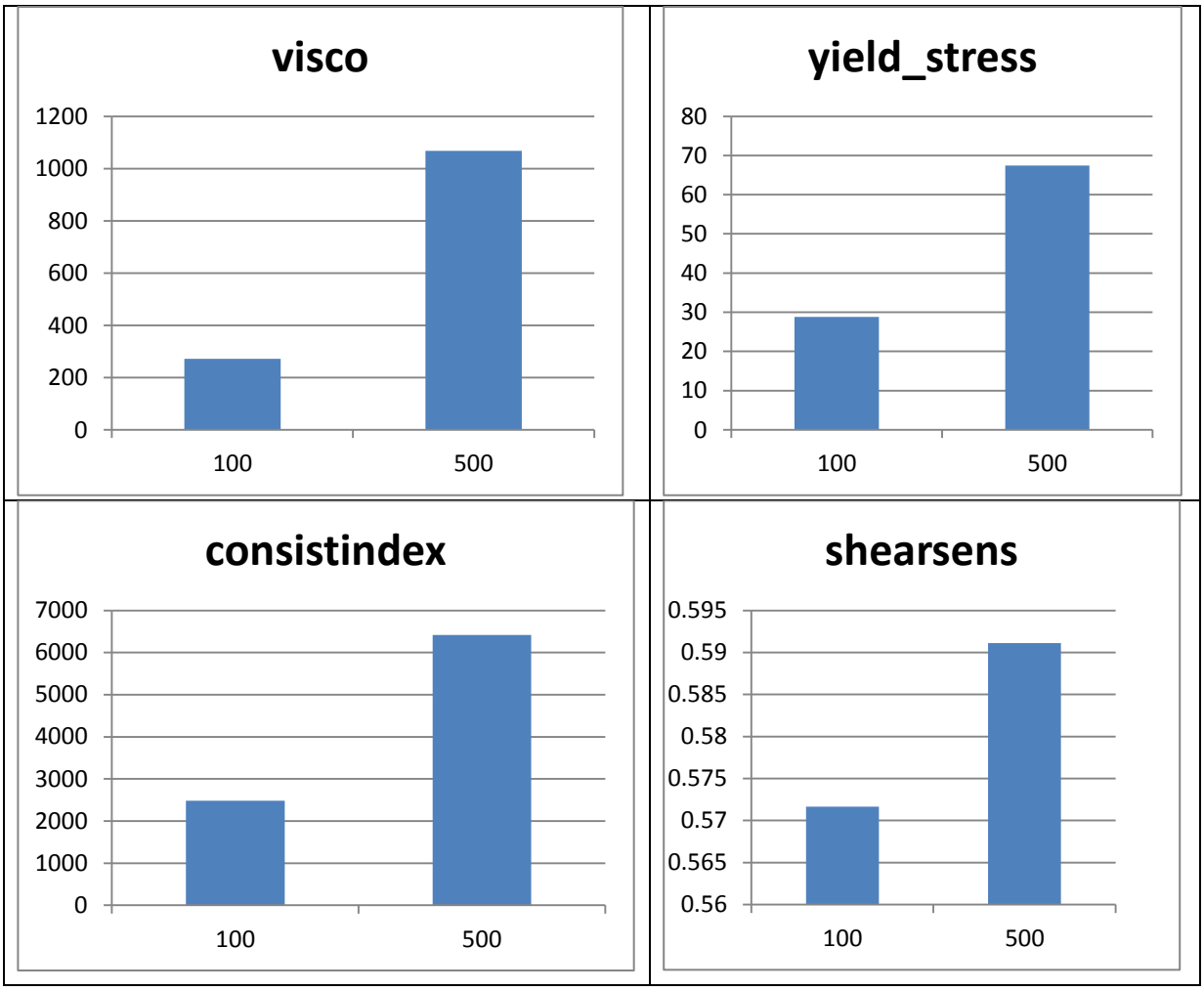

Figure 3.1: Effect of chitosan grade (100 and 500) with constant ferrous salts concentration on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

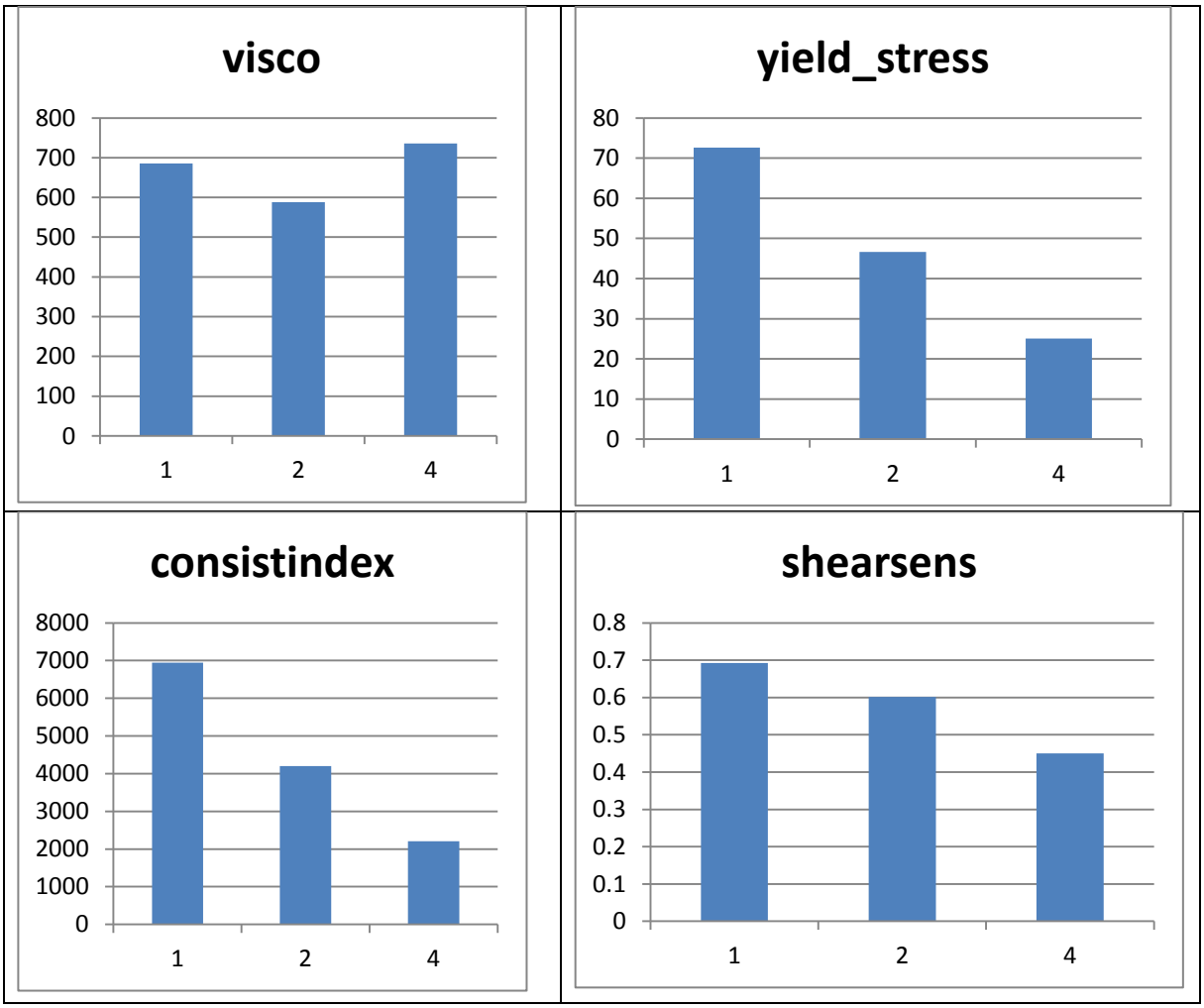

Figure 3.2: Effect of chitosan concentration (1, 2 and 4) with constant ferrous salts concentration on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

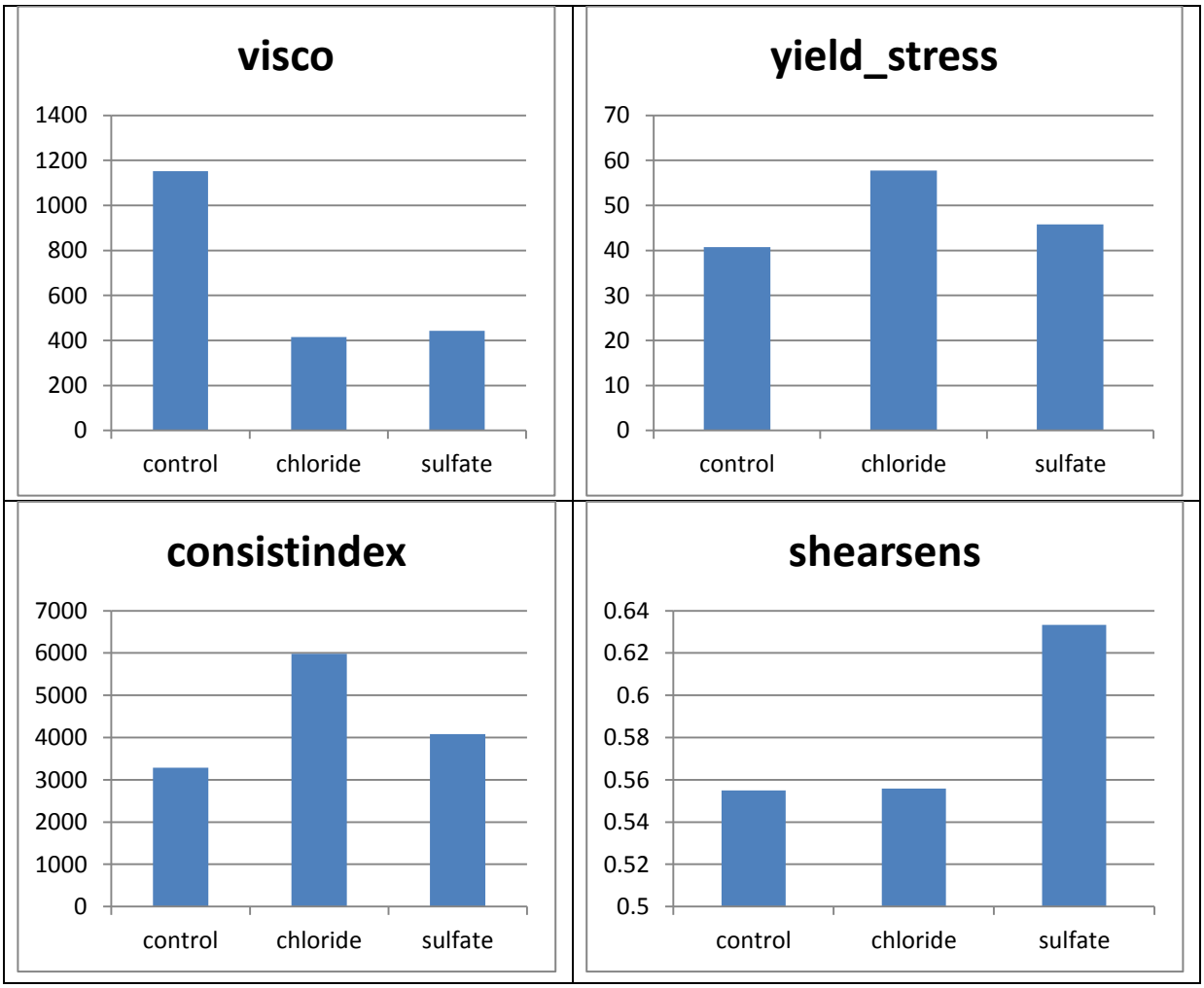

Figure 3.3: Effect of ferrous chloride (chloride) and ferrous sulfate (sulfate) on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

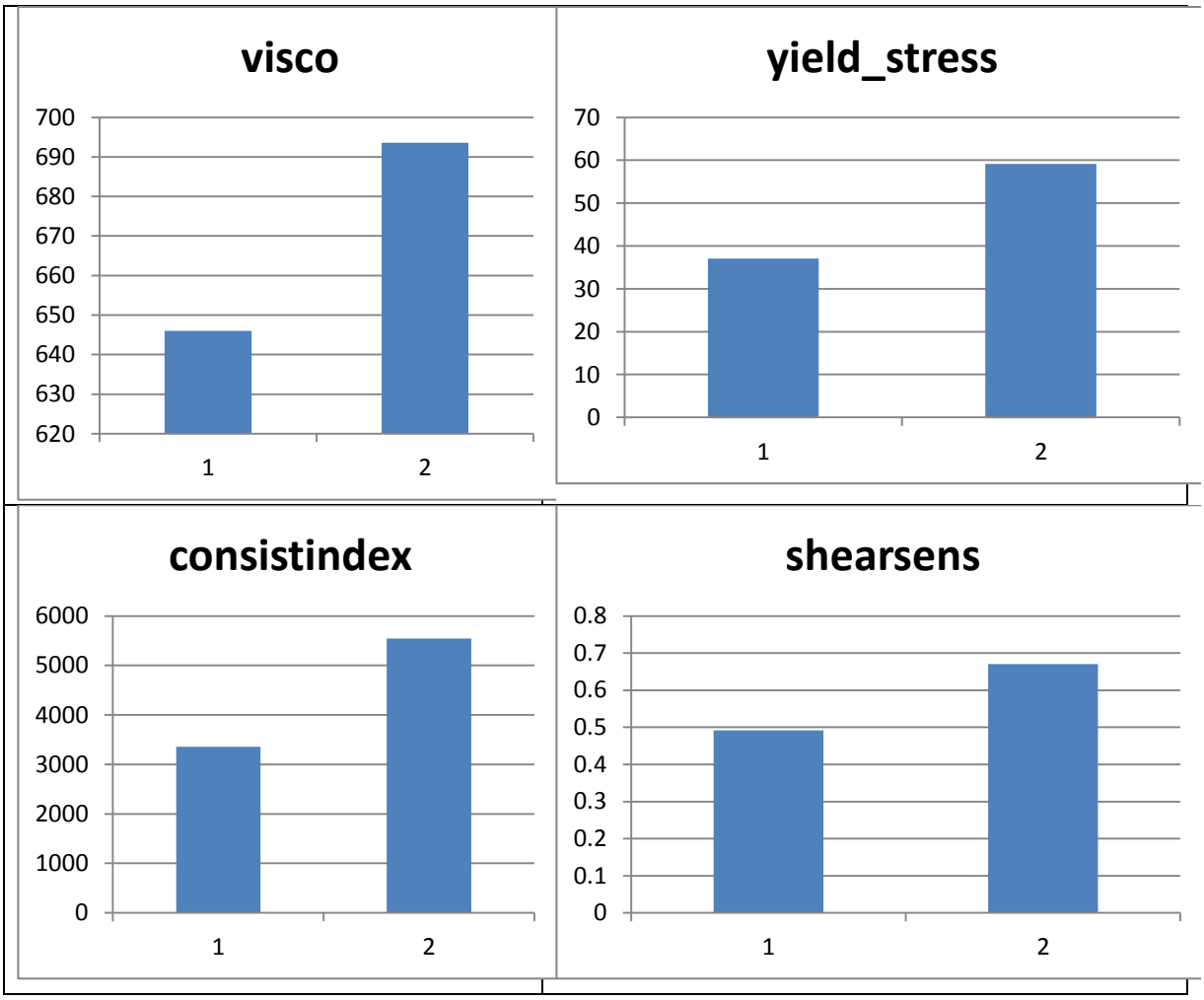

Figure 3.4: Effect of Ferrous salts concentration (1 and 2) on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens).

