TRANSPORT PROPERTIES OF A CATIONIC POLYELECTROLYTE IN DILUTE AND CONCENTRATED SOLUTIONS: CHITOSAN

by

CARLOS ALFREDO KIENZLE-STERZER

S.M., Chemical Engineering, M.I.T. (1982)

S.M., Food Science and Technology, M.I.T. (1980)

Ingeniero Quimico, Universidad Metropolitana (1975)

SUBMITTED TO THE DEPARTMENT OF
NUTRITION AND FOOD SCIENCE
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS OF THE
DEGREE OF

DOCTOR OF PHILOSOPHY

at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

May, 1984

Signature of Author ______________________
Department of Nutrition and Food Science
May 15, 1984

Certified by ______________________
ChoKyun Rha
Thesis Supervisor

Accepted by ______________________
Steven R. Tannenbaum
Chairman, Departmental Graduate Committee

This doctoral thesis has been examined by a Committee of the Department of Nutrition and Food Science as follows:

Professor Marcus Karel ______________________________
Chairman

Professor Charles L. Cooney ______________________________

Professor Toyoichi Tanaka ______________________________

Professor ChoKyun Rha ______________________________
Thesis Supervisor

Transport Properties of a Cationic Polyelectrolyte
in Dilute and Concentrated Solutions:
Chitosan

by

Carlos Alfredo Kienzle-Sterzer

Submitted to the Department of Nutrition and Food Science
on May 15, 1984 in partial fulfillment of
the requirements for the degree of

Doctor of Philosophy

Abstract

This study is an analysis of the effect of the inter- and intramolecular interactions on the rheological respone of a cationic polyelectrolyte. The effect of pH and ionic strength on the charge density and chain flexibility of the polyelectrolyte backbone is related to the flow behavior of charged polysaccharide solutions.

The hydrodynamic volume of the isolated polysaccharide molecule is estimated from the intrinsic viscosity as affected by the degree of ionization and the counterion concentration. The hydrodynamic volume of isolated chitosan molecules decreases with decreasing the degree of ionization and increasing the counterion concentration. This behavior shows the effect of both the repulsive electrostatic interaction between neighboring charged groups, and the counterion shielding process on the spatial configuration of chitosan in dilute solutions.

The rheological response of dilute chitosan solutions suggests that the molecules behave as non-draining equivalent spheres composed of a series of rigid rods with elastic bonding joints. The flexibility of chitosan represents that of $\beta(1-4)$ glucan derivatives as shown by the value of the "characteristic ratio" ($C_\infty = 14.2$) and the "stiffness parameter" ($B = 0.08$).

The potentiometric titration of dilute and concentrated chitosan solutions show that "counterion condensation" occurs for all pKapp values below 3.5 independently of both the polyion and the counterion concentrations.

Concentrated chitosan solutions can be modeled as a "sponge-like" network readily swollen by solvent. This is demonstrated by a general master curve of the relative self-diffusion coefficient of glucose and the overlapping parameter for all pH's and ionic strength.

The rheology of concentrated chitosan solutions indicates that the intermolecular interactions control their flow behavior, and that the contribution of the Brownian motion to the overall viscosity decays as the overlapping parameter increases,leveling for $C[\eta] \geq 1.5$.

Chitosan molecules expand as a result of increasing intermolecular interactions behaving as rod-like molecules at high concentrations as indicated by: 1) the zero shear viscosity which is independent of the salt concentration; 2) the number of counterions associated per monomer which decreases with increases in the overlapping parameter and 3) the self/diffusion coefficient of glucose which is linear with the chitosan concentration.

This study makes a quantitative evaluation of the effect of the solution conditions (pH and ionic strength) on the inter- and intramolecular interactions of chitosan solutions. This provides the basis for the molecular modeling as well as for the prediction of the rheological response of β(1-4) glucan derivatives.

Thesis Supervisor

Dr. ChoKyun Rha
Associate Professor of
Biomaterials Science and
Engineering

ACKNOWLEDGEMENTS

I wish to express my gratitude to my advisor, Professor ChoKyun Rha, for her support, patience, and always stimulating advice. Her character and her ideas made it possible to bring out the best of this work.

I thank the members of my thesis committee: Prof. Marcus Karel (Chairman), Prof. Toyoichi Tanaka and Prof. Charles Cooney for their patience and final understanding of the main purposes of this thesis. Their advice, suggestions and criticisms were always clear and directed with the only idea of improvement and help the final results.

I deeply appreciate the C.O.N.I.C.I.T. (Caracas, Venezuela) for the financial support during my residency at M.I.T.

I am also indebted to all my fellow students in the Biomaterial Science and Engineering Laboratory for their friendship, comments and criticisms.

Also, I wish to express my gratitude to Prof. Anthony Sinskey for his advice and encouragement in all the difficult moments during the development of this work.

I am especially indebted to Ginger Burr for her patience and help in typing my reports and thesis.

I am profoundly grateful to my parents, brother and grandmother because they always believe in me and in my capabilities; they were always present when I needed them.

To my wife, Dolores, she is my special and secret FORCE, always saying "You can do it"; she deserves all the credit. To her and to our baby for whom we are waiting, I want to dedicate this thesis. Thank you.

Finally, I wish to thank all the people who have believed in me and helped me along all my student life. Thank you to all of you.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Chitosan (β1,4-2 amino-2-deoxy glucose) is a hydrophilic polyelectrolyte obtained by deacetylation of chitin (Horawitz et al., 1957; Broussignac, 1968). Chitosan, being the derivative of one of the most abundant, natural polymers along with its unique polycationic nature, makes its industrial application a very appealing goal. In order to generate information on the basic material characteristics, preliminary studies on the rheology of chitosan have been conducted (Kienzle-Sterzer et al., 1980, 1982a, b,and c; Kienzle-Sterzer 1980).

One of the first attempts to study the rheological properties of chitosan was its film forming properties. Chitosan films were prepared by the drying of chitosan solutions in either acetic or propionic acids (Rigby, 1936; Kienzle-Sterzer, 1980). Equilibrium stress-strain experiments, as well as linear stress relaxation experiments were performed (Kienzle-Sterzer et al., 1980, 1982a). The results indicate that:

1. The non-Gaussian behavior of swollen chitosan films is due to the increase in cross-linking density upon elongation.
2. A linear relationship exist between the apparent Young's modulus and chitosan concentration.

3. The mechanical response of chitosan films depends on the type of organic acid used in the casting solution.
4. The relaxation process of the chitosan molecules upon elongation is of physical nature.
5. Electrostatic interactions and hydrogen bond are both important when the molecules aligne under deformation.

In order to evaluate the electrostatic nature of the interactions between neighboring chains in chitosan films, stress-relaxation experiments (both in the linear and non-linear viscoelastic region) as affected by the ionic strength were studied (Kienzle-Sterzer et al ., 1982 b). The results indicated that:

1. A decrease in the internal stresses with increasing ionic strength as a result of the increased charge neutralization effect of counterion interactions.
2. Rearrangement of entanglements and renewal of the random configurations due to electrostatic interactions under deformation.

These studies showed the ionic nature of chitosan molecules in both the casting solution and in the network obtained after film formation, and clearly indicated the influence of the solution conditions on the properties of the final film.

The analysis of viscoelastic properties of semiconcentrated chitosan solutions shows the significant effect of polyion concentration on the entanglement density represented by G_{en} and the local friction coefficient (Kienzle-Sterzer _et_ _al_., 1982c). It is also indicated the importance of the electrostatic repulsive force between charged groups represented by the low values of G_{en} (in the order of 10^2-10^3 dyne/cm^2 compared with the non-charged polymers of similar molecular weigth (in the order of 10^6-10^7 dyne/cm^2). These Gen values together with the high values of the crosslinking density (in order of 1.0 MPa - 2.0 MPa) (Kienzle-Sterzer, 1980) demonstrated the significant influence of the attractive forces between neighboring groups on the neutralized chitosan molecules forming the network.

The results suggest the importance of charges on chitosan backbone on the mechanical properties of chitosan films and globules (Rodriguez and Rha, 1981). Therefore, a more detailed information on the effects of charge on the conformation of chitosan molecules needs to be generated.

In water or other strongly polar solvents, polyelectrolytes ionize forming macroions. Polyelectrolytes can exhibit properties in solution which are quite different from non-ionizable structures, leading to unusual behaviors. Current literature provides partial descriptions of the behavior of selected polyelectrolytes as a phenomenological view of "causes-effects." The lack of understanding of these "causes-effects" makes it difficult and almost impossible to interpret and predict the behavior of polyelectrolytes in solution at different solution conditions.

The solution behavior can be distinguished as follows:

a. The very dilute concentration regime where polymer chains are isolated. Under this condition polymer chains are considered not to interact directly with one another.

b. The concentrated regime where individual polymer chains interact. Entanglement and specific orientations of the macromolecular chains take place and these interactions are affected directly by the ionization of the polyelectrolytes.

OBJECTIVES

The overall objective of this work is to elucidate the mechanisms (cause-effect) responsible for the solution behavior of polyelectrolytes. More importantly, the work establishes and characterizes the nature of the transition from isolated to interacting regime on the rheological and transport properties. In order to determine the mechanism responsible for the solution behavior of polyelectrolytes, the major factors contributing to a particular behavior are identified and the relative importance of each factor assessed.

Rheological and transport properties are used to evaluate size, shape, inter-, and intra-molecular interactions, as well as the polymer and solvent (ions, coions and water) interactions.

A model system selected for this study is chitosan in aqueous acid solution. Chitosan is a linear polysaccharide with a high charge density. In addition, chitosan is a polycation, derived from chitin which is the second most abundant polymer on earth, which makes its evaluation and characterization wessential for a variety of industrial applications.

The hypothesis is that the electrostatic charge on the macroion affects the solution behavior via three major mechanisms:

1. Through intramolecular interactions which determine size and shape of the hydrodynamic domain in an isolated molecule.
2. Through intermolecular interactions which control the viscosity and counterion activity, and
3. Through electrostatic interactions between the macroion and the solvent which in turn control the mechanisms (1) and (2).

SPECIFIC AIMS

The specific aims of this research are:

1. Modeling of a linear polyelectrolyte, by quantitative evaluation of the major factors contributing to the hydrodynamic properties of the isolated molecule.
2. Evaluate the contribution of intramolecular interactions to the viscosity of polyelectrolytes as the degree of ionization and ionic strength is varied.
3. Determine the nature and the dynamics of the transition between isolated and interacting macroion domains. Develop a general analysis to describe such transition.
4. Identify and assess the primary controlling factors on the solution behavior (rheological-transport) of the polyelectrolyte when intermolecular interactions predominate.