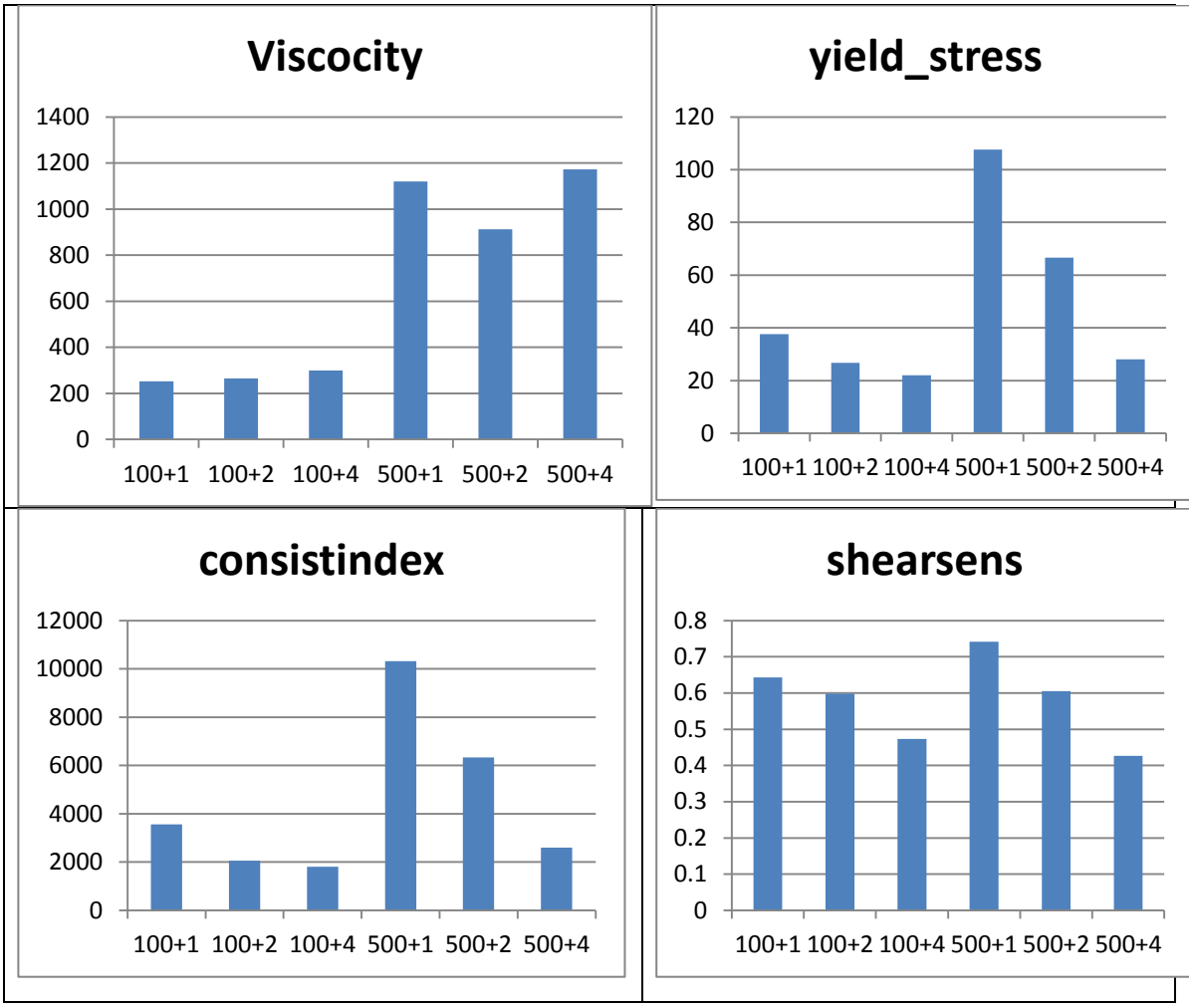

Figure 3.5: Effect of chitosan grade (100 and 500) and concentration (1, 2, and 4) with constant levels of ferrous salts on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

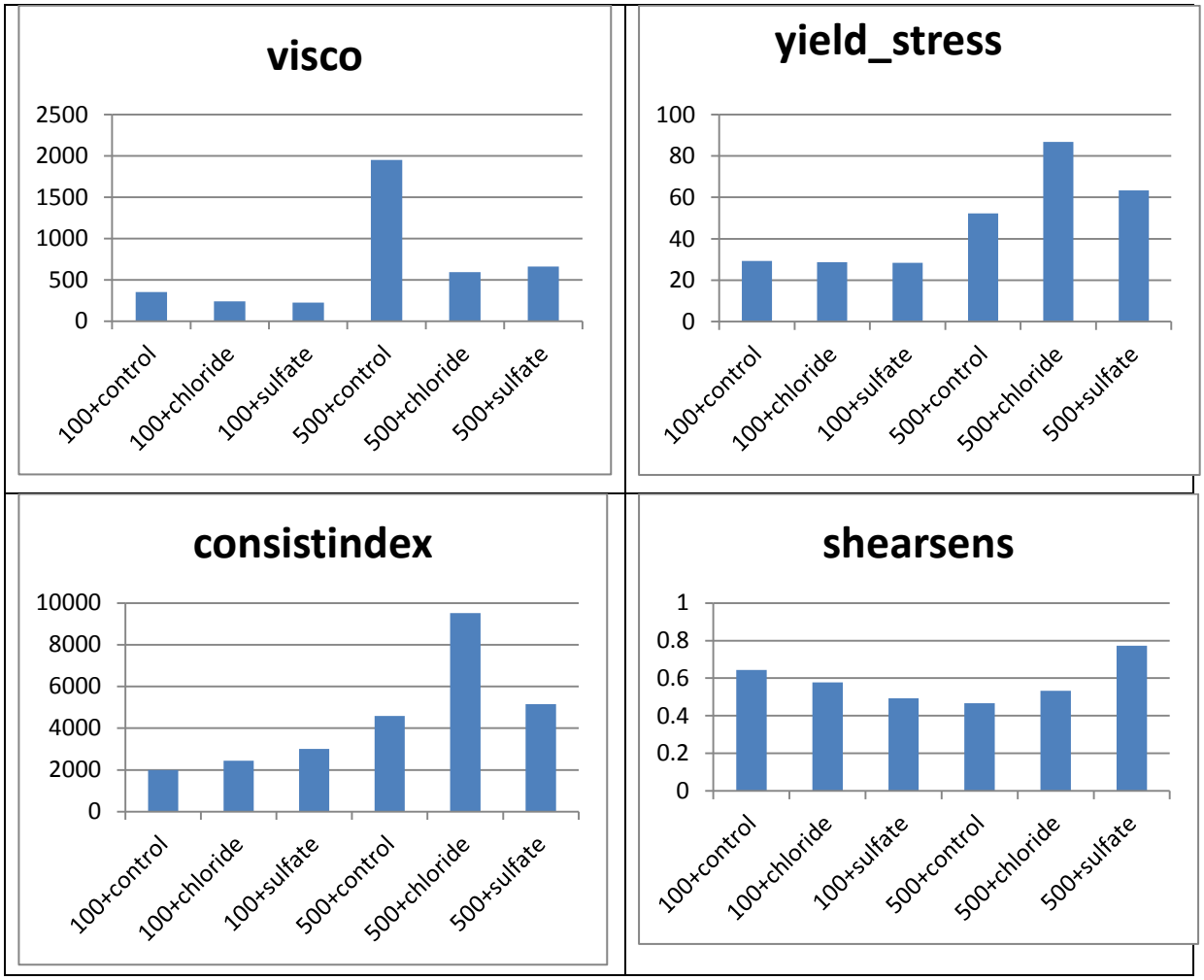

Figure 3.6: Effect of chitosan grade (100 and 500) and salt types (chloride and sulfate) on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

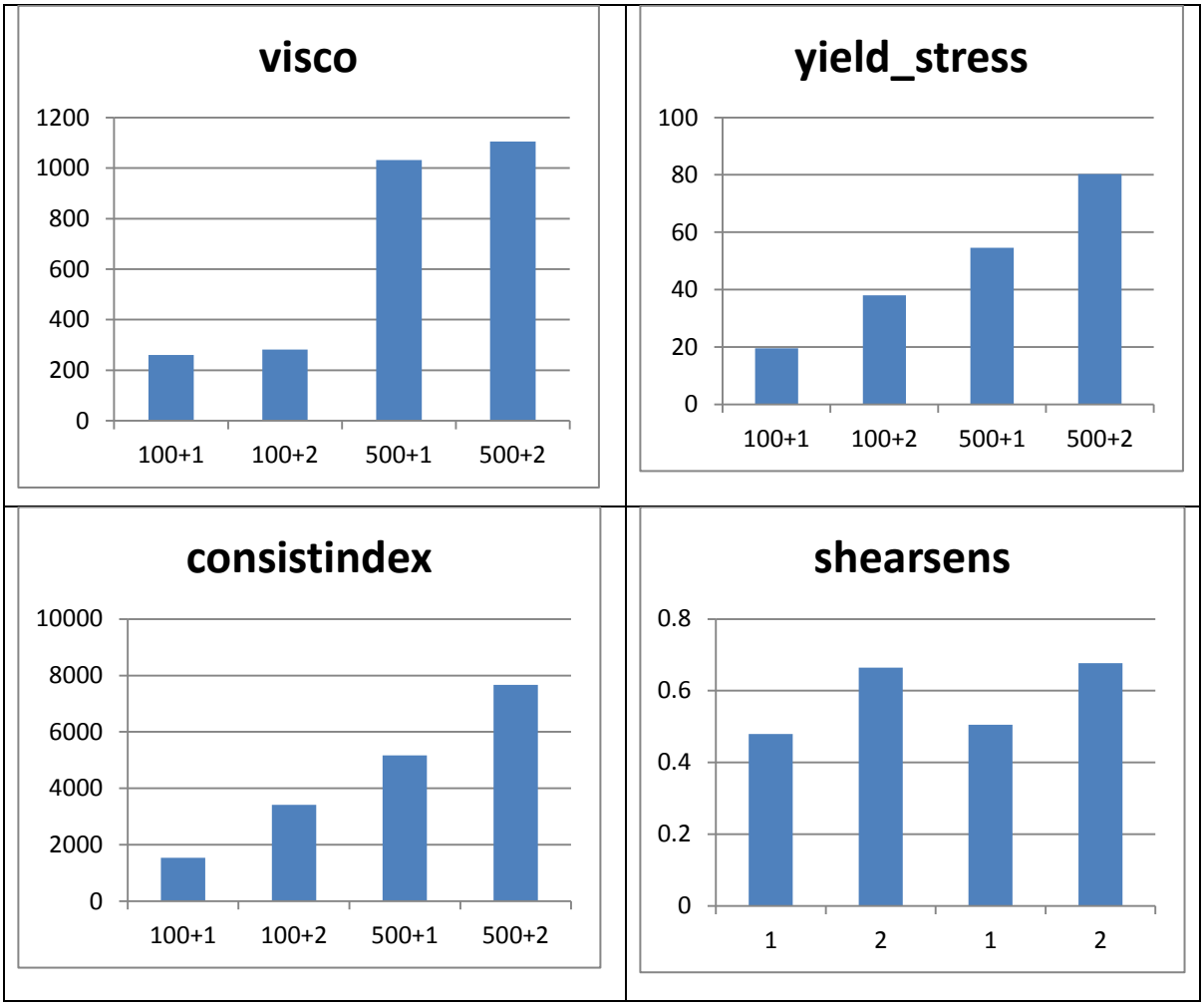

Figure 3.7: Effect of chitosan grade (100 and 500) and ferrous salt concentration (1 and 2) on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

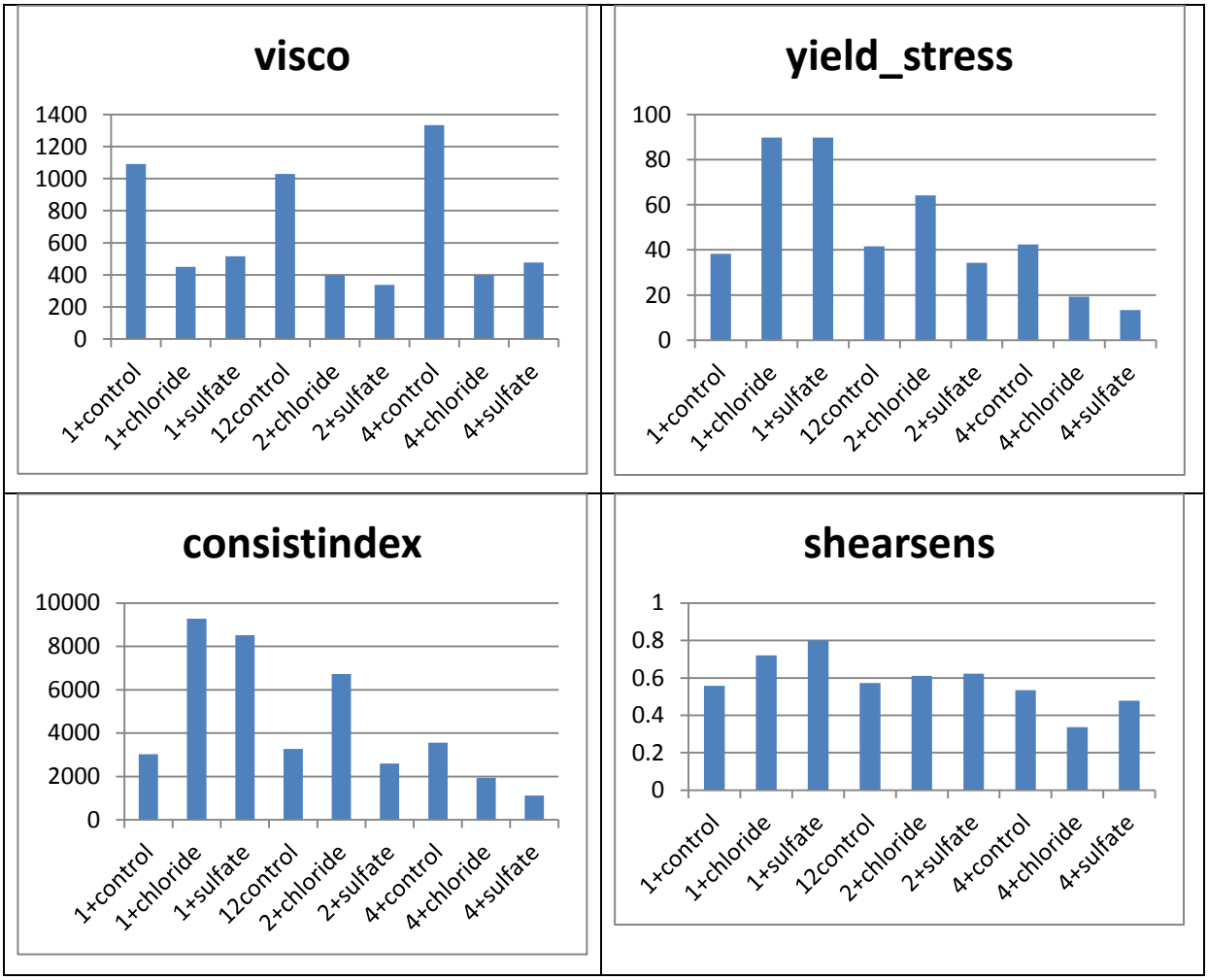

Figure 3.8: Effect of chitosan concentration (1, 2, and 4) and ferrous salt type (chloride and sulfate) on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

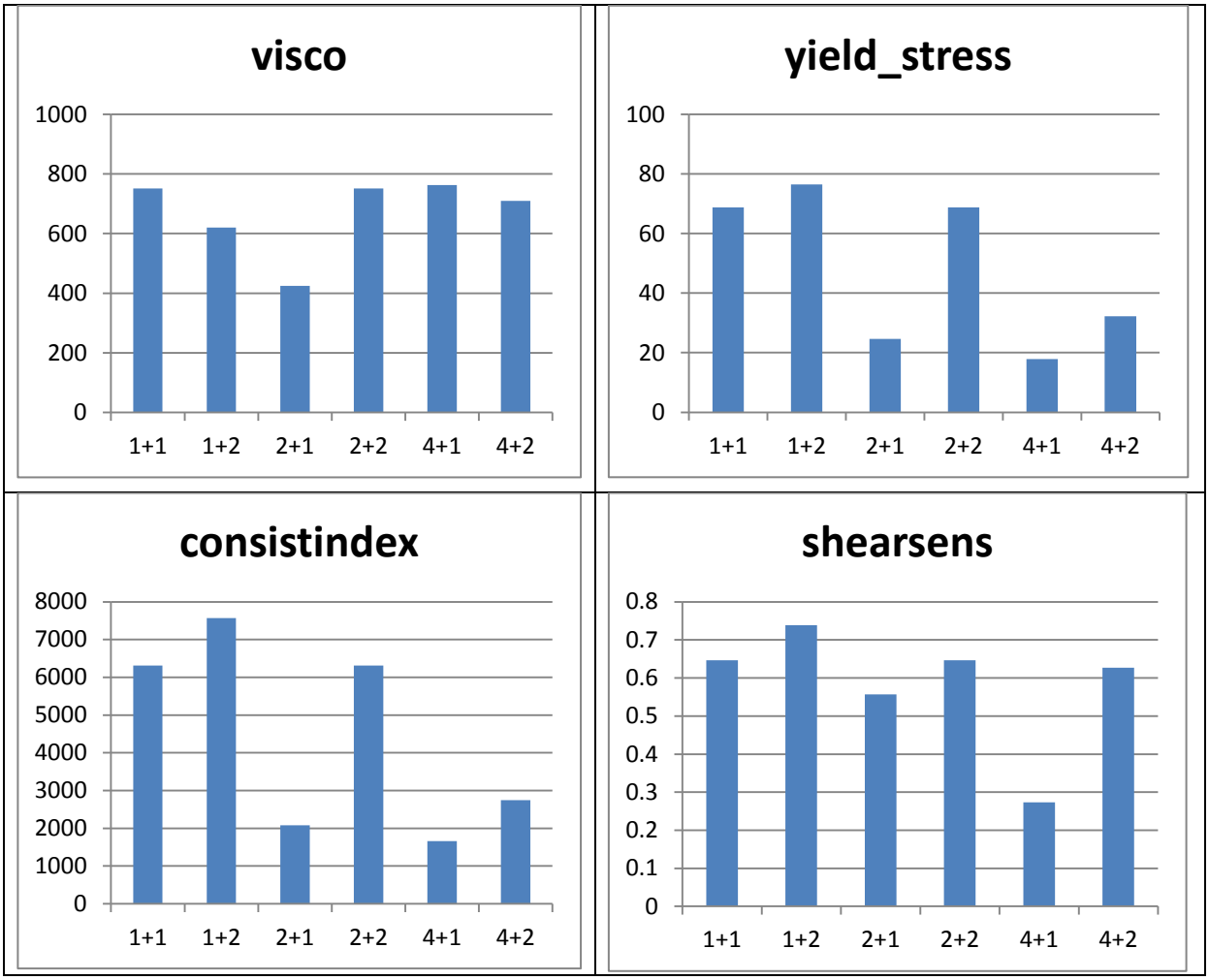

Figure 3.9: Effect of chitosan concentration (1, 2 and 4) and ferrous salt concentration (1, and 2) on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

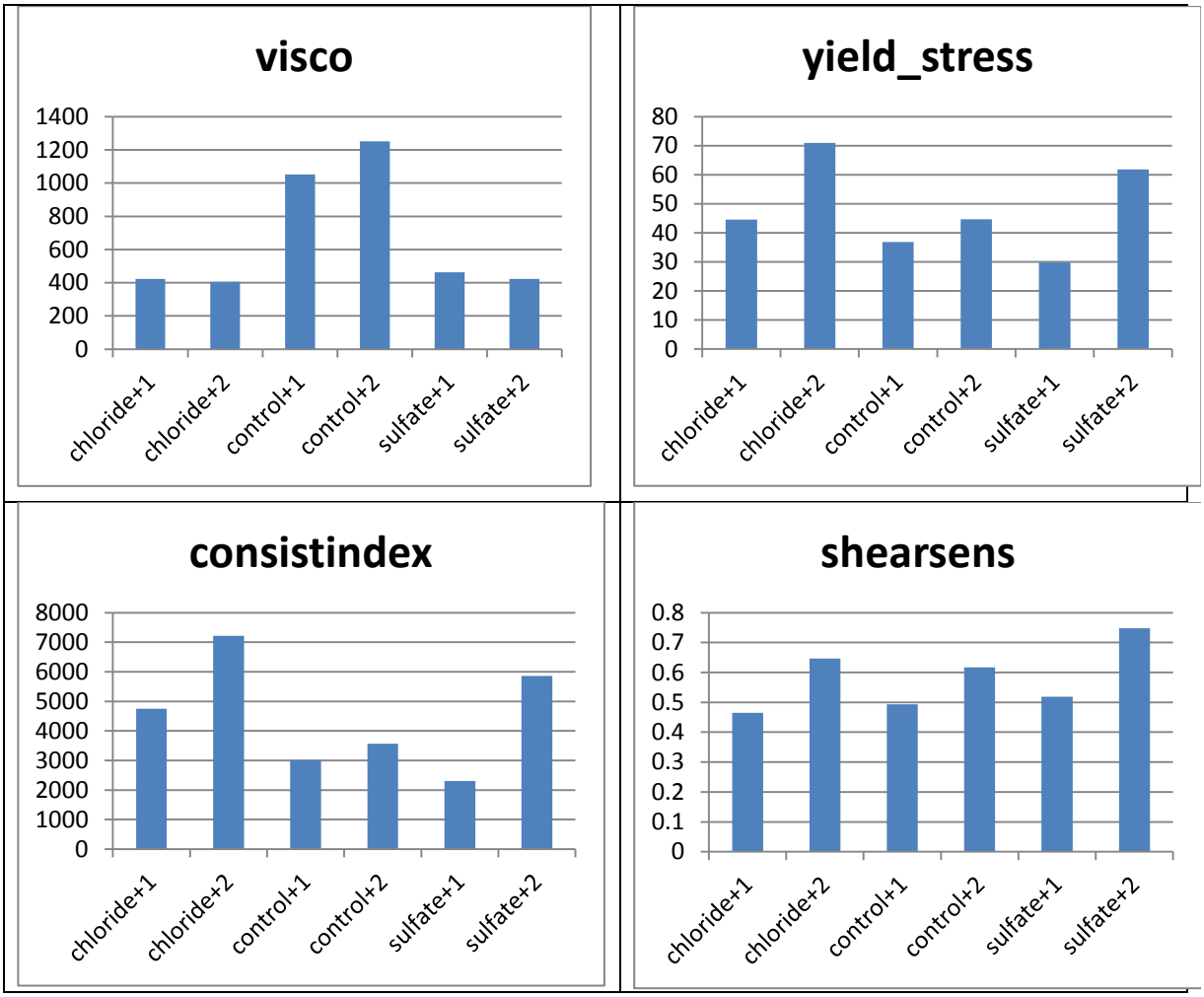

Figure 3.10: Effect of ferrous salt (chloride and sulfate) and concentration (1 and 2) with constant chitosan concentration on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

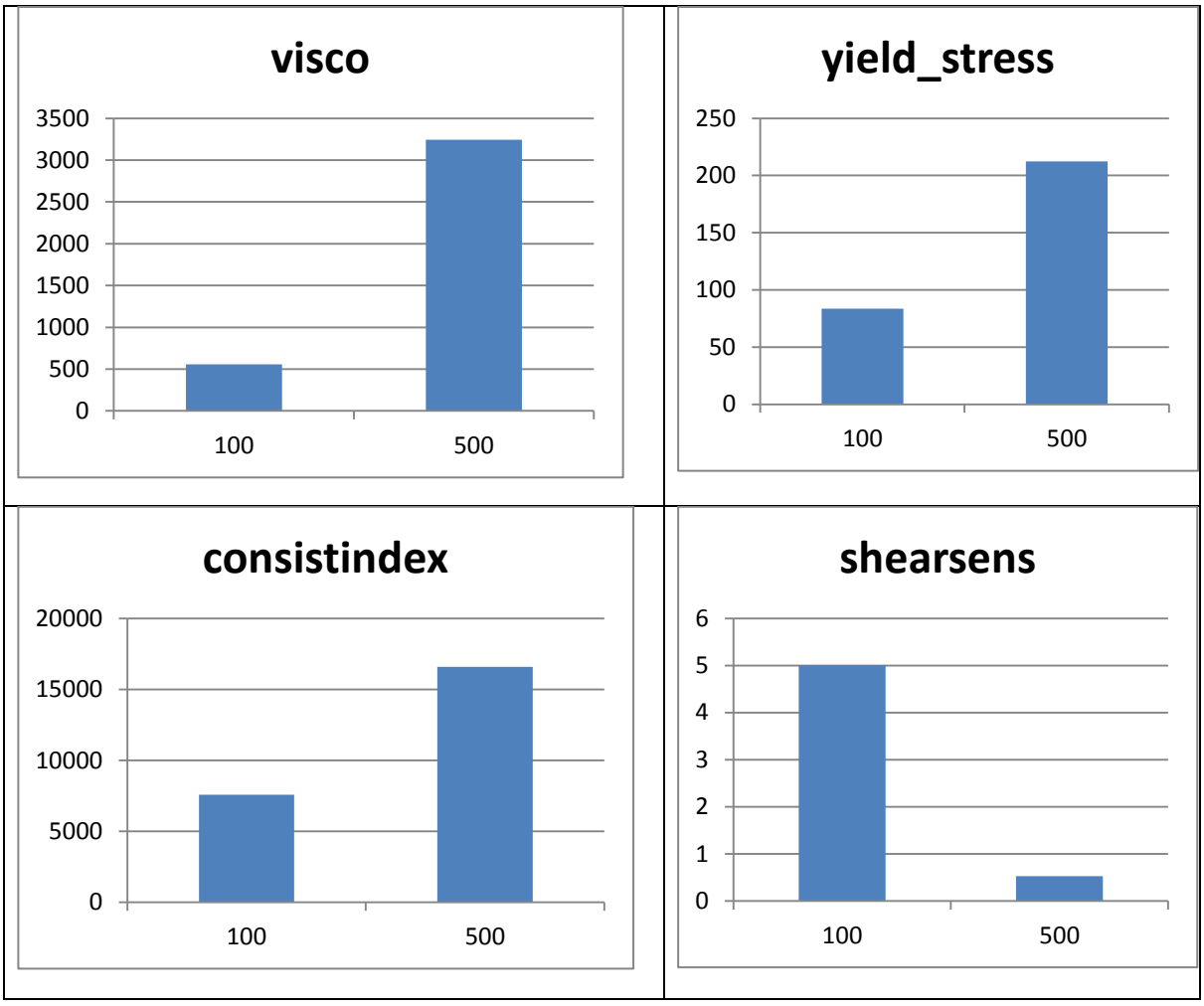

Figure 3.11: Effect of chitosan grade (100, 500) with constant magnesium concentration on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