RESEARCH SCHEME

The following experiments in both dilute and semi-concentrated chitosan solution regime were carried out:

A) Characterization of Chitosan as a Polyelectrolyte Model in Dilute Regime.

1. Potentiometric titration:

Objective: Determine the effect of ionic strength on the relationship between the polyelectrolyte surface charge density and ionization degree.

Assumption: Electroneutrality of the polyion-counterion-solvent system. Debye-Huckel approximation.

2. Intrinsic viscosity:

Objective: Study of the hydrodynamic behavior of chitosan as affected by charge density and ionic strength. Evaluation of the following hydrodynamic parameters: end to end distance, electrostatic expansion, electrostatic contribution to the excluded volume, local viscosity.

Assumption: Free non-draining equivalent sphere composed of a series of stiff cylinders joined by bonds with limited flexibility.

Theories Involved: Flory-Fox for non-draining equivalent sphere; Wormlike and Manning approach for stiff cylinders joint by flexible bonds.

B) Characterization of the Effect of the Polymer - Interaction. (Semiconcentrated regime).

1. Potentiometric titration:

Objective: Evaluation of the relationship between surface charge density and degree of ionization of chitosan as affected by counterion and/or polymer concentration. Establish the electrostatic nature of polyion-polyion interaction.

Assumption: Electroneutrality of the system polyion-counterion-solvent.

2. Activity coefficient of counterions:

Objective: Compare experimental values of the counterion activity coefficient, as affected by pH and ionic strength, with current theories of polyelectrolytes in solutions. Determine the number of counterions per monomer in the solvatation sheath. Effect of the overlapping parameter in the number of counterions bound.

Theories Involved: Manning theory; Iwasa theoretical approach; Gueron theoretical approach.

3. Diffusion coefficient of non-interacting small molecules:

Objective: Relationship between the self-diffusion coefficient of glucose and ionic strength, pH and polymer concentration. Effect of the overlapping parameter on the hydration layer around the polyion.

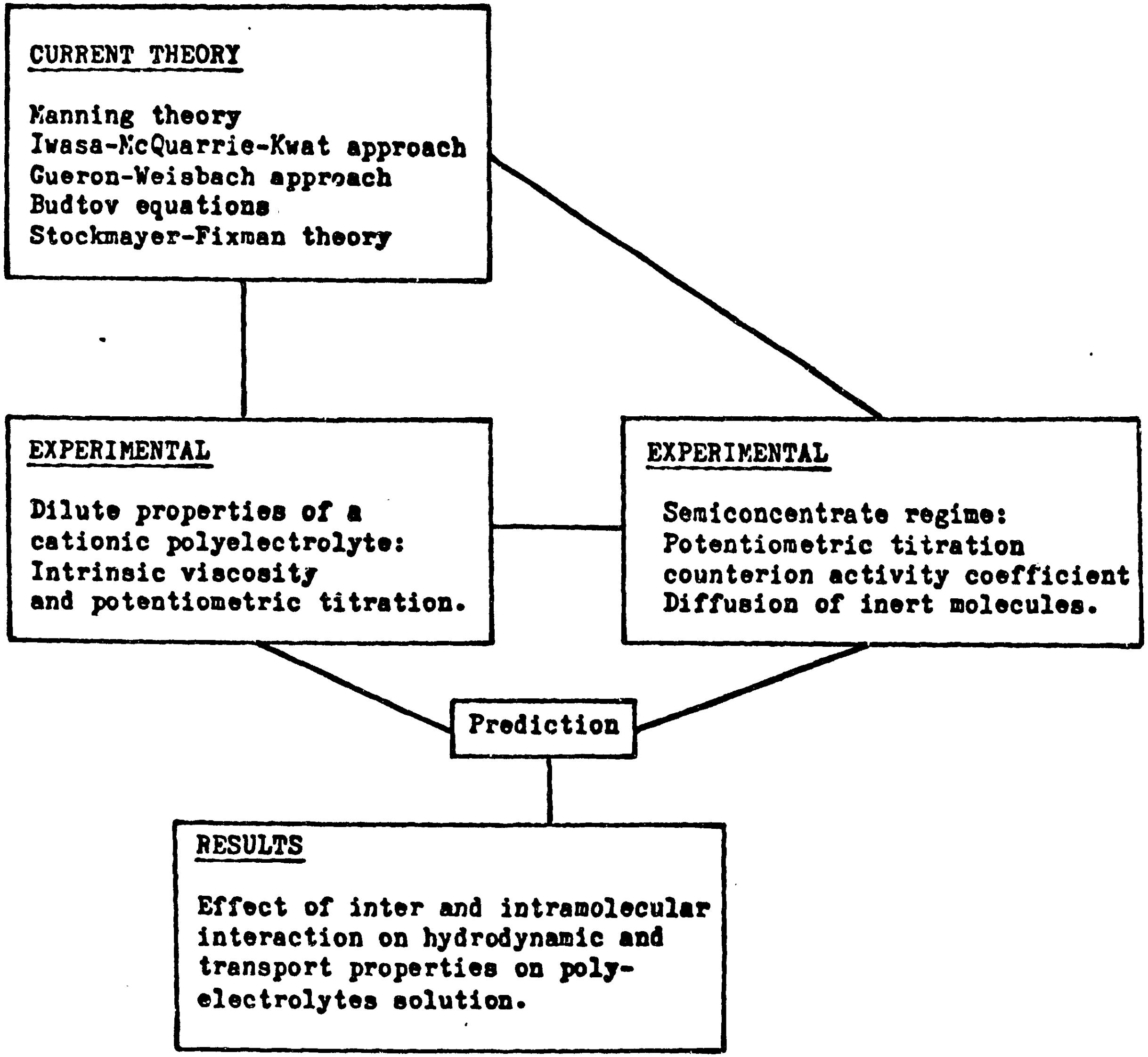
Theory Involved: Wang theory for the determination of the amount of solvent bound per monomer.

C) Rheology of Concentrated Chitosan Solutions

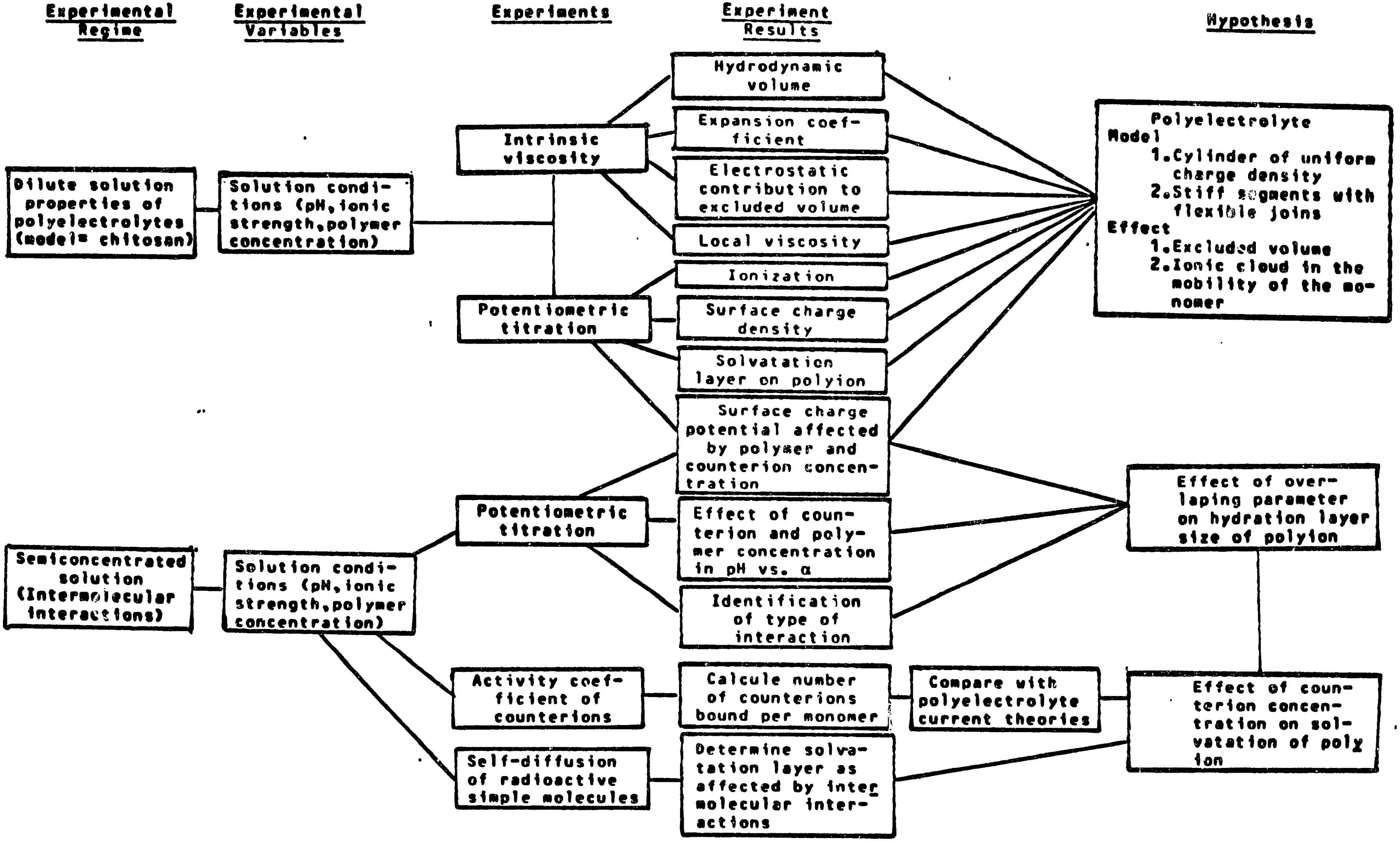
Objective: The study of the effect of intermolecular interactions on the rheological response of concentrated chitosan solutions. The effect of pH and ionic strength of the media will be established.

Theory Involved: Martin's equation.

Using this experiments, this research will establish the nature of intermolecular interactions on the semiconcentrated regime, their influence on the thermodynamic and, hydrodynamic properties. In order to isolate and evaluate the contribution of the intermolecular interactions to the transport properties of counterions and the hydrodynamic volume volume of the polyion, the experimental rtesults on the semiconcentrated regime will be directly compared with those estimated with current polyelectrolyte theories in the dilute regime using the chitosan as a model polyelecrolyte molecule.