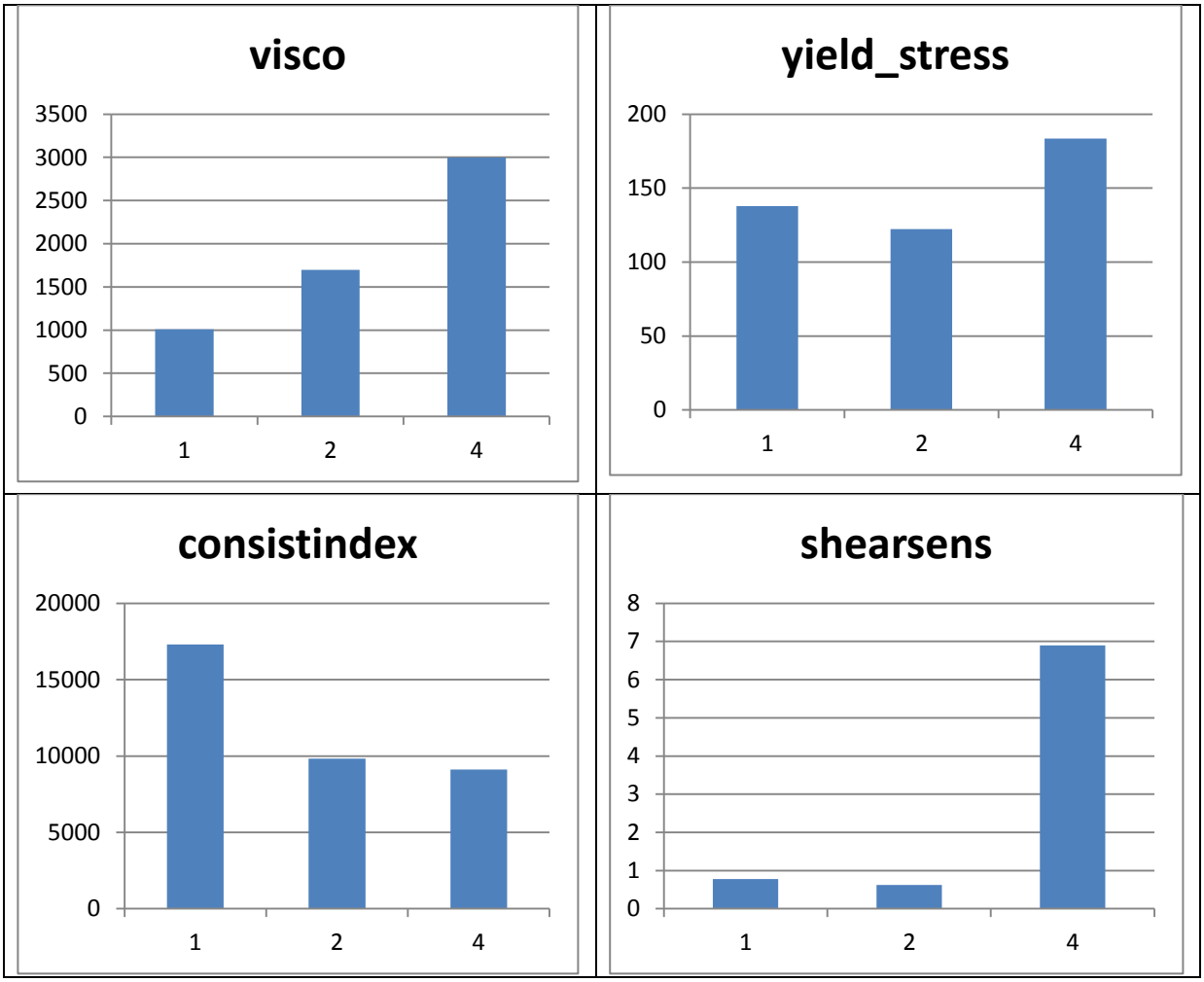

Figure 3.12: Effect of chitosan concentration (1, 2 and 4) with constant magnesium concentration on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

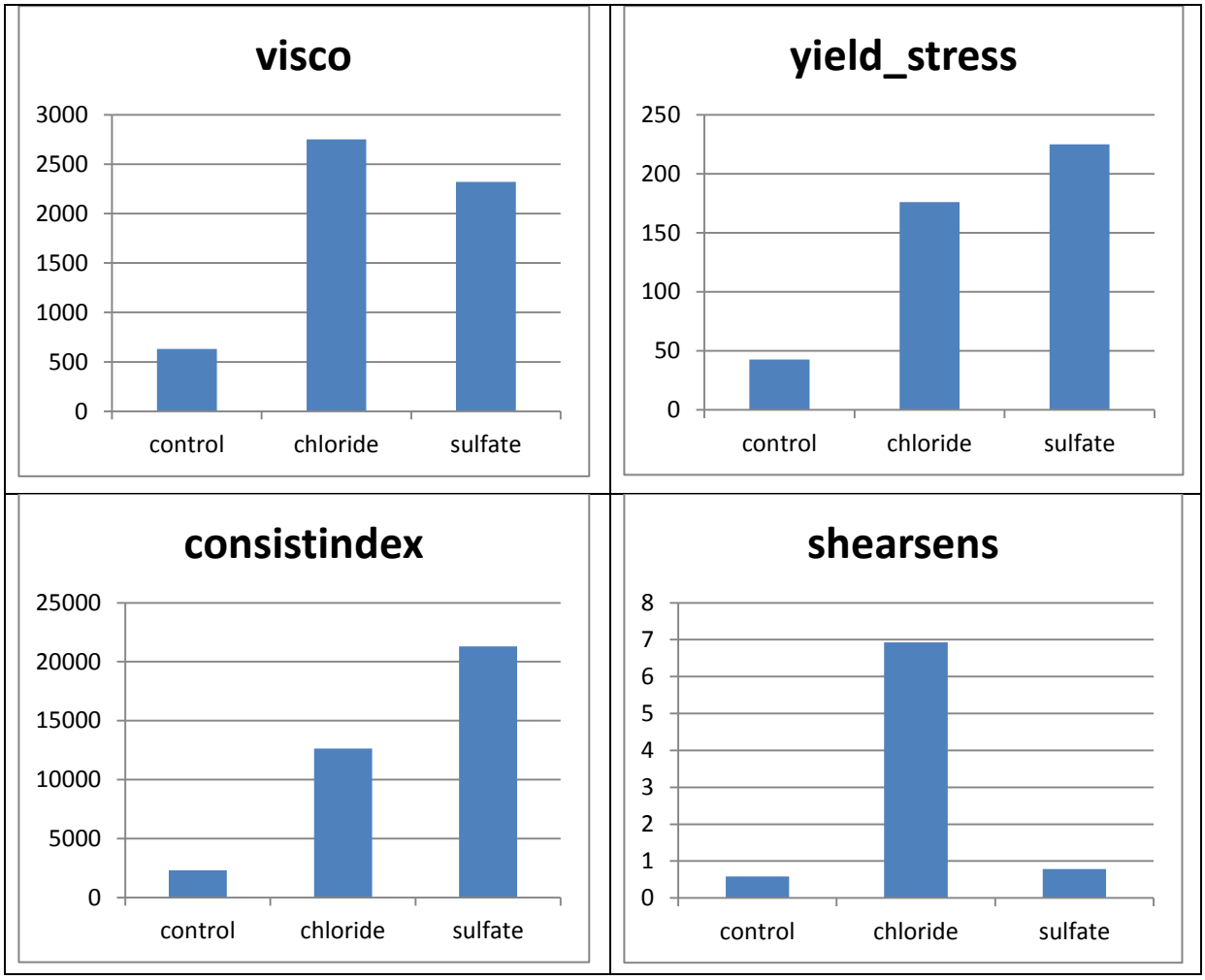

Figure 3.13: Effect of magnesium salt (chloride and sulfate) with constant chitosan concentration on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

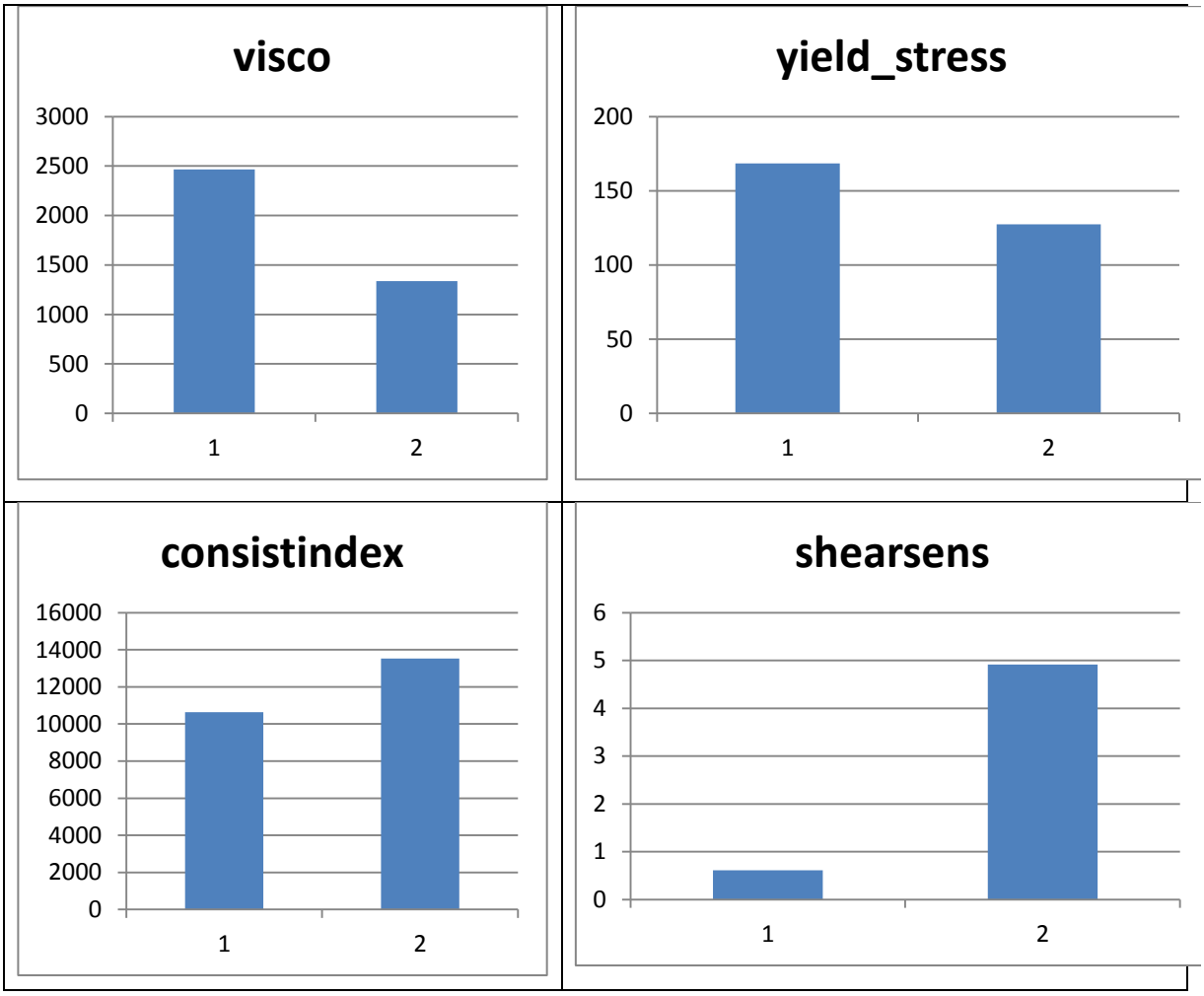

Figure 3.14: Effect of magnesium salt concentration (1 and 2) with constant chitosan concentration on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

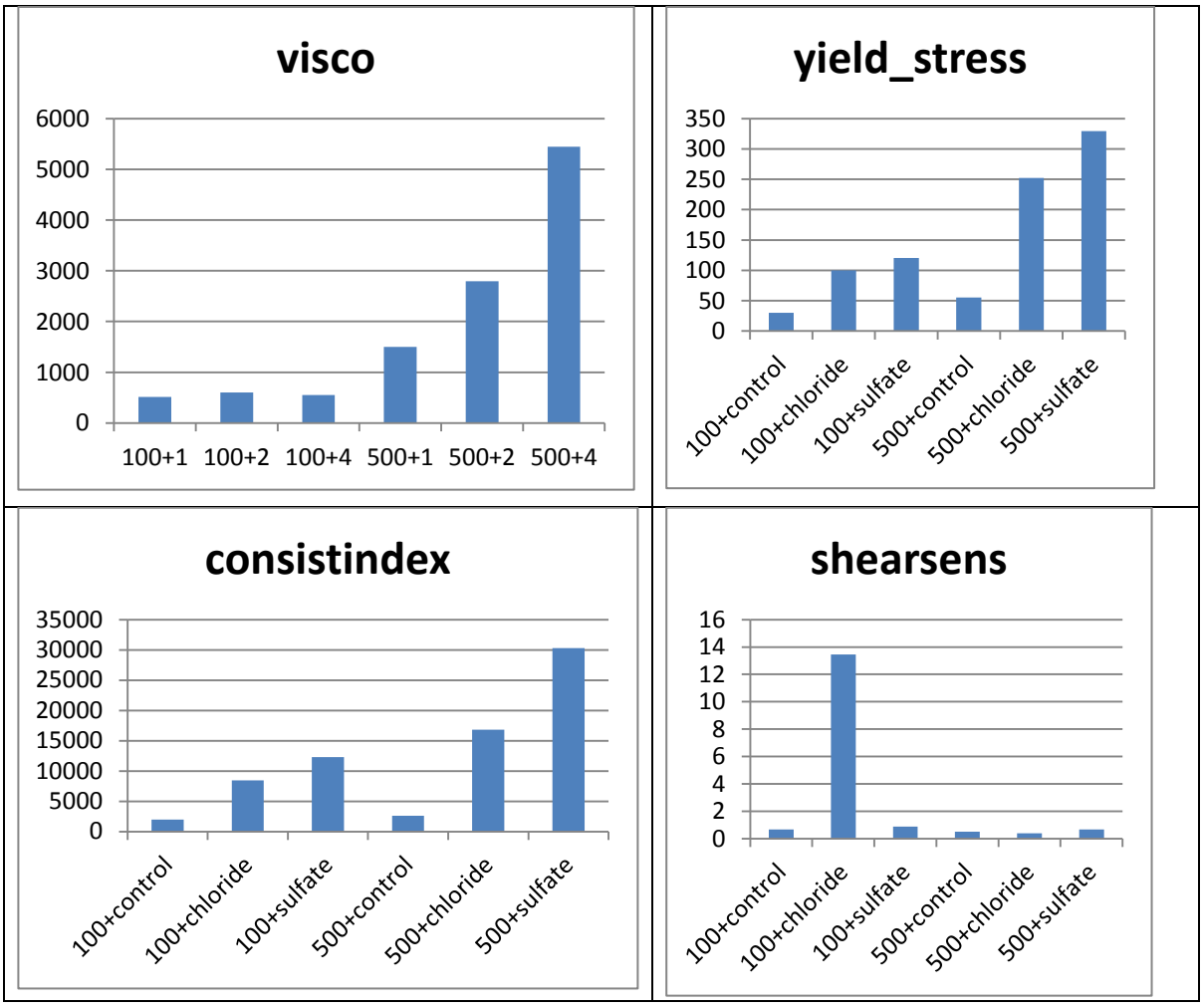

Figure 3.15: Effect of chitosan grade and concentration with constant magnesium concentration on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

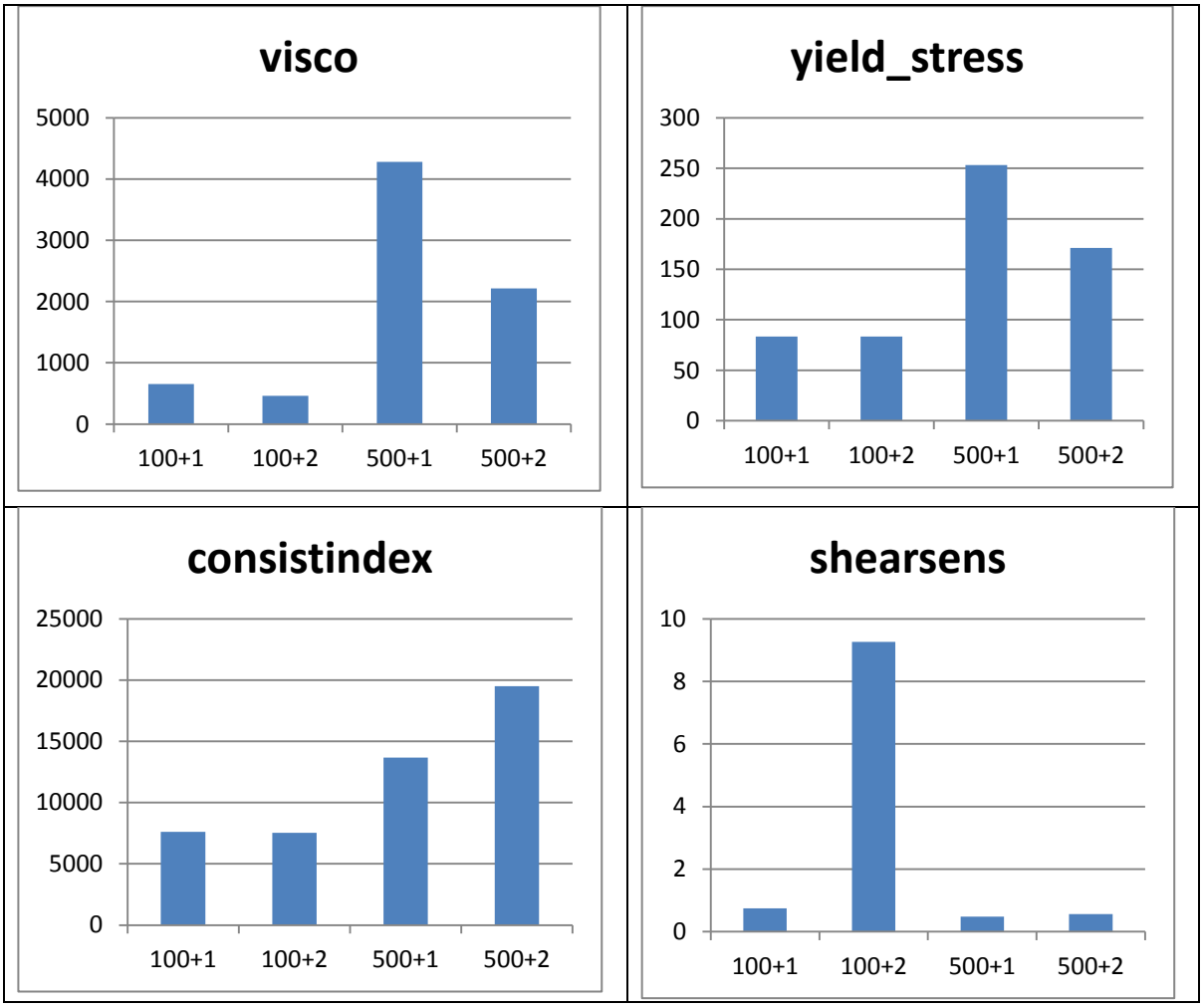

Figure 3.16: Effect of chitosan grade (100 and 500) and magnesium salt concentration (1 and 2) on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

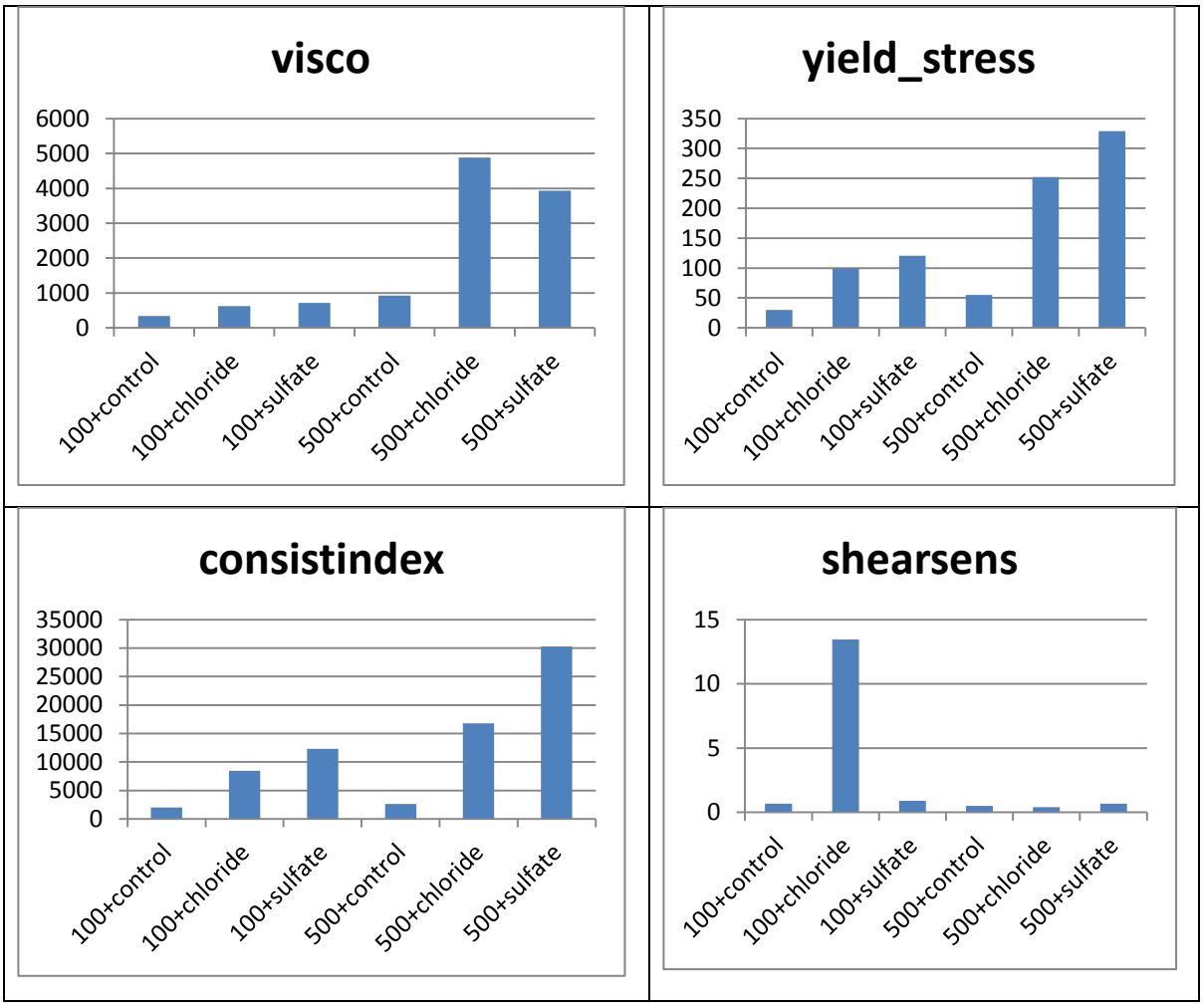

Figure 3.17: Effect of chitosan grade (100 and 500) and magnesium salt type (chloride and sulfate) on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

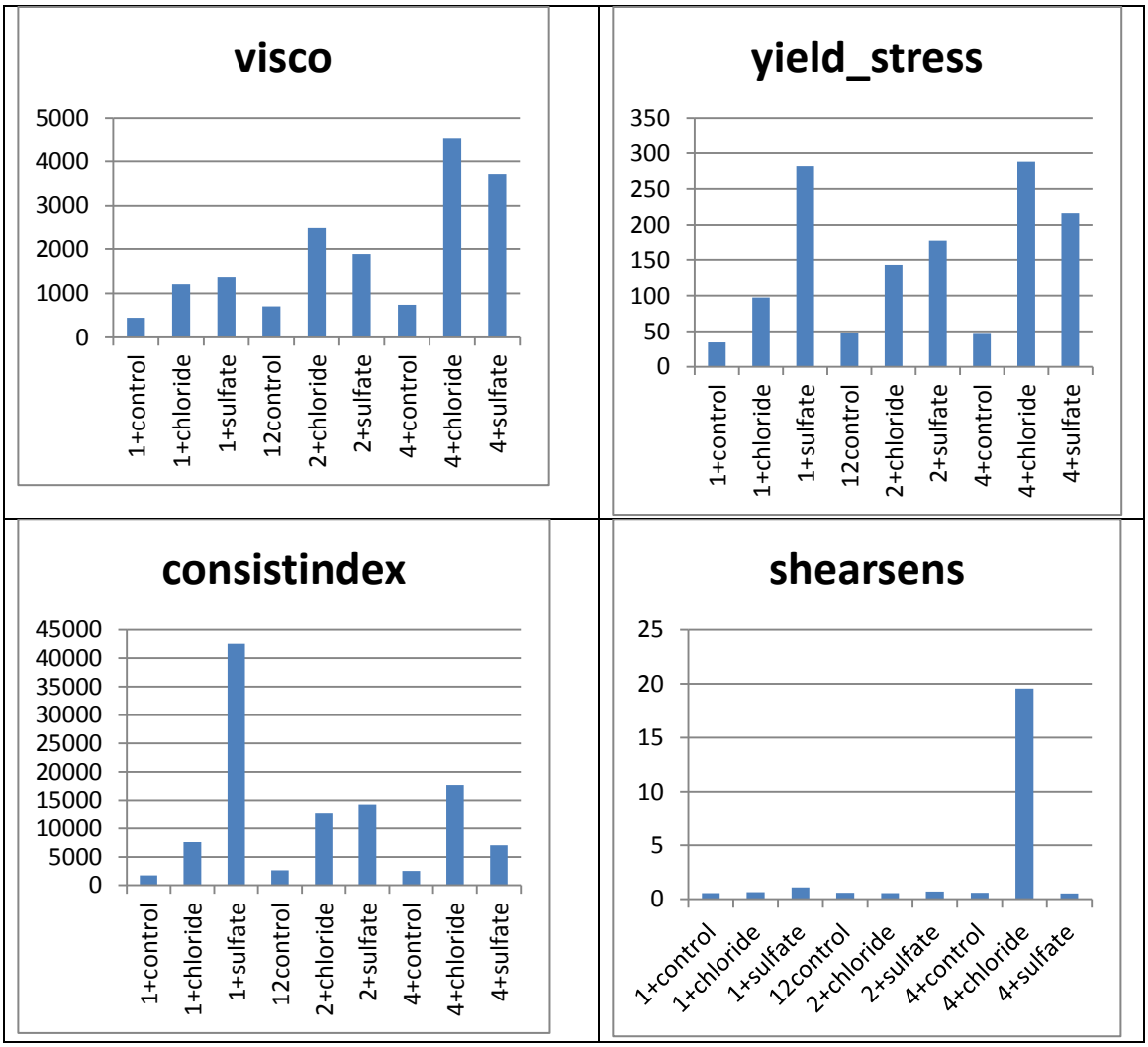

Figure 3.18: Effect of chitosan concentration (1,2 and 4) and magnesium salt type (chloride and sulfate) on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

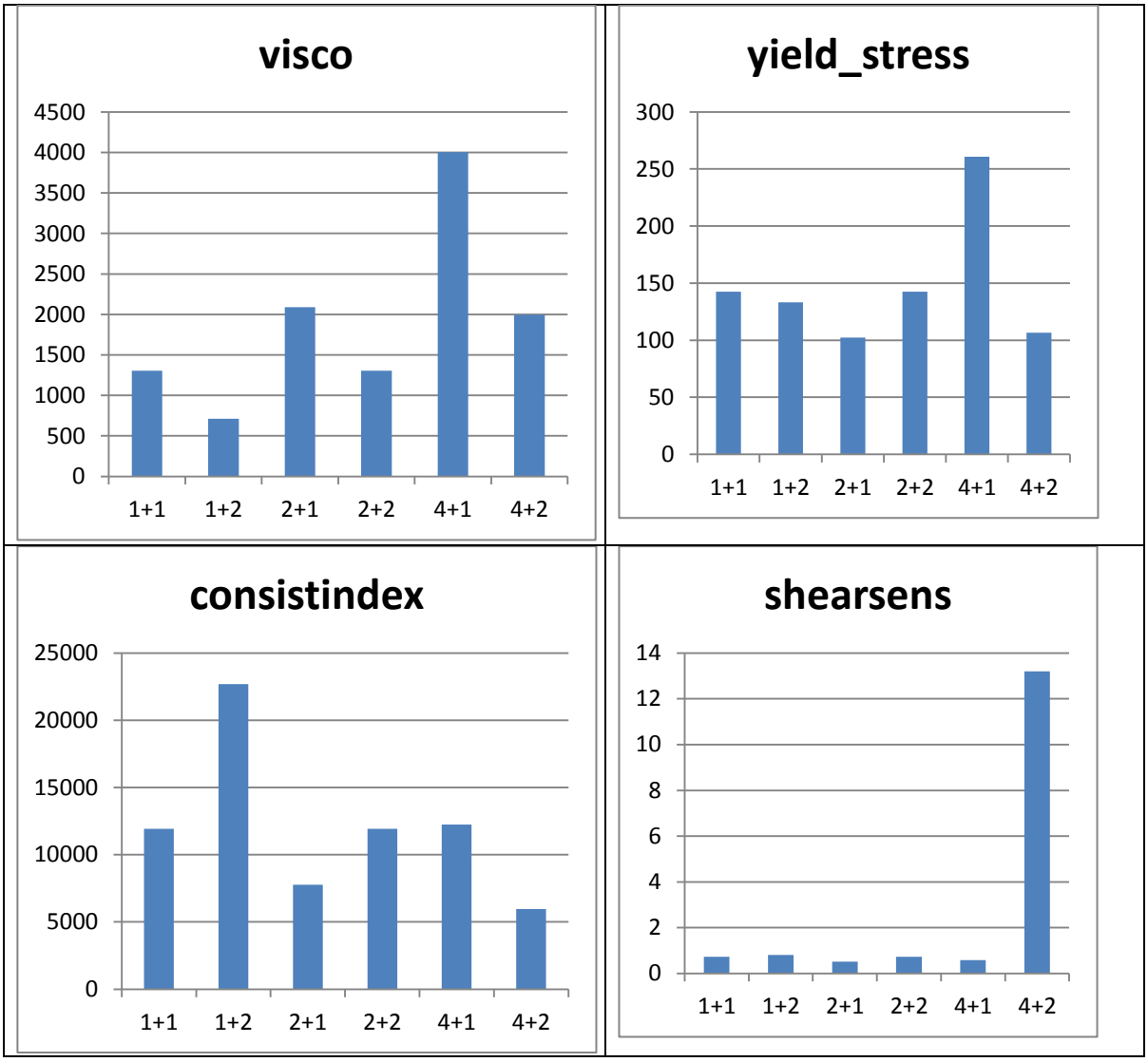

Figure 3.19: Effect of chitosan concentration (1, 2 amd 4) and magnesium salt type (chloride and sulfate) on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