CURRENT THEORY
Manning theory
Iwasa-McQuarrie-Kwat approach
Gueron-Weisbach approach
Budtov equations
Stockmayer-Fixman theory
EXPERIMENTAL
Dilute properties of a cationic polyelectrolyte: Intrinsic viscosity and potentiometric titration.
EXPERIMENTAL
Semiconcentrate regime:
Potentiometric titration
counterion activity coefficient
Diffusion of inert molecules.
Prediction
RESULTS
Effect of inter and intramolecular interaction on hydrodynamic and transport properties on polyelectrolytes solution.

EXPERIMENTAL SCHEME



2. LITERATURE REVIEW

2.1 Chitin and Chitosan

2.1.1 Chitin

2.1.1.1 Sources and Occurrences

Chitin, one of the most widespread polysaccharides amongst lower forms of plants and invertebrates, consists of unbranched chains of β(1-4) 2-acetamido-2-deoxy-D glucan residues. As its name indicated chitin (from the Greek word, χιτων , tunic or covering) is the principal component of the exoskeletons of many crustaceans and insects and, in less degree of importance, it is a minor component of the cell walls of fungi and other microorganisms (Austin *et al*., 1981; Ashford *et al*., 1977; and Rudall, 1969). A compilation of the reported occurrence of chitin in selected crustacea, insects and fungi is presented in Table 1. From this diversity of sources it is observed that recent studies (Swanson *et al*., 1980; Revah-Moiseev and Carroad, 1981) estimated that the potential annual production of chitin in the United States alone is between 5.3×10^6 Kg to 7.8×10^6 Kg. Table 2 shows the total global estimates for chitin production which

Table 1

Chitin Content of Selected Crustacea, Insects, Molluscan Organs, and Fungi (Kong, 1975; Naczk *et al.*, 1981)

Type	Chitin Content (%)	Type	Chitin Content (%)
Crustacea		**Insects (continued)**	
Cancer (crab)	72.1[c]	May beetle	16[b]
Carcinus (crab)	0.4-3.3[a]	Diptera (true fly)	54.8[c]
	8.29[b]	Pieris (sulfur butterfly)	64[c]
	64.2[c]	Grasshopper	2-4[a]
			20[c]
Paralithodes (King crab)	35[b]	Bombyx (silkworm)	44.2[c]
Callinectes (blue crab)	14[a]	Calleria (wax worm)	33.7[c]
Pleuroncodes (red crab)	1.3-1.8[b]		
		Molluscan Organs	
Crangon (shrimp)	5.8[b]		
	69.1[c]	Clamshell	6.1
Alaskan shrimp	28[d]	Oyster shell	3.6
Nephrops (lobster)	69.8[c]	Squid, skeletalpen	41.0
	6.7[b]	Krill, deproteinized shell	40.2±5.2
Homarus (lobster)	60.8-77.0[c]		
Lepas (barnacles)	58.3[c]		
		Fungi	
		Aspergillus niger	42.0[e]
Insects		Penicillium notatum	18.5[e]
Periplaneta (cockroach)	2.0[o]	Penicillium chyrsogenium	20.1[e]
Blatella (cockroach)	18.4[c]		
	10[b]	Saccharomyces cerevisiae (bakers yeast)	2.9[e]
	35[c]		
Colcoptera (beetle)	5-15[b]		
	27-35[c]	Mucor rouxii	44.5
Tenebrio (beetle)	2.1[a]	Lactarius vellereus (mushroom)	19.0
	4.9[b]		
	31.3[c]		

---

[a] Wet body weight
[b] Dry body weight
[c] Organic weight of cuticle
[d] Total dry weight of cuticle
[e] Dry weight of the cell wall

Table 2

Global Estimates of Annually Accessible Chitinaceous Materials as Potential Chitin Sources[a] (Allan *et al.*, 1978)

		Chitinaceous Waste				
Chitin Resources	Quantity Harvest[b]	As Fraction of Harvest (%)	Wet Weight	Solids Content (%)	Dry Weight	Chitin Potential[c]
Shellfish[d,e]	1,700	50-60	468	30-35	154	39
Krill[f]	18,200	40	3,640	22	801	56
Clam/ Oysters[e]	1,390	65-85	521	90-95	482	22
Squid[e,g]	660	20-40	99	21	21	1
Fungi[h]	790	100	790	20-26	182	32
Insects	negligible	--	--	21-56	--	--
Total	22,740		5,118		1,640	150

[a] Estimates are based upon mean values and are given in 10^3 metric tons

[b] It is assumed that only half of the harvested organisms are processed, except for fungi where the entire production is already processed for the principal product of the fermentation.

[c] Calculated using mean chitin content values

[d] Shellfish includes crab, shrimp, prawn, lobster & crayfish

[e] Five-year (1970-1974) average landings

[f] FOA (Rome) projection of potential landings

[g] Calculated on the assumption that the pen is 50% water

[h] By-products of citric acid and antibiotic manufacture; it is assumed that the quantity of citric acid and its mycelium are equivalent, antibiotic waste is 50% nonfungal and world production is twice that of the U.S.

amount to a total of near 150×10^6 Kg for 1978.

2.1.1.2 Isolation and Production

Chitin exists in nature in close association with other materials such as proteins and minerals (calcium carbonate among others). Therefore, strong chemical and thermal treatments must be used to its obtention and purification. Several procedures have been applied for the preparation of chitin:

i. Hackman Method (Hackman, 1954)

220 g of cleaned and dry shells are digested for 5 hr with 110 g of 2N-hydrochloric acid at room temperature. After treatment the shells are washed and ground finely and the product is extracted for 2 days with half liter of 2N-hydrochloric acid at 0°C under vigorous stirring. The insoluble material is then washed and extracted with 1N-sodium hydroxide at 100°C. The chitin yield is 17%.

ii. Horowitz Method (Horowitz *et al.*, 1957)

10 g of decalcified and cleaned shells are shaken with 100 ml of concentrated formic acid for 18 hr at room temperature. After filtration the solid material is treated with half liter of 10% sodium hydroxide solution for 2.5 hr under steam. The chitin yield is 60-70%.

iii. Takeda and Abe Method (Takeda and Abe, 1962)

After decalcification of the shells with EDTA at pH 10.0 and room temperature, the dry shells are digested with tuna proteinase at pH 8.6 and/or papain pH's between 5.5 and 6.0 at 37.5°C. The chitin obtained by this method can have about 5% of protein.

From these processes we can observe that the production of chitin from crustacean shells waste consists of two steps: (1) protein separation, and (2) calcium carbonate separation. A simplified flow diagram of chitin and chitosan processing is shown in Figure 1, and an industrial flowsheet for industrial obtention of chitin-chitosan in Figure 2.

2.1.1.3 Properties of Chitin

2.1.1.3.1 Conformation

Chitin occurs in three different polymorphic forms as shown by x-ray spectra (Rudall, 1963 and Rudall and Kenchington, 1973): α-chitin, the most tightly compacted where the chains are arranged in an antiparallel fashion; β-chitin, where the chains are arranged in parallel fashion; and γ-chitin is the polymorphic form where two chains are "up" for every one "down." The projection for

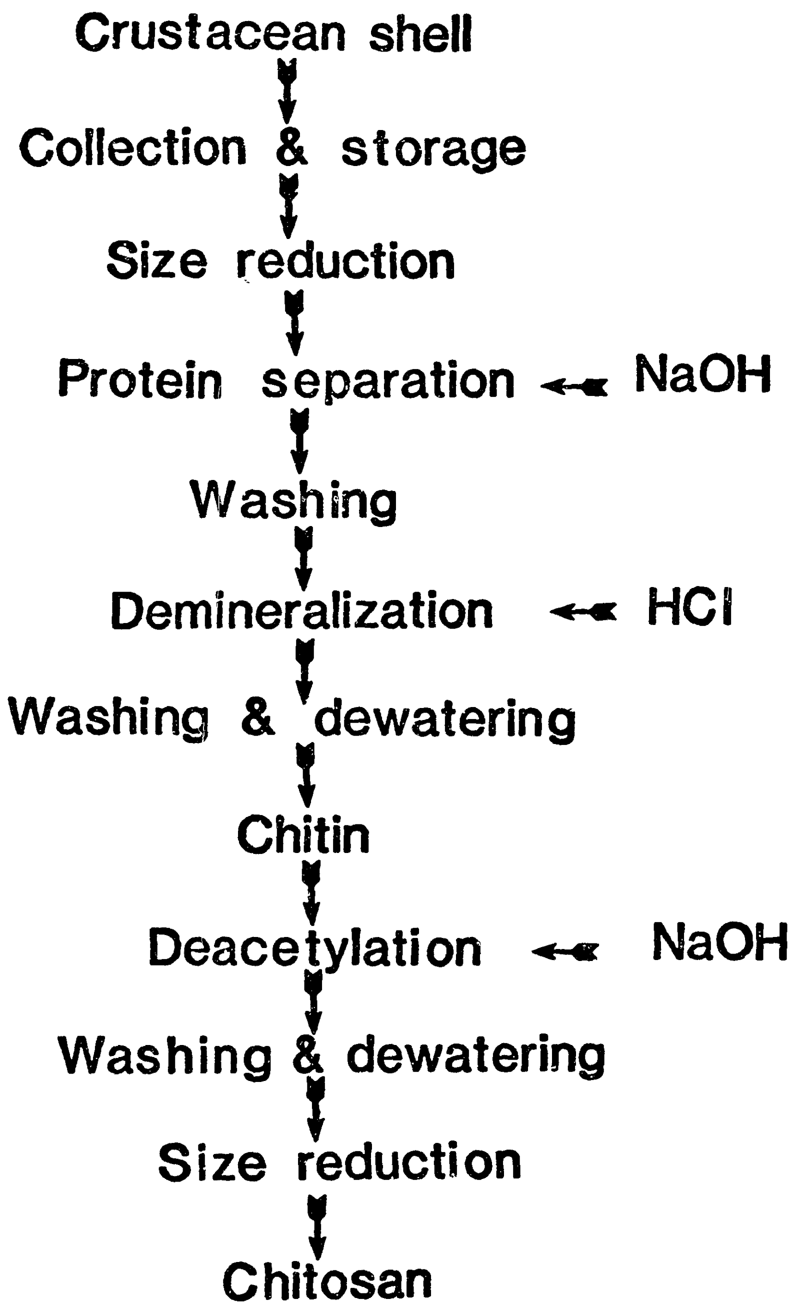


Figure 1. Flow diagram of chitin and chitosan processing.