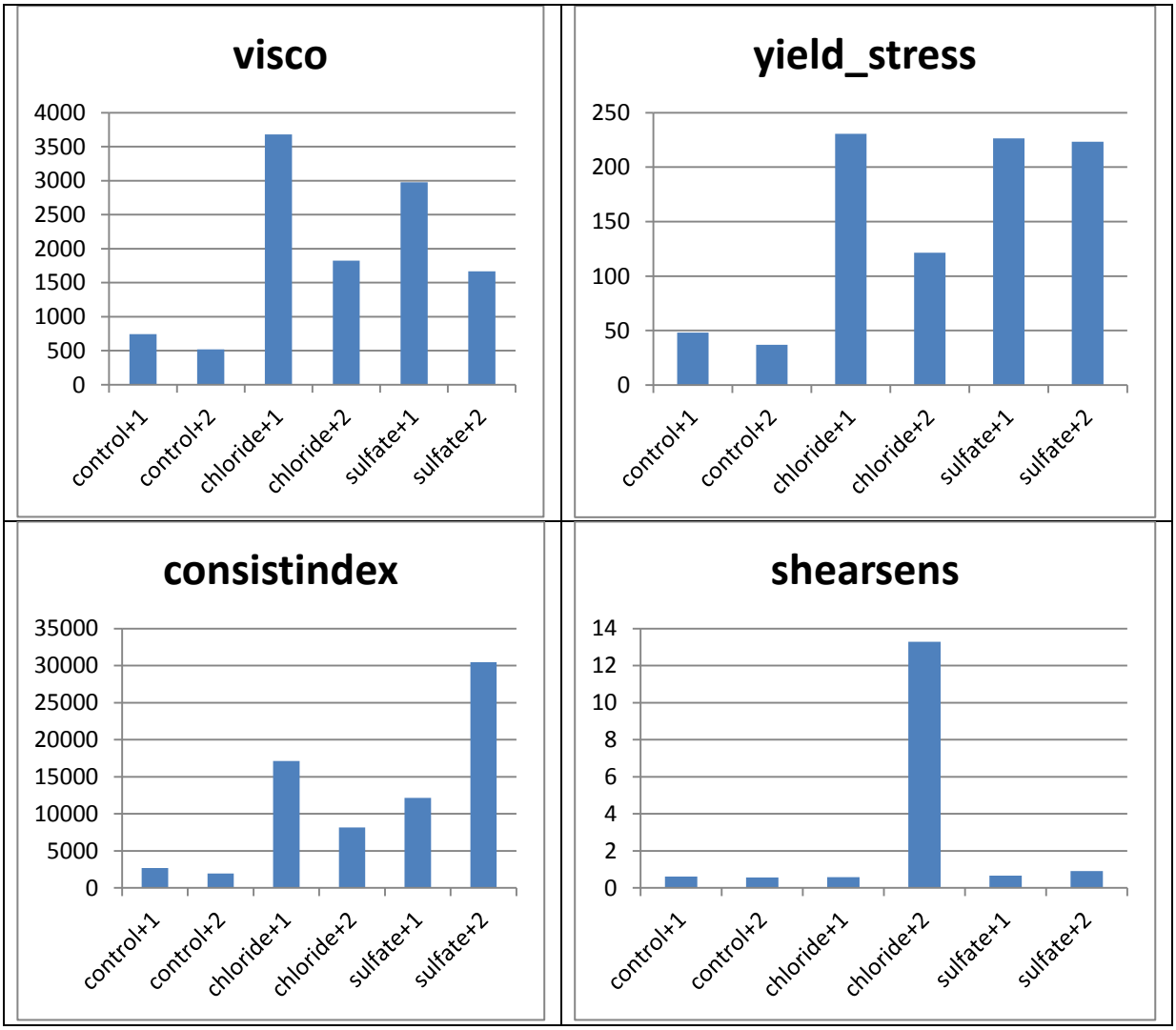

Figure 3.20: Effect of magnesium salt type (chloride and sulfate) and concentration (1 and 2) with constant chitosan concentration on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

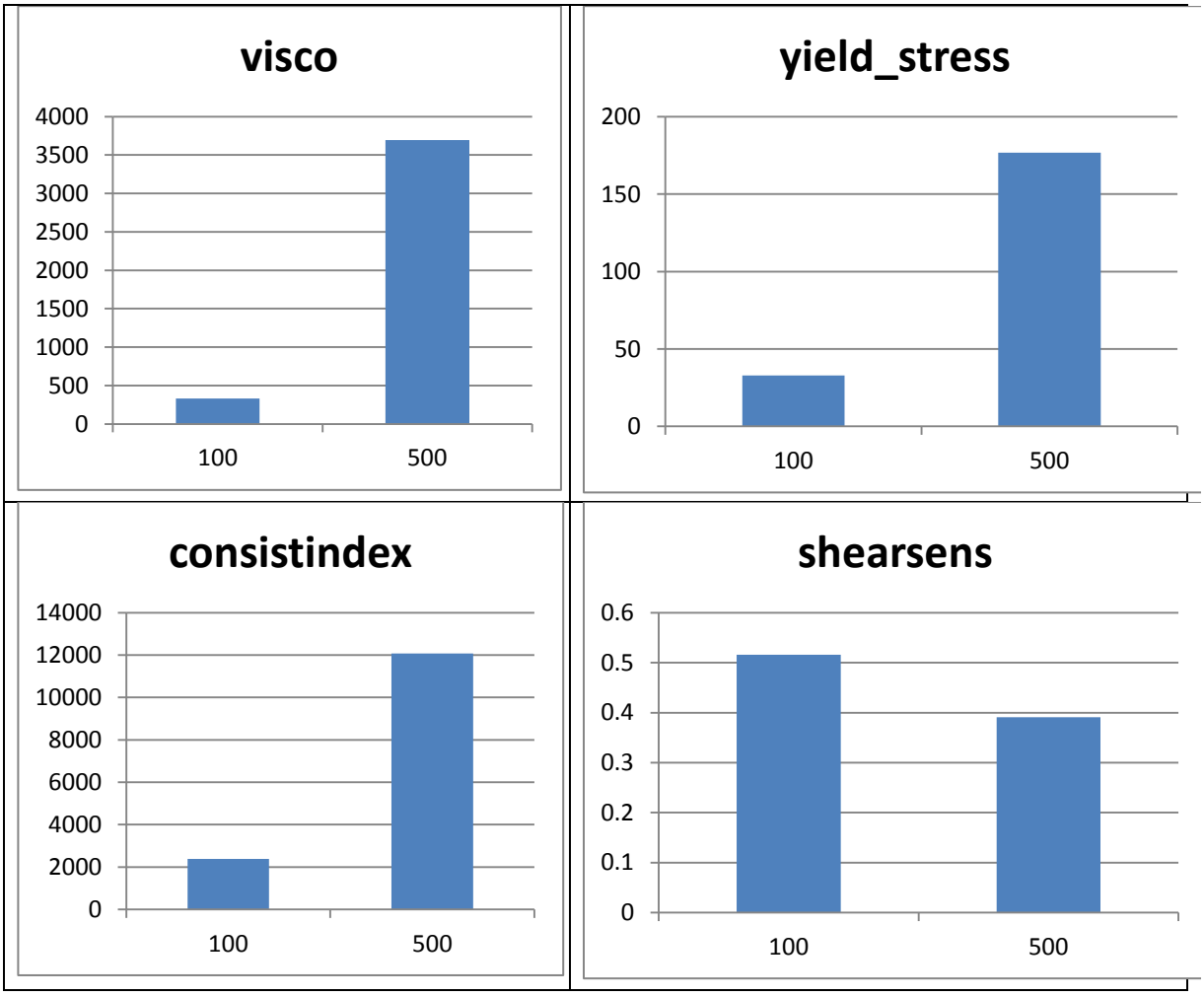

Figure 3.21: Effect of chitosan grade (100 and 500) with constant zinc concentration on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

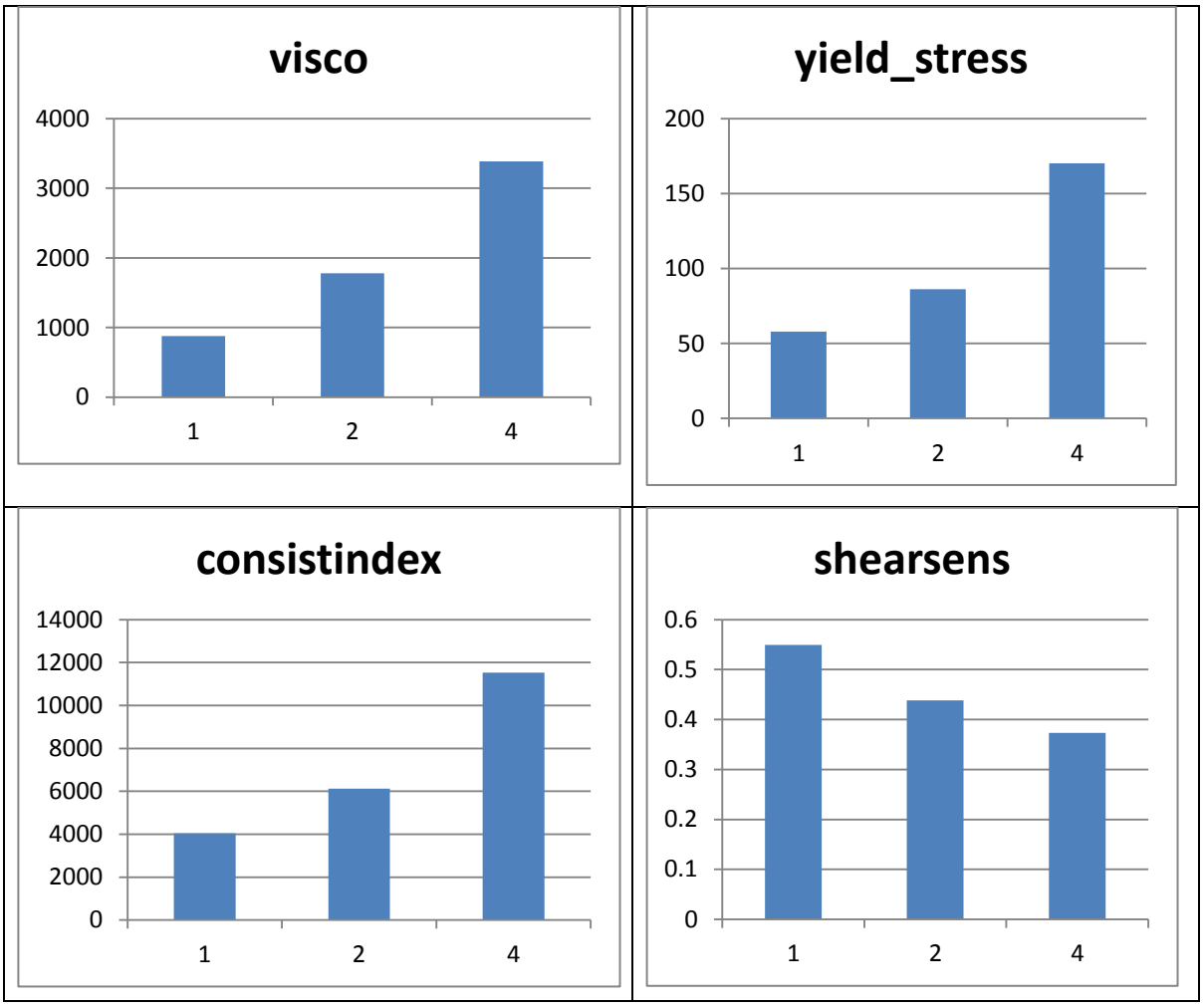

Figure 3.22: Effect of chitosan concentration (1, 2 and 4) with constant zinc concentration on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

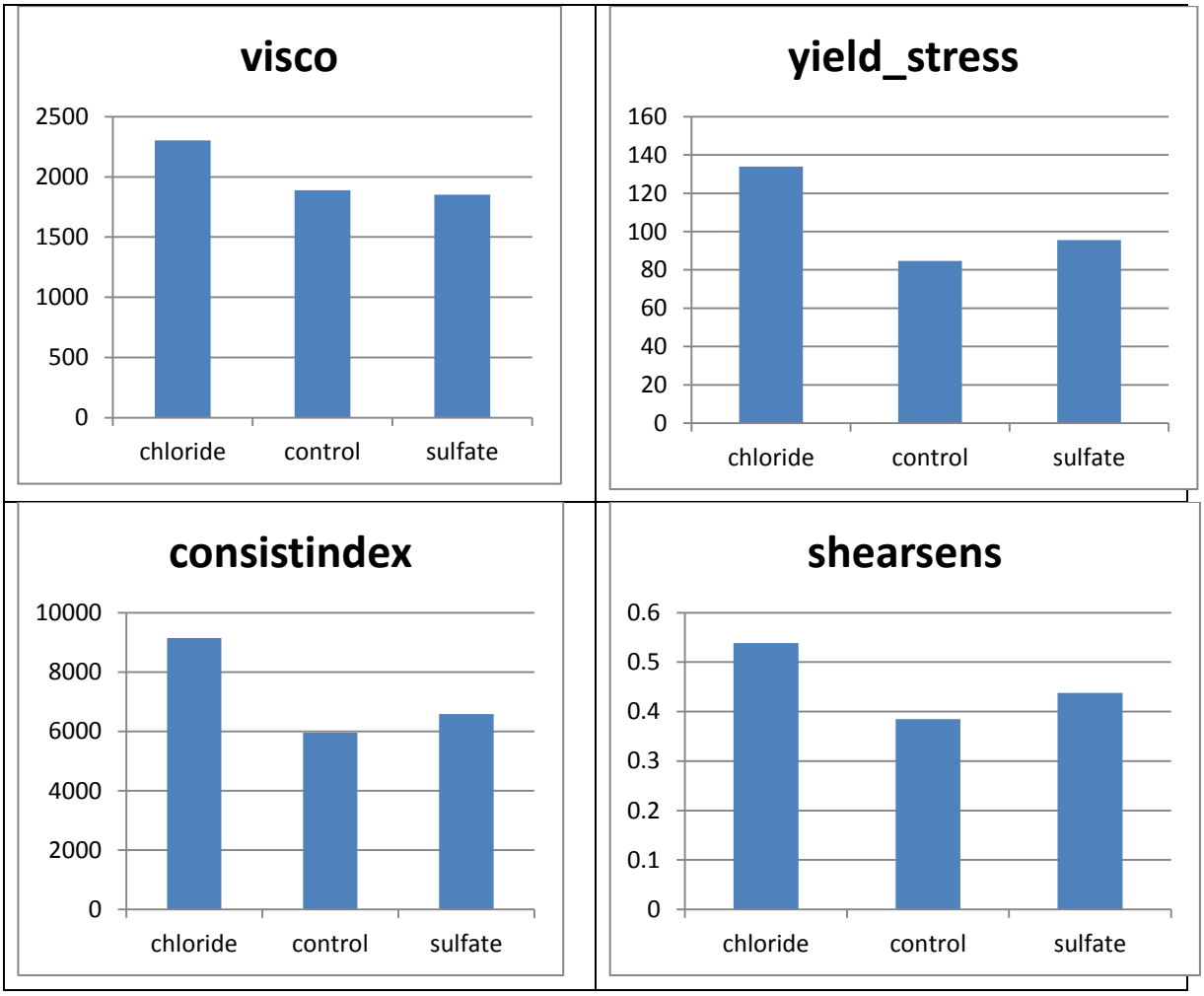

Figure 3.23: Effect of zinc salt type (chloride and sulfate) with constant chitosan concentration on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

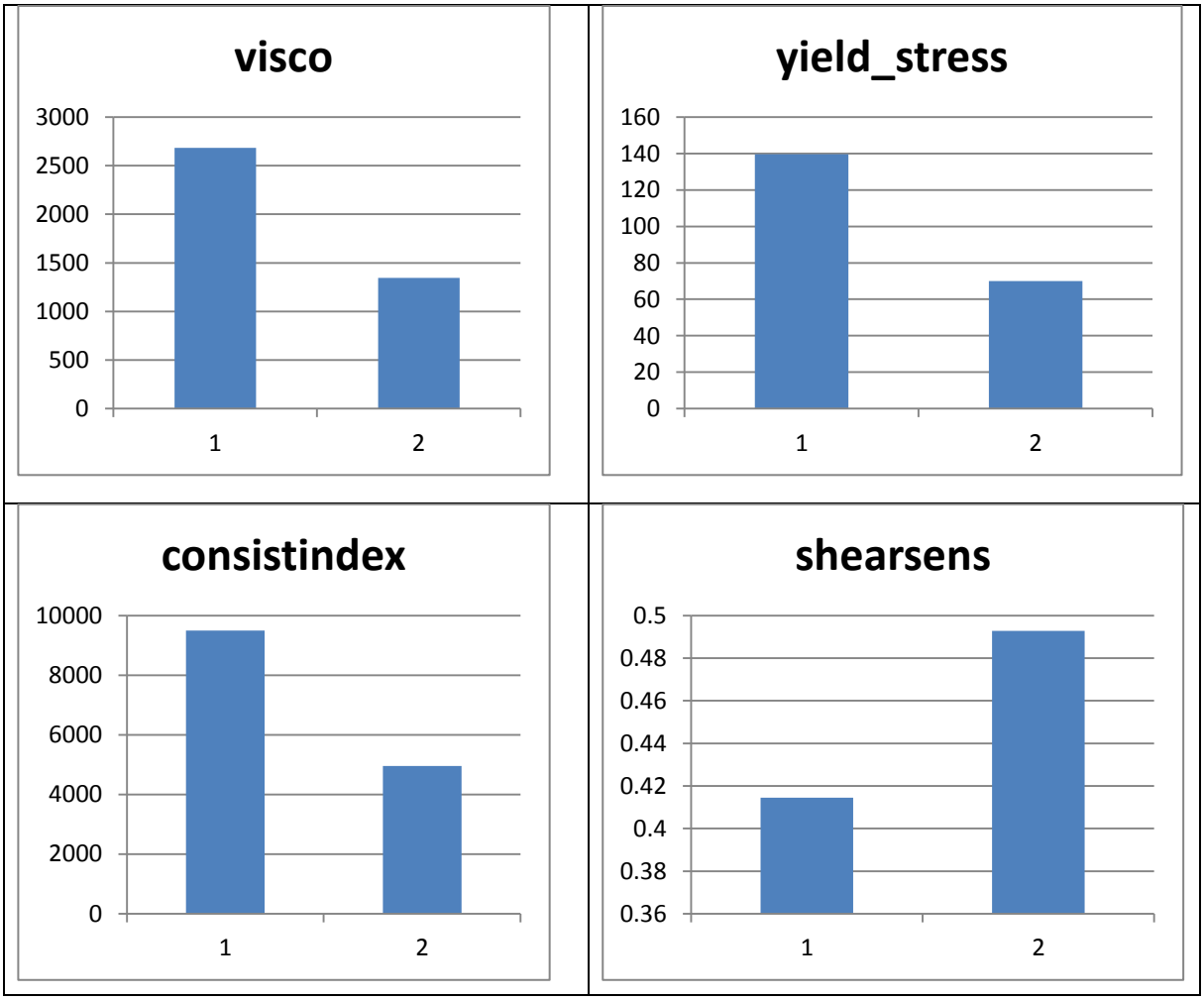

Figure 3.24: Effect of zinc salt concentration (1 and 2) with constant chitosan concentration on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

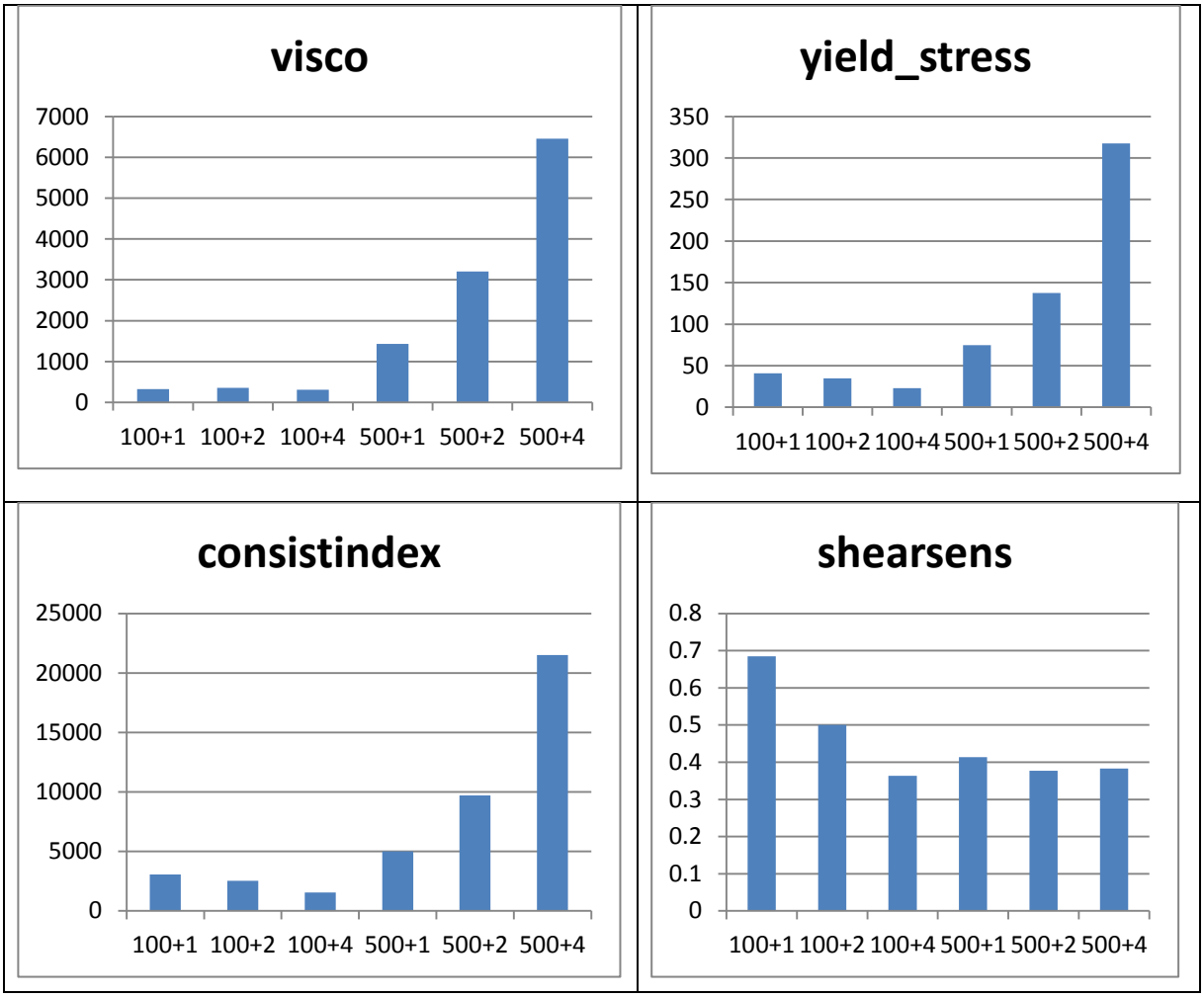

Figure 3.25: Effect of chitosan grade (100 and 500) and concentration (1, 2, and 4) with constant zinc concentration on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

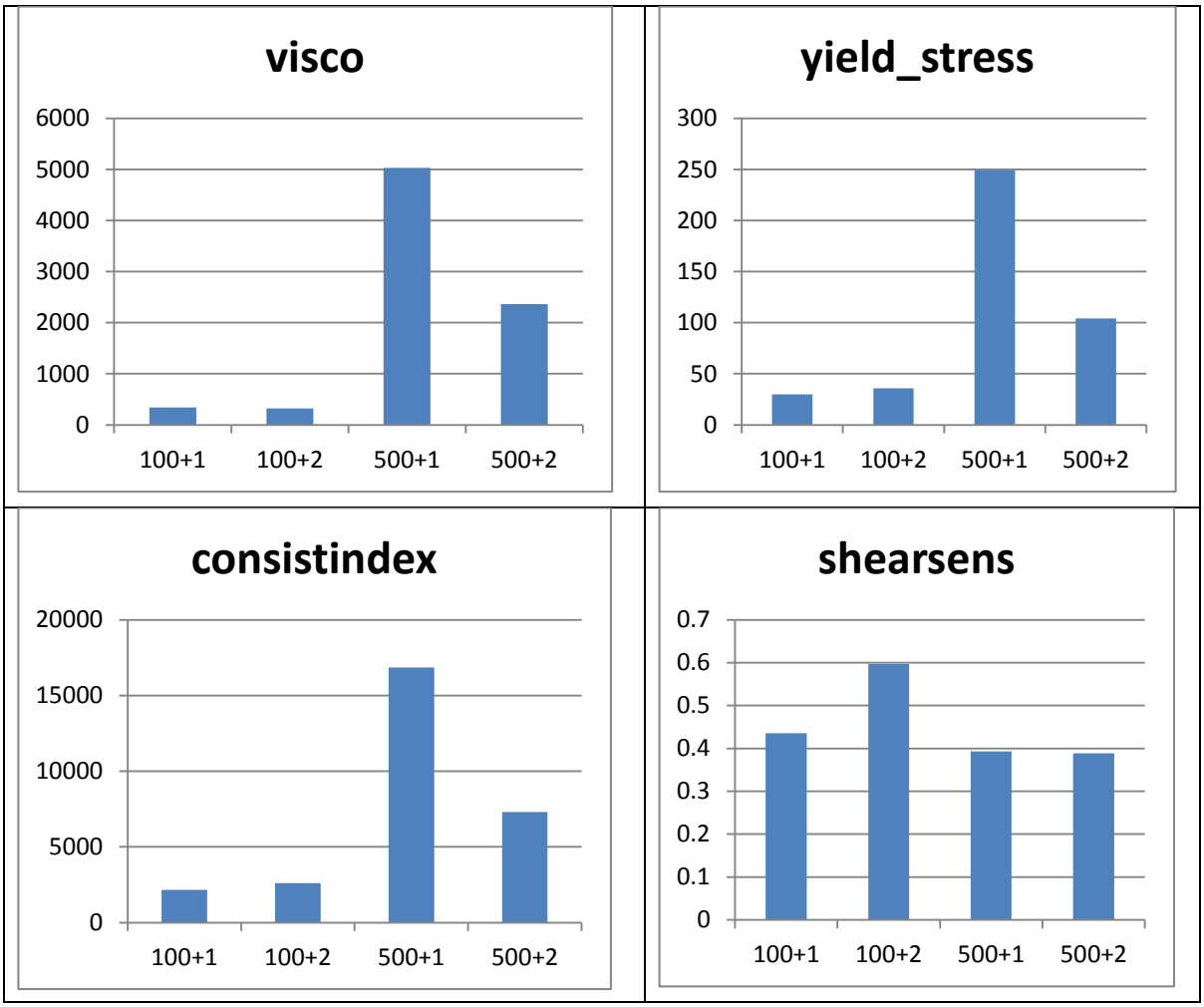

Figure 3.26: Effect of chitosan grade (100 and 500) and zinc concentration (1 and 2) on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