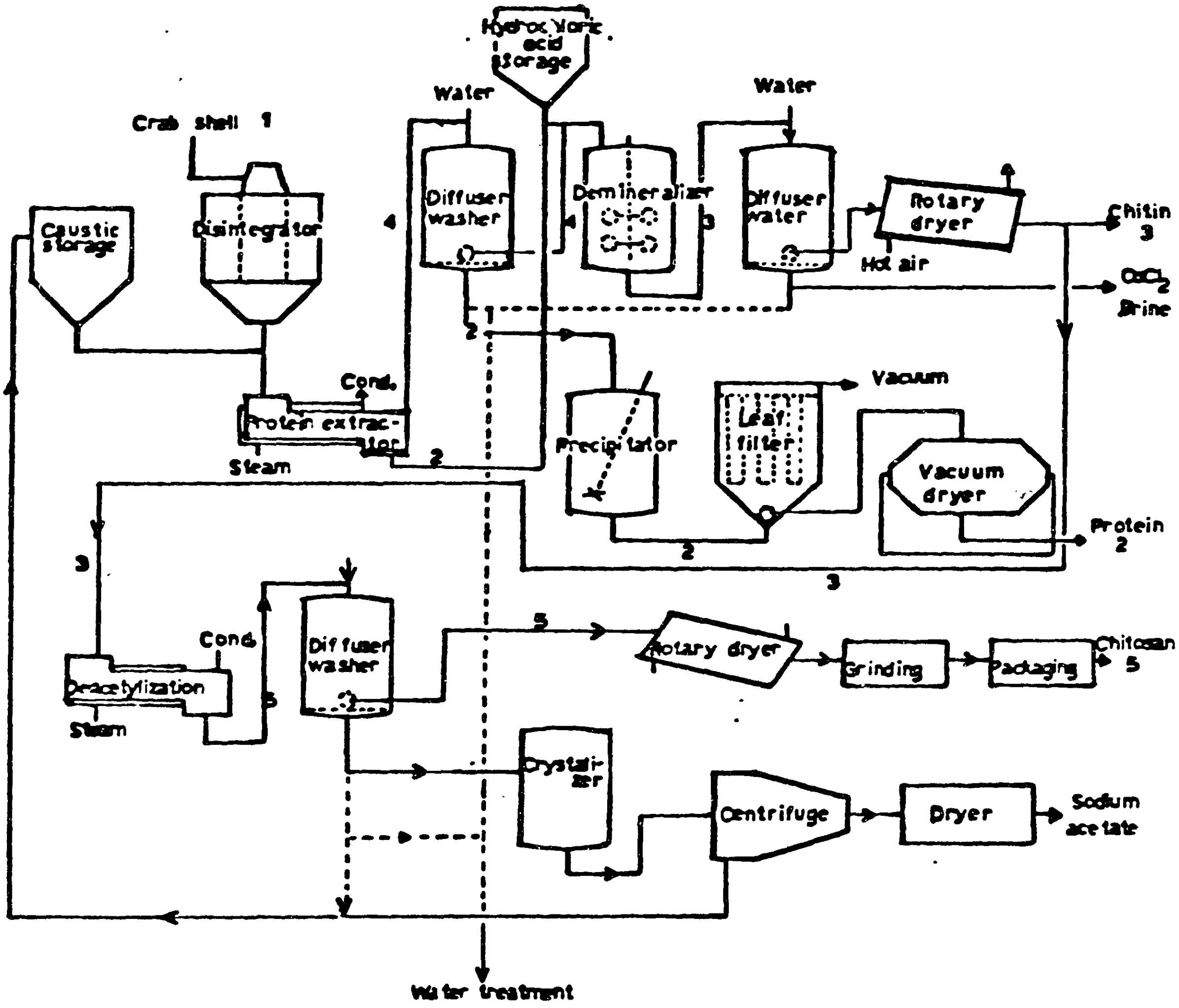


Figure 2. Industrial Process of Chitosan. (Muzzarelli, 1973)

the proposed model of α-chitin and β-chitin by Muzzarelli (1977) are shown in Figures 3 and 4.

The -chitin chain can be considered as a helix. Based on the x-ray diffraction photographs of this polymer. Ramakrishman and Prasad (1972) found that (1) the values of the unit translation as 0.515 nm with 2 residues per turn and (2) that the unit cell contains two chains running in opposite directions, each conformed of 2 screw units of monomer residues.

Meanwhile, the unit cell of β-chitin conformation has been modeled as a typical monoclinic that contains two monomer residues related to a two-fold screw axis (Blackwell *et al.*, 1967; Gardner and Blackwell, 1975). This model for β-chitin shows numerous points of similarity with native cellulose, both structures are conformed of parallel chains structured as an array of hydrogen bonded sheets (Gardner and Blackwell, 1974).

2.1.1.3.2 Solubility

Chitin can be dissolved in a variety of solvents. The choice of the most appropriated solvent will be based on the following criteria: rate of disolution, rate of degradation and viscosity of both the solvent and the

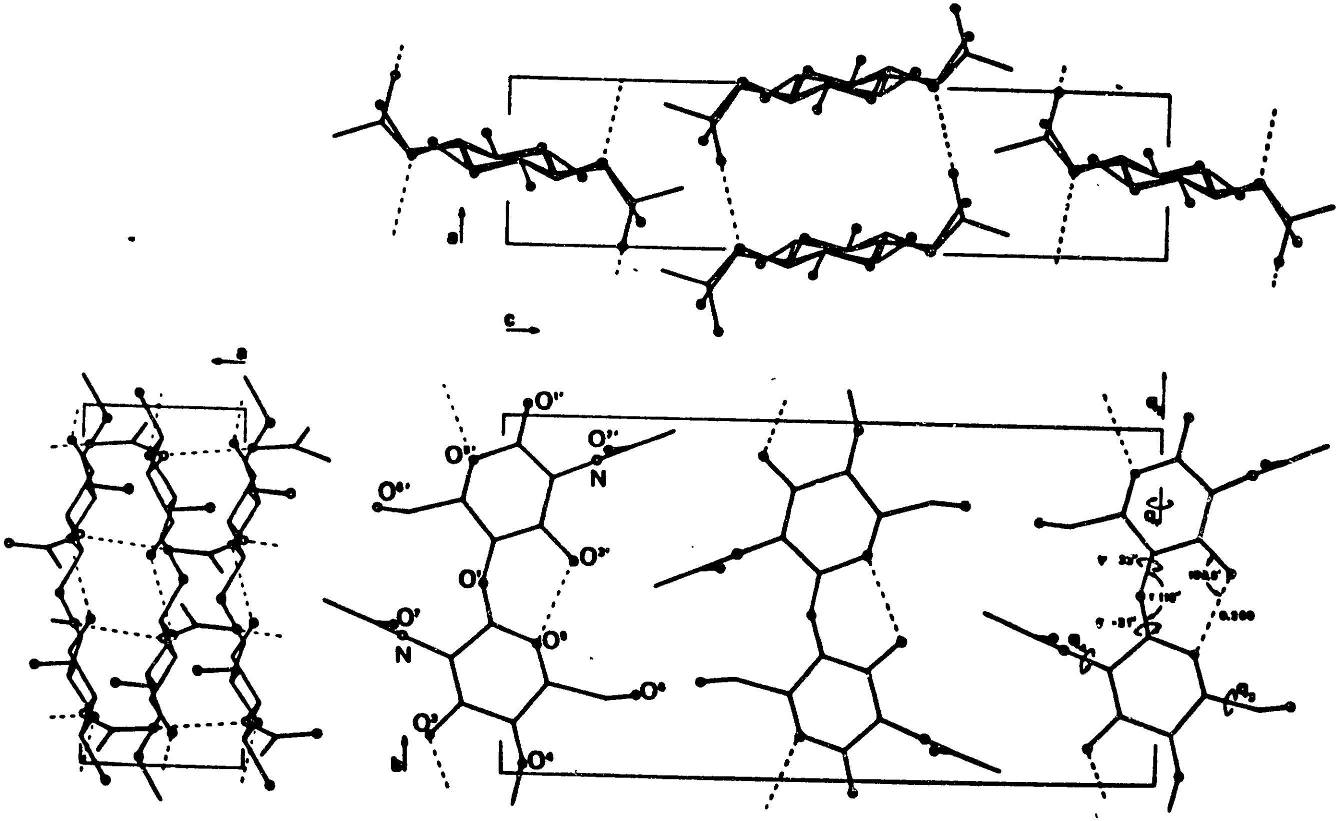


Figure 3. Projections of the proposed model for α-chitin. (Muzzarelli,1977)

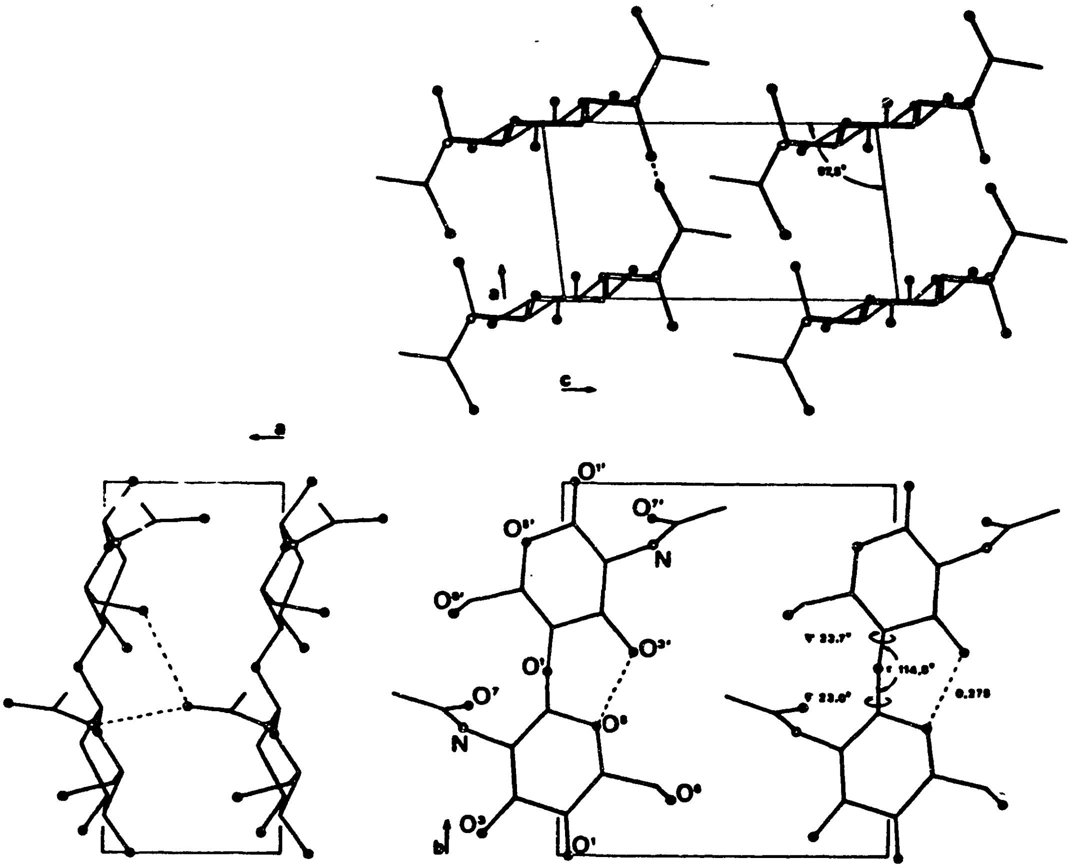


Figure 4. Projections of proposed model for β-chitin. (Muzzarelli, 1977).

final solution. In the last few years, several solvents have been used to solubilize chitins. Among them the most used are: Hexafluoroisopropanal, hexafluoroacetone sesquihydrate and N-methylpyrrolidinone for the obtention of very viscous dopes (Capozza, 1975; Unitika Ltd., 1983). It has been shown (Austin, 1975) that mixtures of chloroalcohols and aqueous solutions of mineral acids such as hydrochloric, sulfuric or nitric acids are good solvents for chitin in any form, i.e., native or crystalline chitin. Meanwhile, partial solubility of chitin (5%) and total solubilization of chitosan (100%) is achieved with dimethylformamide with nitrogen tetroxide (Allan _et al._, 1971).

However, polar aprotic solvents such as dimethylsulfoxide are totally ineffective on α-chitin, and are only able to swell β-chitin (Lee, 1974).

At this point, it must be observed that some degradation is obtained when chitin is dissolved in mineral acids, for example x-ray diffraction studies showed that when chitin is dissolved in hydrochloric acid, hydrolysis of the glycosidic links and the removal of the acetyl groups commences immediately (Danilov and Plisko, 1954; Clark and Smith, 1935).

Colloidal solutions of chitin can be prepared by dispersion in hot, concentrated aqueous solutions of neutral salts (von Weimarn, 1927; Smirnoff, 1975). The dispersal efficiency follows the order LiCNS>LiI>LiBr> LiCl. Meanwhile, dispersion of chitin in calcium chloride is very difficult (Muzzarelli, 1975).

2.1.1.3.3 Reactivity and Reactions

The presence of the 3-acetylamino group in chitin has a very strong influence in its reactivity if compared with that of cellulose.

Acetates

Chitin may be acetylated with acetic anhydride with continuous flow of dry HCl to produce a 2.99 acetyl groups per glucose residue (Schorigin and Hait, 1935). The acetate chitin is soluble in formic acid and concentrated nitric acid, but insoluble in other organic solvents.

Sulfate esters

Sulfate diester of chitin has been prepared in view of obtaining heparin-like blood anticoagulants. The sulfating agent is chlorosulfonic acid in pyridina and chlorosulfonic acid in 1,2-dichloroethane (Bergstrom, 1936; Cushing *et al.*, 1954; Cushing and Kratovil, 1956). Chitin disulfates can show up to 20% of the anticoagulant potency of heparin, with only slight toxicity (Astrup *et al.*, 1944; Roth *et al.*, 1954).

Methyl Ethers

The monomethyl ether of chitin has been prepared by treatment with dimethylsulfate and sodium hydroxide (Wolfrom *et al.*, 1964). However, the methylation of chitin was found to be very difficult, because of its inability to swell in alkali, and the di- and trimethyl ethers were not obtained (Schorigin and Makarova-Semlyanskaya, 1935).

Carboxymethylation

Sodium carboxymethyl chitin may be prepared by treating chitin with sodium chloroacetate in sodium hydroxide solution (Okimasu, 1958), or with dimethylsulfoxide in an alcohol-sodium hydroxide solution (Trujillo, 1968). Sodium carboxymethylchitin is a hygroscopic substance. Carboxymethylchitin containing more than 6.13% of sodium dissolves in water and subsequent heating does not cause coagulation.

The viscosity behavior of carboxymethylchitin is very similar to that of carboxymethylcellulose, there is an increase in the relative viscosity with dilution (Danilov and Plisko, 1961; Muzzarelli, 1977).

2.1.2 Chitosan

2.1.2.1 Sources and Preparation

Chitosan is the best known chitin derivative. Chitosan is deacetylated chitin, it consists of unbranched chains of β(1-4) 2-amino-2-deoxy-D glucan residues (Figure 5). Generally, the production of chitosan from chitin involves the hot alkali treatment of chitin. It must be pointed out, that any alkali treatment of chitinous materials produce less pronounced effects upon the macromolecular length than the acid treatment (Lusena and Rose, 1953; Numazaki and Kito, 1975).

A number of methods have been developed to obtain chitosan. For example:

i. Horowitz Method (Horowitz *et al.*, 1957)

Chitin is melted with a mixture of solid potassium hydroxide and nickel under nitrogen environment at 180°C. After half an hour the melt is precipitated in ethanol and washed with water. This method renders almost 95%

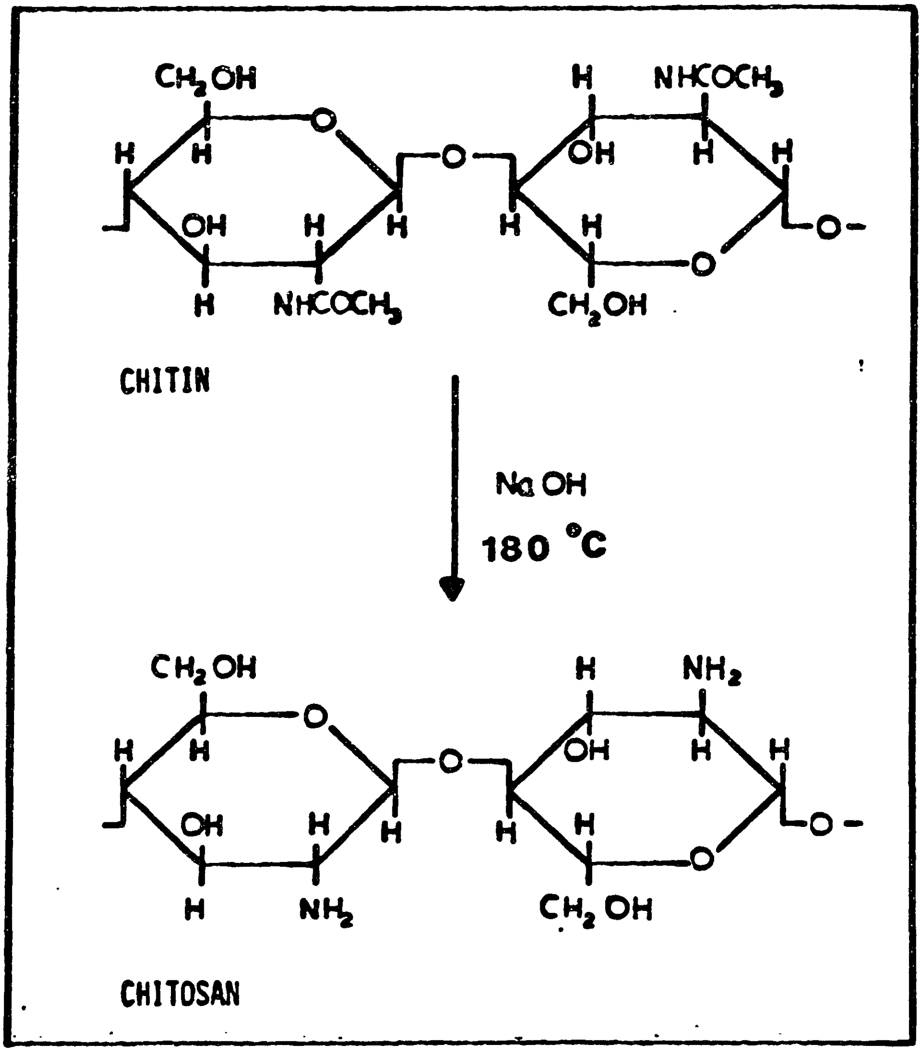


Figure 5. Chitin and Chitosan Molecules.

deacetylation and very low molecular weight chitosan.

ii. Extraction with NaOH (Ribgy, 1936)

Chitin is treated with 40% NaOH solution at 115°C for 6 hours under nitrogen atmosphere. After cooling, the mixture is filtered and washed with water until neutralized. An 82% deacetylated chitosan is obtained with this method.

iii. Deacetylation with Mixed Solvents
(Broussignac, 1968)

Chitin is deacetylated by a mixture of potassium hydroxyde (50%), plus 96° ethanol (25%) and monoethylenglycol (25%) at boiling temperature (120°C). After 16 hours, the mixture is filtered and washed with water. Chitosan with approximately 83% of deacetylation is obtained.

2.1.2.2 Properties of chitosan

2.1.2.2.1 Solubility

Chitosan is a hydrophilic polysaccharide that is insoluble in water and in organic solvents, alkali and in some mineral acids such as phosphoric and sulfuric acids. However, it is soluble in dilute aqueous organic acid solutions (Filar and Wirick, 1978; Muzzarelli, 1973). In the process of solubilization the 2-amino group is protonated, thus providing the polycationic nature of chitosan molecules in solution.

In the presence of a limited amount of acid, chitosan is soluble in water-methanol, water-ethanol, water-acetone, and other mixtures (Muzzarelli, 1973).

The solubility of chitin and chitosan in dimethylformamide and dinitrogen tetroxide as compared with other polysaccharides is shown in Table 3 (Allan et al., 1971). Infrared analysis of the starting material and products indicated the absence of chemical modifications. For the mechanism by which solution occur, it was suggested that hydrogen bonds are disrupted by NO^+ ions (Muzzarelli, 1973).

2.1.1.3.3 Reactivity and Reactions

Oxidative Deamination

Several methods have been used for the deamination of chitosan. Deamination of chitosan can be achieved by reacting chitosan with hydrochloric acid-silver nitrate mixture (Matsushima and Fujii, 1957), or with periodate at

Table 3

Solubility of Marine Polysaccharides in Dimethylformamide and Dinitrogen Tetroxide (Allan *et al.*, 1971)

Polysaccharide	Fraction Dissolved (%)
Alginic acid	70
Agar	100
Chitin	5 (swell)
Chitosan	100
Laminaran	100
Mucilage of *Uva lactura*	75
Propylene glycol alginate	100

25°C and pH's between 5.0 and 3.0 (Jeanloz and Forchielli, 1950).