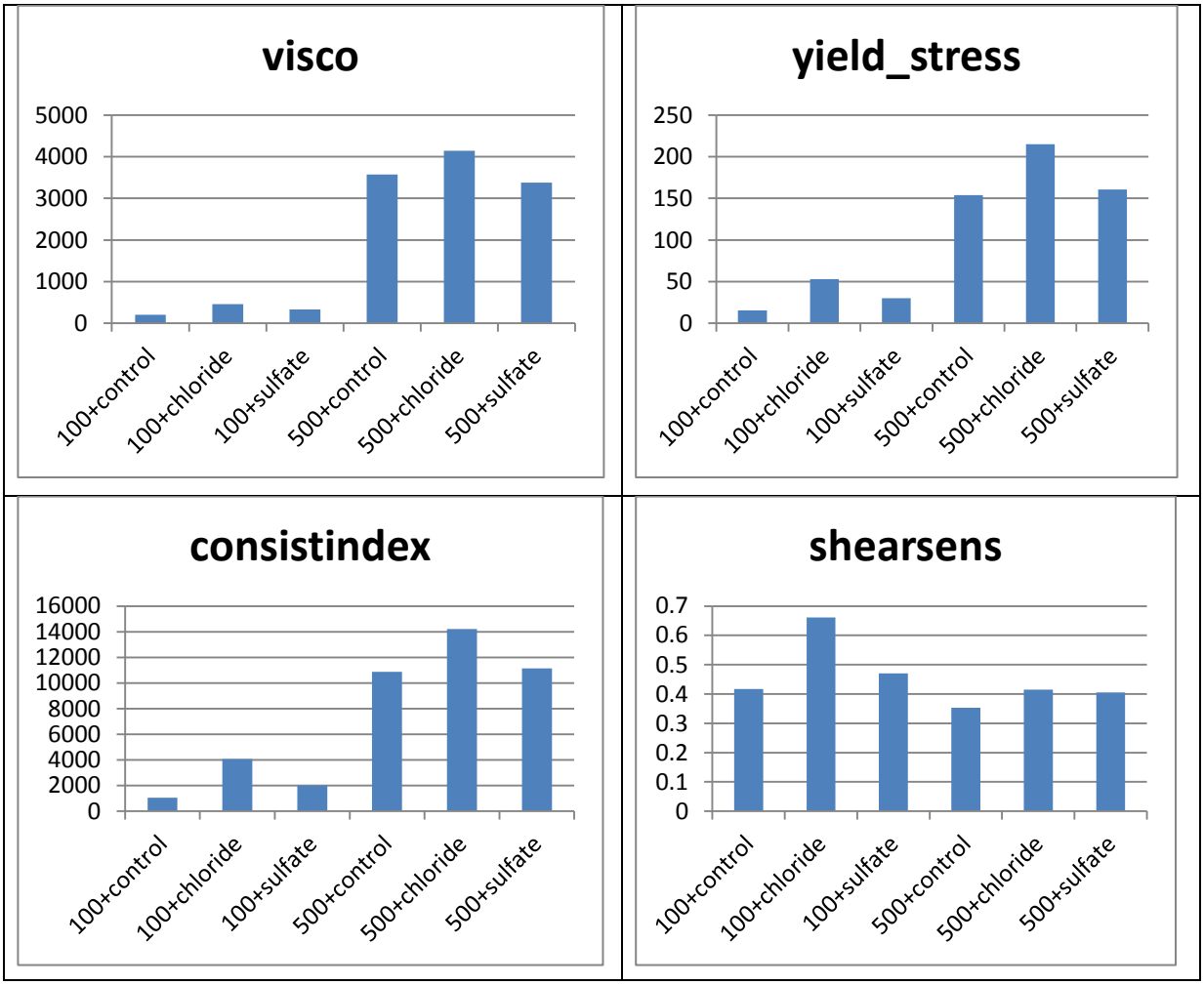

Figure 3.27: Effect of chitosan grade (100 and 500) and zinc salt type (chloride and sulfate) on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

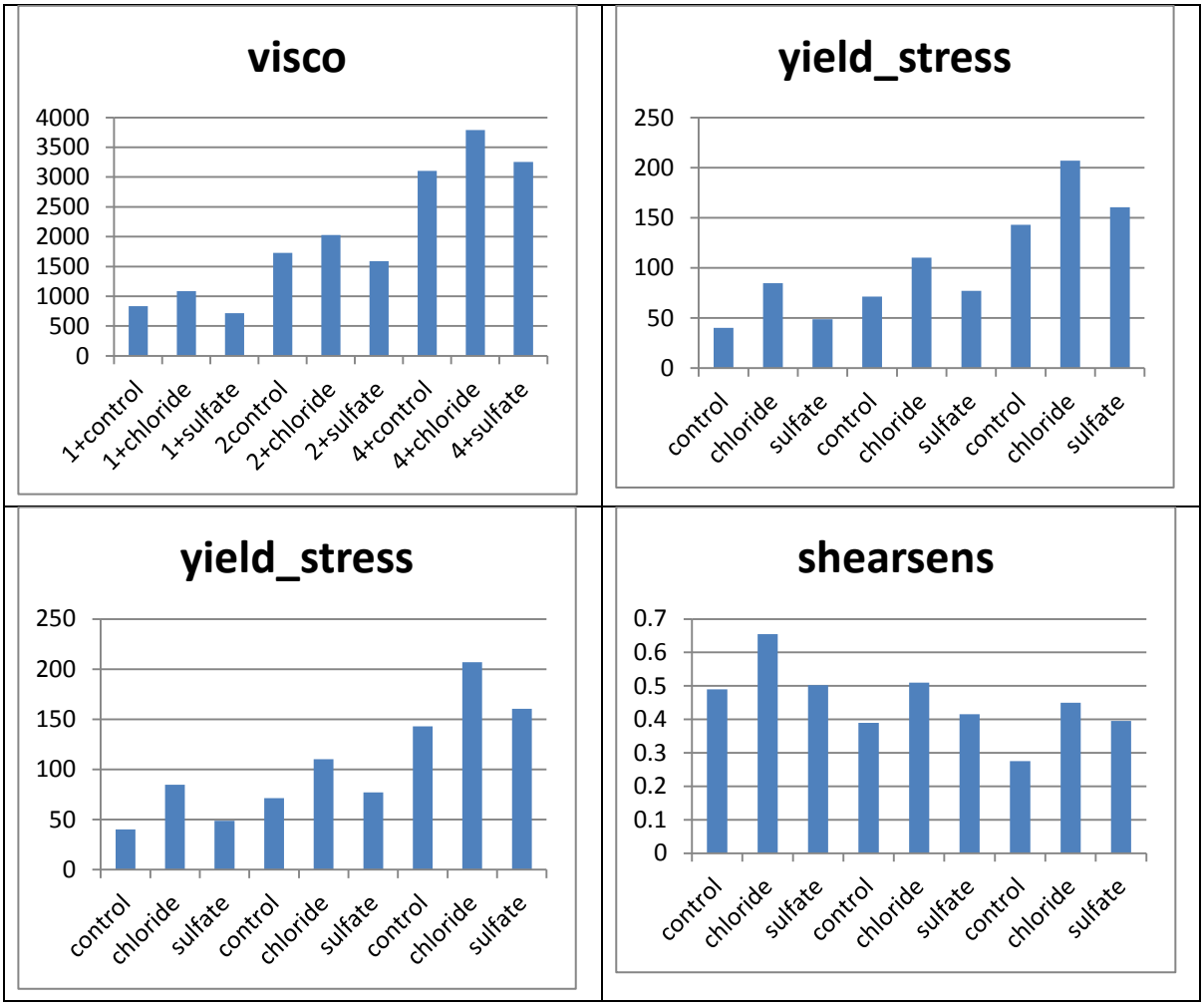

Figure 3.28: Effect of chitosan concentration (1, 2 and 4) and zinc salt type (chloride and sulfate) on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

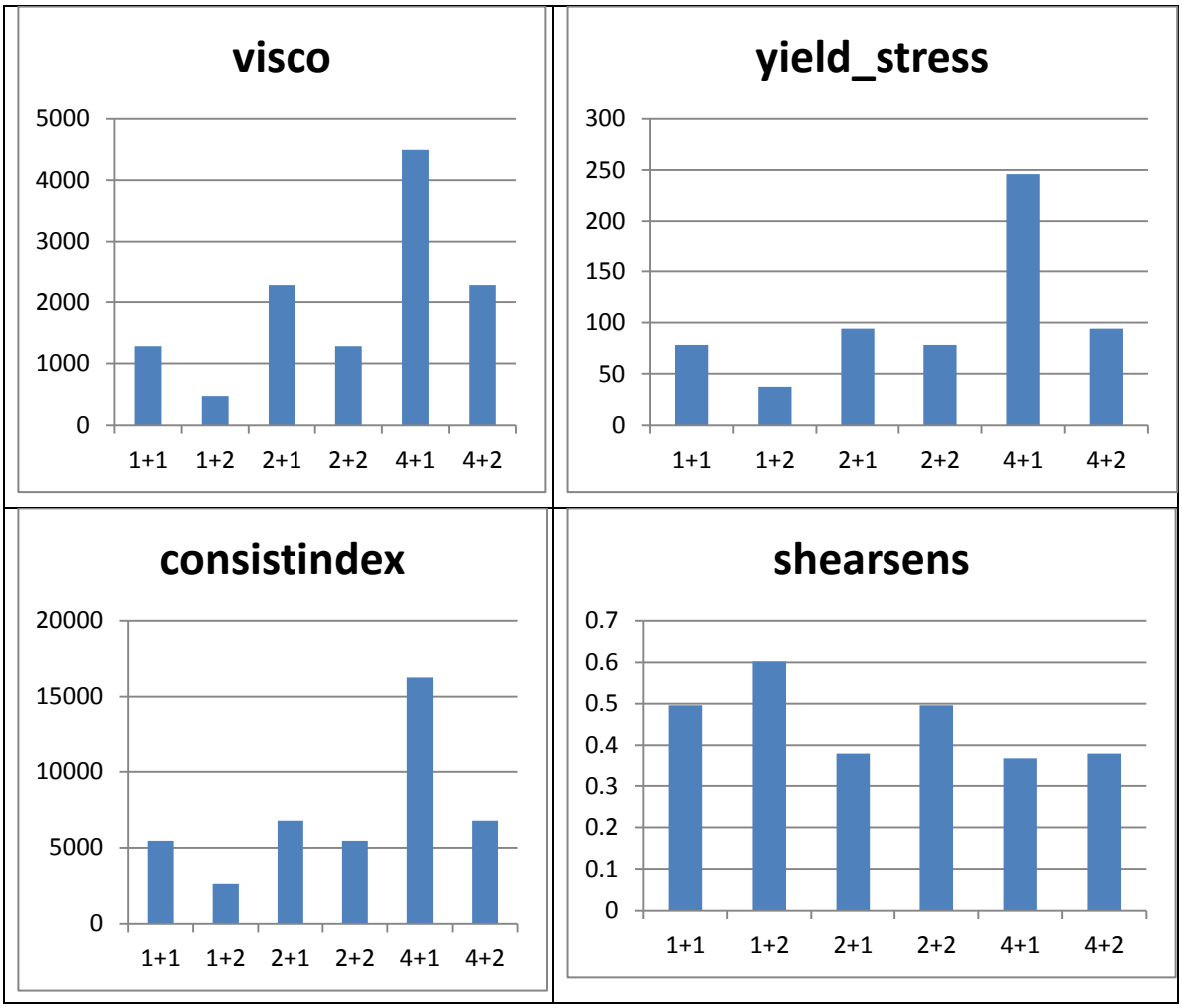

Figure 3.29: Effect of chitosan concentration (1, 2 and 4) and zinc salt type (chloride and sulfate) on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)

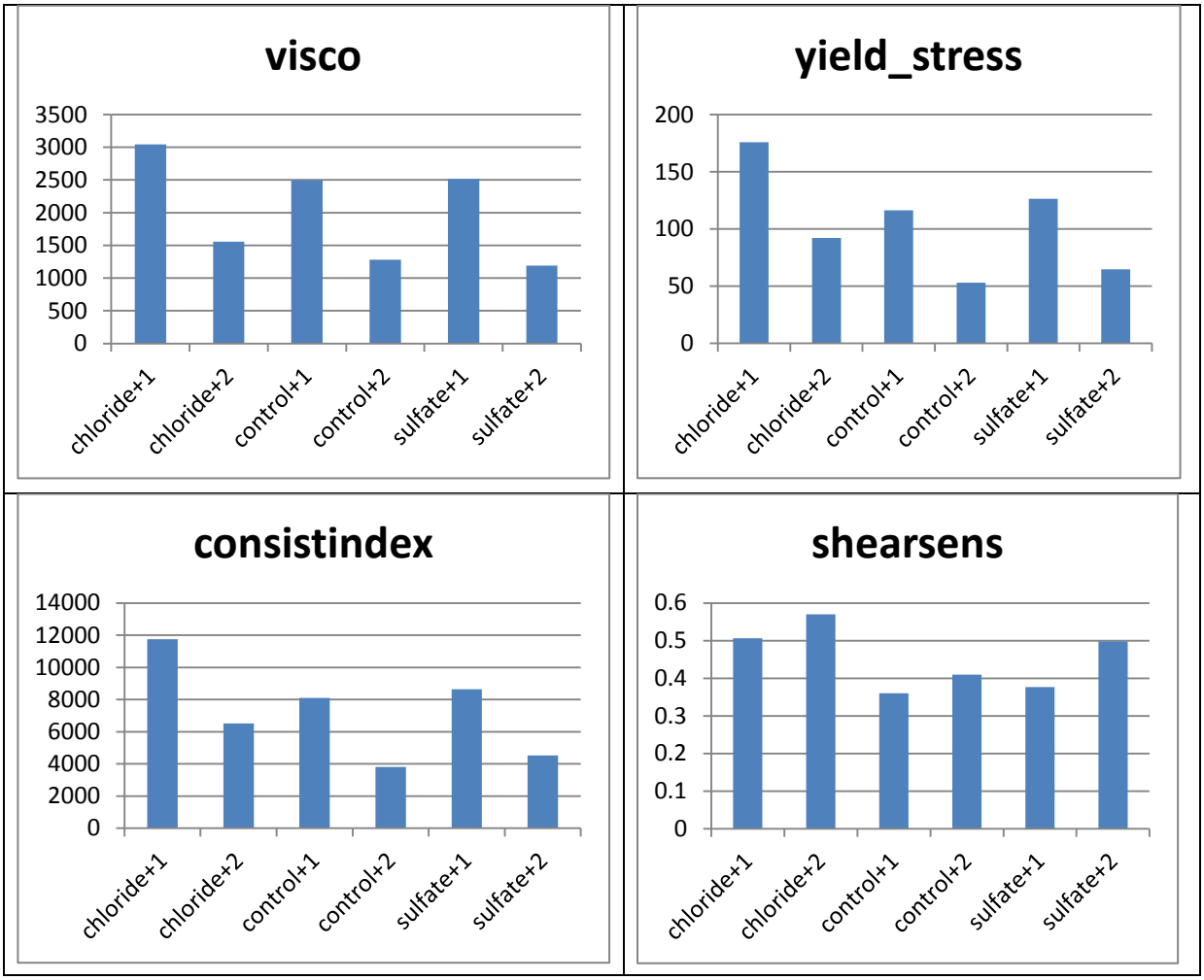

Figure 3.30: Effect of zinc salt type (chloride and sulfate) and concentration (1 and 2) with constant chitosan concentration on viscosity (visco), yield stress, consistency index (consistindex) and shear sensitivity (shearsens)