The deaminated chitosan has been studied as an inhibitor of tumor growth, and it has been tested *in vitro* against Leukemia L-1210 cells. The results concluded that deaminated chitosan and the 6C carboxilated analog inhibited cell growth by 50% at concentration of 0.6 mM (Sirica and Woodman, 1971).

Carboxymethylation

Carboxymethylchitosan has been obtained as sodium salt through deamination and carboxymethylation of chitin (Okimasu, 1958). Carboxymethylchitosan is soluble in 2% acetic acid, but if the degree of substitution is near one it is soluble in water. Carboxymethylchitosan has the isoelectric point at pH 6.3 and its solution behavior resembles typical polyelectrolyte solutions, with the viscosity strongly affected by the salt concentration of the media.

An important possibility for commercial application of carboxymethylchitosan is its expected reactivity with metal ions in water treatment plants and mining (Muzzarelli, 1983).

Sulfation

Reactions for the production of chitosan sulfate have been carried on with sulfuric acid (Nagasawa and Tanoura, 1972) and sulfur trioxide-N, N-dimethylformamide complex (Hoffman-LaRoche, 1957). Due to the stabilizing effect of the free amino groups, chitosan is more stable than chitin to depolimerization, thus making chitosan a better substrate than chitin.

Both chitin and chitosan sulfate have been studied in connection with their anticoagulant activity and compared with a well known anticoagulant such as heparin (Wolfrom, 1958).

2.1.2.3 Film Properties

The mechanical properties of chitosan films have been related to the preparing procedure and to the film structure. The mechanical responses of swollen chitosan films show that chitosan films do not behave as an ideal rubber-like material. The non-ideality of chitosan films has been represented by a negative slope on the plots of the reduce stress versus the reciprocal of the elongation (Kienzle-Sterzer, 1980). This negative deviation suggested that the intermolecular interactions increased with increasing strain (Kienzle-Sterzer, 1980; Kienzle-Sterzer *et al.*, 1980). This orientation of the chitosan molecules upon elongation was confirmed by x-ray diffraction (Samuels, 1981).

The results of the stress-relaxation and the dynamical tests performed on chitosan films showed the ionic characteristic of the interactions responsible for the network formation (Kienzle-Sterzer, 1982b; Ogura *et al.*, 1980). Stress-relaxation experiments of swollen chitosan films in aqueous medium of different ionic strength (0-2.0 M NaCl) showed that the elasticity modulus increases with increasing ionic strength. Also, the relaxation times in the order of 10^4 to 10^5 seconds (Kienzle-Sterzer *et al.*, 1982a,b) show the electrostatic nature of the inter- and intra-molecular interactions in chitosan networks.

2.1.3 Industrial Applications of Chitin and Chitosan

Polysaccharides play important roles in many biotechnological processes and their use will increase. For example, polysaccharides are used:

- in recovery and delivery processes
- as materials in supports for bioreactors
- as precursors and/or raw materials for a variety of processes.

Critical to the use of polysaccharides in such applications are:

1) the type of sugar backbone and its chemical linkage
2) the charge density of the polymer
3) the molecular weight of the polysaccharide.

Chitosan is deacetylated chitin. The chitin sugar backbone structure is poly-β(1-4)-N-acetyl-D-glucosamine. Both chitin and chitosan are processed industrially from crustacean shell wastes (Knorr, 1984). Chitin is also found in insects, fungi and yeast, and wholly deacetylated chitin is found in various fungi. The availability of chitin has been recently summarized by Knorr (1984) in Table 1, indicating that chitin is widely available from a wide variety of sources. Chitosan can be chemically modified. The uses of a number of novel chitosan derivatives have been reviewed by Muzzarelli (1983) (see Table 4).

Chitosan is one of the few natural cationic polyelectrolytes. This property in itself results in many useful applications of chitosan and some will be described below.

A variety of investigators have been determining the functional properties of chitin and chitosan (Knorr, 1984; Muzzarelli, 1983; Kienzle-Sterzer *et al*., 1982; Robinson-Lang *et al*., 1982). Properties that have been investigated include:

Table 4. Chitosan Derivatives and Proposed Uses (Muzzarelli,1983).

<u>N-Acyl chitosans</u>: acetyl, propionyl, butryl, hexanoyl, octanoyl, tetradecanoyl, lauroyl, myristoyl, palmitoyl, stearoyl, benzoyl, dichloroacetyl, carbamoyl (textiles, membranes).

<u>N-Carboxyalkyl chitosans:</u> N-carboxymethyl (metal ion collection).

<u>N-Carboxyacyl chitosans</u>: from anhydrides such as: maleic, itaconic, (acetylthio)succinic, glutaric, cyclohexane-1, 2-dicarboxylic, phtalic, cis-tetrahydrophtalic, 5-norbornene-2,3-dicarboxylic, diphenic, salicyl.

<u>O-Carboxyalkyl chitosans</u>: O-carboxymethyl, epichlorohydrin-cross-linked O-carboxymethyl (membranes, molecular sieves).

<u>Deoxyglycit-l-yl chitosans</u>: l-deoxygalactit-l-yl, l-deoxyglucit-l-yl, l-deoxymelibiit-l-yl, l-lactit-l-yl (gels, drilling muds).

<u>Metal ions - chitosan chelates</u>: palladium, copper, silver, iodine (catalysis, photography, health products, insectisides).

Table 4. (Continued)

Semi-synthetic resins of chitosan: Methyl methacrylate, poly(urea-urethane), poly(amide-esther), acrylamide-maleic, anhydride-chitosan copolymer.

Natural polysaccharide complex: chitosan-glucan (flocculation, metal ion chelation).

Miscellaneous: alkali chitin (intermediate), benzyl chitin (serine protease purification), hydroxybuthyl chitosan (desalting), cyanoethyl chitosan (filtration, dialysis, insulating papers), glycol chitosan (dialysis, special papers), glutaraldehyde chitosan (enzyme immobilization), linoleic acid-chitosan complex (food additive, anti-cholesterolemic), uracyl chitosan, theophilline chitosan, adenine chitosan, chitosan salts of acidic polysaccharides.

- Hydrophilic properties
- Interphasic properties
- Intermolecular properties
- Sensory properties
- Biodegradability
- Chelating properties
- Rheological properties.

These studies have allowed investigators to explore applications of chitosan for a variety of high technology, as well as more conventional applications.

Industrial applications include:

- As a coagulating agent,for example recovery of protein from waste products (Shinoda _et al_., 1979; Wu _et al_., 1978).
- As a chelating material, such as removal of heavy metals from water (Randall _et al_.,1979; Hall and Yalpani, 1980).
- As an adsorbent (Unitika Ltd., 1982).

High technology applications include:

Biomaterials for medical applications;

- Wound healing (Muzzarelli, 1983)
- Hemostatic (Malette _et al_., 1983)

- Artificial kidney membranes (Hirano *et al.*, 1981)
- Contact lenses (Ivani, 1982)
- Dermatology (Gross *et al.*, 1982)

Drug delivery;

- Drug compounding (Sawayanagi *et al.*, 1982a)
- Sustained release (Sawayanagi *et al.*, 1982b)

Support;

- Immobilized enzymes (Muzzarelli, 1980)
- Metal catalysts (Arena, 1983)
- Immobilization of animal cells (Mosbach *et al.*, 1982)

Composites;

- Antistatic agent in fibers (Kao Soap Co., 1982)
- Collagen matrix as a sausage casing (Kosugi *et al.*, 1981)
- Texturizing agent such as a complex with acidic polysaccharides (Bomstein, 1974)
- Graft copolymer for paper (Sinkovitz and Slagel, 1973)
- Magnetic particles (Landel *et al.*, 1981)

Films;

- Diffusion transfer film system (Berger et al., 1983)
- Diazo copying layer (Ricoh Co., 1983)
- Dialysis (Ando and Kataoka, 1979)
- Photography (Scott, 1977)

Fiber;

- Hollow-fibers (Pittalis et al., 1983)
- Textile industries (Tokura et al., 1979)

Critical to these broad applications is a fundamental understanding of the solution properties of chitosan and how they may be controlled.

Entrapment and Encapsulation with Chitosan

Immobilization techniques for living organisms and biocatalysts such as enzymes offer solutions to a variety of problems in biotechnology. For example, advantages can include:

- Improvements in cell separation and product recovery
- Improved productivity of bioprocesses

- Continuous processes
- Improved cell stability

The principles for entrapment and encapsulation processes with chitosan have been established (Rodriguez-Sanchez and Rha, 1981 and 1984). Entrapment methods are commonly based on the inclusion of cells within a network. Cells can not diffuse out of the network while nutrients can diffuse into the matrix. A variety of materials are widely used for entrapment processes. As examples: polyacrylamide gels (Shimizu *et al.*, 1975; Saif *et al.*, 1975; Ohmiya *et al.*, 1977); collagen (Vieth *et al.*, 1973; Saini and Vieth, 1975); agar gels (Toda, 1975; Weetall and Bennett 1976); Ca-alginate (Kierstan and Bucke, 1977; Jones and Veliky, 1981; Birnbaum *et al.*, 1981); cellulose triacetate (Fleming *et al.*, 1974); gelatin (Nilsson and Moshach, 1980).

Many of these immobilization processes involve severe fabrication conditions that may not be suitable for some biological systems. For example, the use of organic solvents, synthetic polymeric materials, high temperatures, or extreme pH values may cause inactivation of cells (lysis or death) as well as damage of their metabolic capabilites or the loss of activity in chemically or biologically active components.

In Table 5 the cell loading capacities for several entrapment or immobilization methods are compared to those

Table 5

Cell Loading Capacity of Various Supports

Support	Loading Capacity mg (dry cell)/ g (support)	Reference
Natural polymers		
Chitosan - alginate	279	Bokanga, 1984
Chitosan - polyphosphate	65-374	Bokanga, 1984
Chitosan polyphosphate	376	Vorlop and Klein, 1981
TGCI - KPVS complex*	83	Kokufuta *et al.*, 1982
Carrageenan	119	Chibata and Tosa, 1980
Synthetic polymers		
Dualite A-162	9	Durand and Navarro, 1978
Dulaite A-101	17	Durand and Navarro, 1978
Dowex	21	Durand and Navarro, 1978
Polyvinylchloride	80	Durand and Navarro, 1978
Inorganic supports		
Fritted glass	18	Messing, 1979
Cordierite ceramic	21	Messing, 1979
Zirconia ceramic	22	Messing, 1979

* TGCI = trimethylammonium glycol chitosan iodide
KPVS = potassium poly(vinylalcohol) sulfate

of chitosan matrices. As can be seen in Table 5, high cell loadings are achieved with chitosan-alginate and chitosan-polyphosphate systems.

The flow sheet describing the immobilization processes is summarized in Figure 6. The chitosan-alginate procedure results in capsules, while the cells are suspended in a liquid core. The structural features of these complexes are summarized schematically in Figure 7.

The growth rates for some tested microorganisms (*Bacillus subtilis* and *Bacillus lichenformis*) are found to be dependent on the selected encapsulation/entrapment procedure (Table 6). Cells in the chitosan-polyphosphate system are found to grow at a reduced rate. The rate was about one-third that of the control while the cells in the chitosan-alginate system are able to achieve growth rates comparable to those of the free cells. The explanation for these differences is thought to be due to the resulting differences in the physical properties of the chitosan structure.

Physical Properties of Chitosan Matrices

The fabrication methodology in Figure 6 demonstrates that chitosan matrices can be formulated as globules or as capsules. Both types of matrices are based on the elec-

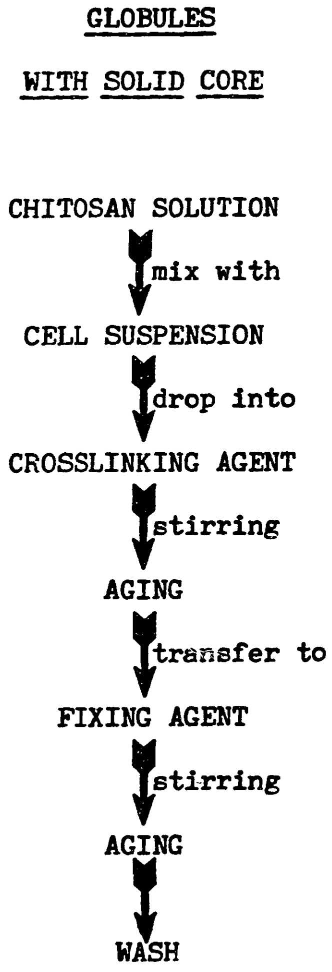
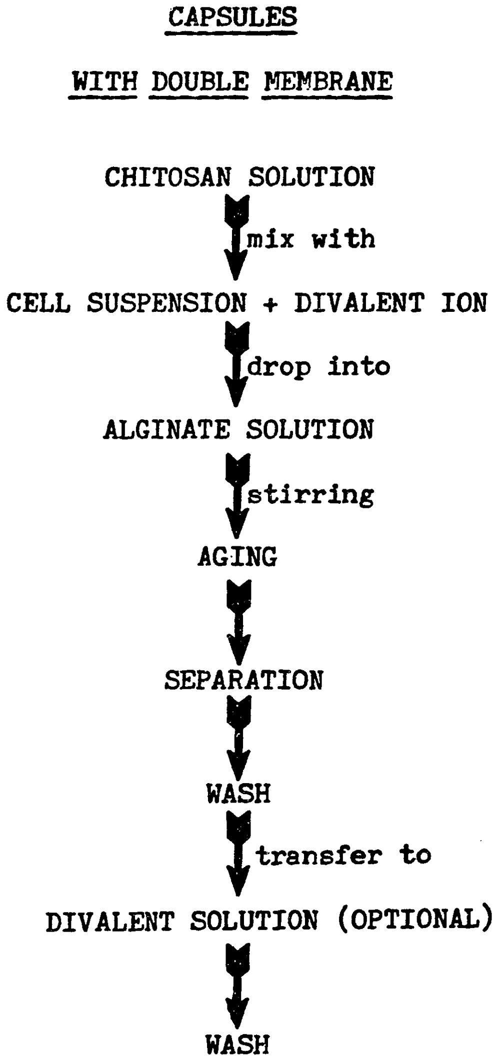


Figure 6. Immobilization process via encapsulation. (Rodriguez-Sanchez and Rha, 1984)



CAPSULES

WITH DOUBLE MEMBRANE

Figure 6. (Continued). Immobilization process via encapsulation. (Rodriguez-Sanchez and Rha, 1984).

CAPSULES

WITH INTERFACIAL POLYION-POLYION MEMBRANE

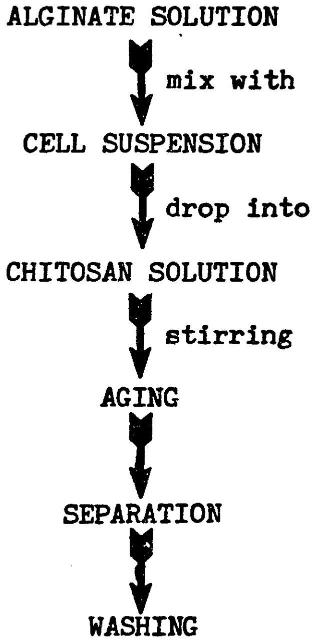


Figure 6. (Continue). Immobilization process via encapsulation. (Rodriguez-Sanchez and Rha, 1984).

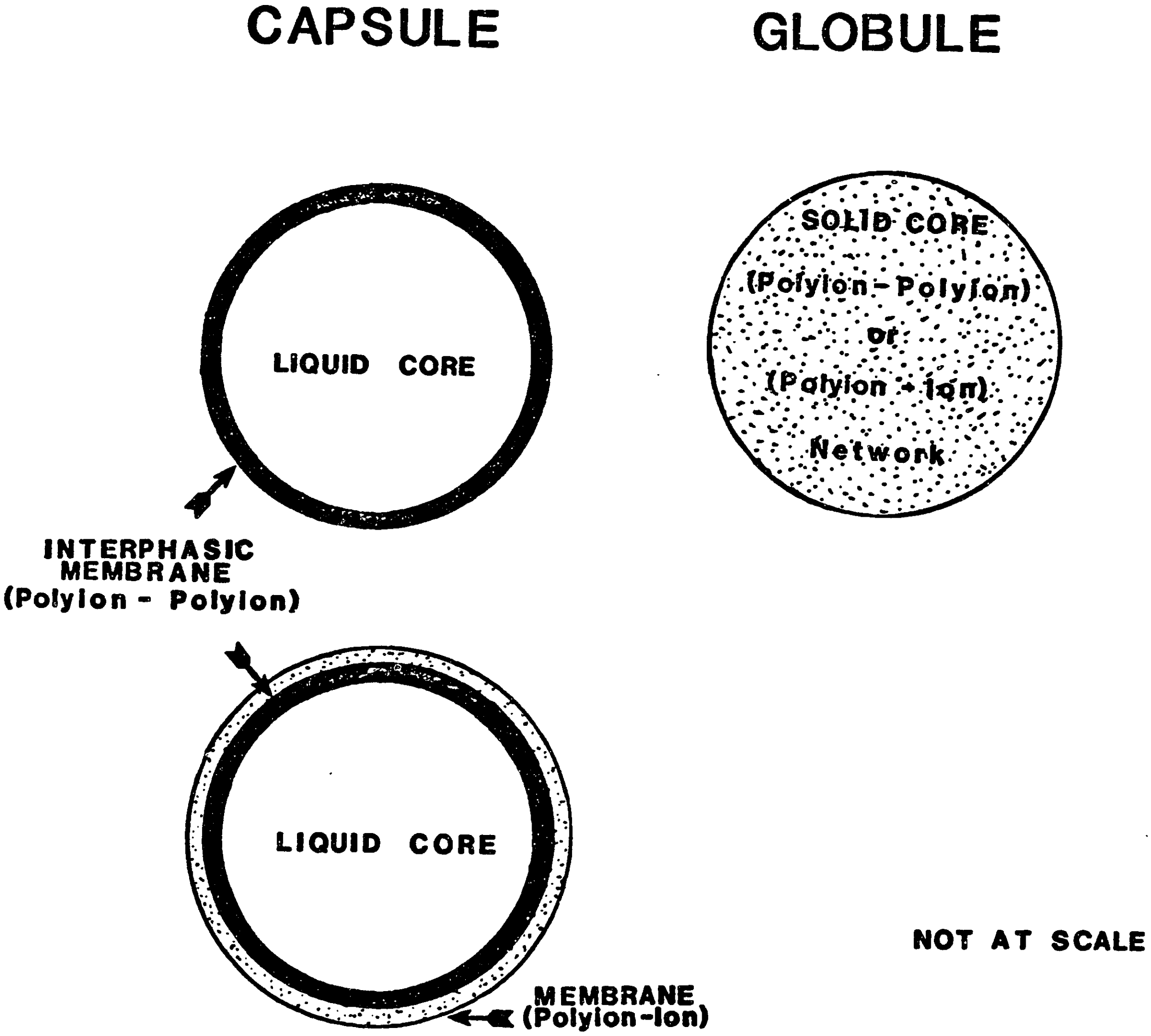


Figure 7. Structural Features of Capsules and Globules. (Rodriguez-Sanchez and Rha, 1984).

Table 6

Growth Rates of Free and Immobilized Cells (Bokanga, 1984)

	Growth rates (h^{-1})		
	Free cells	Immobilized Cells	
Microorganism		Chitosan polyphosphate	Chitosan alginate
B. subtilis	0.423	0.145 (34%)	0.413 (98%)
B. licheniformis	0.405	0.146 (36%)	0.294 (73%)

(Between brackets are given percentages of free cells' growth rates) trostatic interaction between the positive charges on the chitosan backbone and the negative charges on the anionic polymer. The mechanical properties of the chitosan globules and chitosan-alginate capsules have been evaluated (Rodriguez-Sanchez and Rha, 1981; Bokanga, 1984). These studies indicate that the capsules, as well as globules can withstand compression forces of up to 2000 g., and can undergo a 90% deformation without rupture. This fact makes the system suitable for industrial applications since the resistance to mechanical or handling stresses, that may be imposed in several separation or fermentation processes, is high.

Also, the properties of the chitosan-alginate capsules as influenced by the type and concentration of divalent ions, polymer concentration, extent of reaction time for polymer-polymer complex formation, pH and ionic strength has been evaluated. For example, it has been found that by replacing Ca^{++} ions by Ba^{++} ions the mechanical strength of the capsule is increased. Moreover, by controlling the concentration of the divalent ions the rupture strength may be varied from near 800 g/cm^2 to almost 2000 g/cm^2 when Ba^{++} concentration varies from 0.01 M to 0.27 M (Bokanga, 1984).

Studies on the role of both the type of the solvent and the chitosan concentration on the mechanical properties of chitosan matrices have been previously reported (Rodriguez-Sanchez and Rha, 1981; Kienzle-Sterzer et al., 1980, 1982a,b). Moreover, by manipulating the configuration of chitosan molecules in solution, which in turn controls the entanglement or crosslinking density of chitosan network, the mechanical and diffusional properties may be controlled. Therefore, the principles for predicting and designing the functional performance of the chitosan polymer complex can be rationally pursued for a variety of applications.

One primary interest is the use of the capsules (Rodriguez-Sanchez and Rha, 1984) for the encapsulation of living cells, mammalian cells (hybridoma cells), for the production of monoclonal antibodies. Currently, the the diffusion of monoclonal antibodies and other biological active components through the interphasic polyion-polyion membrane (Figure 7), is under evaluation.

In addition, the suitability of using chitosan matrices in other processes, including controlled delivery of both small and large molecules is being examined.

2.2 Polyelectrolytes

2.2.1 General Theory

Polyelectrolytes are polymers with potentially ionizable groups. In polar solvents they dissociated into polyions and counterions, and the strong electric interaction between them is the source of the characteristic properties of polyions in solution.

In order to understand the physic involved in the electrostatic interactions between polyion-counterions, as well as its affect in the spatial configuration of polyions in solution, several models have been developed such as the spherical model and the rod-like model (Oosawa, 1971). Even though these models are geometrically different, both represent the polyelectrolyte in solution as a charged colloidal particle. Therefore, the general equations used in colloidal science can be applied in this particular situation.

i. Spherical Model

In this model the polyelectrolyte molecule is idealized as a charged sphere immersed in a fluid of dielectric constant ε . Then, if we assume that the polyelectrolyte molecule is isolated, and that the charge density in the electrolyte solution creates an electric double layer, then the electric potential at a distance "r" from the surface will be expressed by the Poisson-Boltzman equation as:

$$\Delta\Psi = \frac{1}{r^2}\frac{d}{dr}\left(r^2\frac{d\Psi}{dr}\right) = \frac{1}{\varepsilon}\sum_i n_i^o Z_i \exp\left(\frac{-Z_i e\Psi}{KT}\right) \qquad (1)$$

where: Ψ = electric potential

n_i^o = number of ions of type "i" per unit volume

z_i = valence of ions of type "i"

e = charge of the electron = 1.6×10^{-19} coulomb

K = Boltzman constant

T = absolute temperature

Obviously, this equation cannot be solved analytically for complex situations such as polyelectrolytes, and simplifications such as the Debye-Huckel approximation must be used for small values of the potential (Adamson, 1976; Hiemenz, 1977). Then, the Poisson-Boltzman equation simplifies to:

$$\frac{1}{r^2}\frac{d}{dr}(r^2\frac{d\Psi}{dr}) = K^2\Psi \qquad (2)$$

where

$$K^2 = \frac{4\pi e^2}{\varepsilon KT}\sum_i n_i^o Z_i \qquad (3)$$

and with the boundary conditions:

$\Psi = \Psi_0$ when $r = 0$

$\Psi = 0$ when $r = \infty$

Then equation "2" has the solution:

$$\Psi = \frac{\Psi_o a}{r} \exp[-K(r-a)] \qquad (4)$$

where a = the radius of the particle

The Debye-Huckel approximation is strictly applicable only in the case of low surface potential; such as at 25^oC:

$$\Psi_{o,max} = \frac{KT}{e} = 25.7 \text{ mv.} \qquad (5)$$

Nonetheless, there are several reasons why the importance of the Debye-Huckel approximation ought to be fully understood:

1. It is the limiting result that all others more general approximations are reduced in the boundaries of low potentials.
2. The effects of valence and electrolyte concentration in this approximation are consistent with the experimental results.

ii. Rod-like Model

The rod-like model or better known as the cylindrical model of polyelectrolyte in very dilute solutions is based on the following assumptions (Manning, 1969a):

1. It must first be assumed that the Debye-Huckel limiting law is valid for both the polyelectrolyte solution and any other ionic species in solution. Therefore, the Heltzmon free energy, F^{ex}, of the solution at low concentrations is given by (Fowler and Guggenheim, 1960):

$$\frac{F^{ex}}{VKT} = -(\frac{4\pi e^2}{\varepsilon KT} \sum_i n_i z_i)^{1.5} (12\pi)^{-1} \qquad (6)$$

where V is the volume, e the protonic charge, ε the dielectric constant of the pure solvent, z_i the ions valence and n_i the ions concentration.

2. The real polyelectrolyte chain is replaced by an infinite line charge with density β given by:

$$\beta = \frac{Z_p eP}{L} \qquad (7)$$

where L is the countour length of the polyelectrolyte, P the number of charged groups on the polyelectrolyte backbone of valence Z_p.

3. Intermolecular interactions are neglected regardless of the ratio of polyelectrolyte concentration to that of added simple salt.

4. The dielectric constant ε is taken as that of the pure bulk solvent.

5. Sufficiently many counterions will "condense" on the polyion to lower the charge density parameter to the value of one for 1:1 salts.

The key problem in the evaluation of the electrostatic interactions between polyion and counterions is the obtention of the solution of the Poisson-Boltzmann equation. In general, the Poisson-Boltzmann equation in cylindrical coordinates can be written (Fouss et al., 1951; Alfrey et al., 1951):

$$\frac{1}{r}\frac{d}{dr}\left(r\frac{d\Psi}{dr}\right) = f(\Psi) \qquad (8)$$

with the boundering conditions:

$$\Psi = \Psi_0 \qquad \text{at} \qquad r = 0$$

and

$$\Psi = 0 \qquad \text{at} \qquad r = \infty$$

The difficulty in the solution of this equation is based in the selection of $f(\Psi)$. Several theoretical papers have been published in this area (Manning, 1969a, 1977, 1978; Anderson and Record, 1980; Lampert and Crandall, 1980; Imai and Sasaki, 1980; van Dijk et al., 1981; Deville and Laszlo, 1983; Odijk, 1983).

The most simple and most commonly used simplification for the solution of the Poisson-Boltzmann equation is the Debye-Huckel approximation, thus equation (8) transforms to:

$$\frac{1}{r}\frac{d}{dr}\left(r\frac{d\Psi}{dr}\right) = K^2\Psi \tag{9}$$

The solution of this equation is:

$$\Psi = \Psi_o \exp(-Kr) \tag{10}$$

The simplest approximation to the solution of the Poisson-Boltzmann equation for dilute polyelectrolyte solutions has been widely used (Flory, 1953; Oosawa, 1971; Fixman, 1964, and 1979).

A critical topic in the modern cylindrical model for dilute polyelectrolyte solutions is the "condensation phenomenon." It is well known that highly charged polyions whose linear charge density exceeds a certain threshold, bind with counterions present in their vicinity. In fact, the concept of counterion condensation is quite similar to that arising from the condensation of two dimensional coulomb gas formulated by Kosterlitz and Thoules (1973) and Hauge and Hemmer (1971). The counterion condensation phenomenon was first described by Oosawa (1957) and later elaborated by Manning (1969a,b).

In typical cases, counterions are not bound to polyions by covalent bonds. Nevertheless, in favorable cases, frequently found in practice, the close approach between a counterion and a polyion occurs with mutual perturbation of their hydration layers (Manning, 1979). The perturbation of the hydration layers around the polyelectrolyte and counterions results in changes in volume that can be measured by volumetric techniques (Strauss, 1974) or probed by ultrasonic absorption (Tondre et al., 1978) or refraction index measurements (Ikegami, 1964).

In the counterion condensation phenomenon the counterions moving from a state of free hydration to an environment of perturbed hydration can be called "bound" to the polyion (Anderson and Record, 1980; Boyd and Wilson, 1982; Kerenzi et al., 1978).

Counterions bound to macroions can be classified into two types; nonlocalized, bound but mobile, and localize. If the counterions are tightly bound to individual groups, the water molecules around the polyion must be wholly rearranged. But, if the counterions are not localized but mobile around the polyion, the influence on the water structure in the neighborhood of the microion may be very small (Oosawa, 1971; Manning, 1979).

The phenomenon of counterion condensation at present appears to be evaluated remarkably in a general fashion. It has been confirmed for univalent, divalent and tri-

valent counterions in both dilute and semidilute regimes (Koene et al., 1983; Russel, 1982; Rinaudo and Milas, 1976; Tan and Marcus, 1976; Wells, 1973).
Therefore, we can conclude that increasing the number of charges, the electrical field surronding the polyion becomes stronger and more counterions are retained in the volume of the macroion. Moreover when the number of charges increases beyond a critical value, the apparent degree of dissociation decreases until it becomes equal to one for monovalent counterions, which leads to the minimum separation allows between the charges on the polyion backbone of about 7.33 Å. This characteristic phenomenon of polyelectrolyte solutions is called "counterion condensation".

2.2.2 Intermolecular Interactions

To understand the interaction between a pair of isolated molecules, it is convenient to study the potential energy (ϕ) instead of the resultant force between the molecules.
Table 7 presents a partial list of the main interactions between a pair of isolated molecules and/or ions (Hiemenz, 1977).

In general, the combined effects of the van der Waals attraction and interparticle repulsion can be represented by the equation:

TABLE 7. *Partial list of interactions between pairs of isolated ions and/or molecules. Functions which describe the potential energy versus separation are listed along with the appropriate proportionality constants.* (Hiemenz, 1977).

Description	Φ	Definitions and restrictions	Attributed to	Value of n in $\Phi \propto x^{-n}$
Ion 1–ion 2	$\frac{(ze)_1(ze)_2}{x}$	z = valence, e = electron charge under vacuum (otherwise ε in denominator) (sign depends on the z value)	Coulomb	1
Ion 1–permanent dipole 2	$\frac{(ze)_1\mu_2 \cos\theta}{x^2}$	μ = dipole moment, θ = angle between line of centers and axis of dipole. Length of dipole small compared to x (sign depends on z and orientation)	(Coulomb)	2
Permanent dipole 1–permanent dipole 2	$(\text{const})\frac{\mu_1\mu_2}{x^3}$	Numerical constant (including sign) depends on orientation. Const = $\sqrt{2}$ for average over all orientations. Const = +2 for parallel and −2 for antiparallel alignment	(Coulomb)	3
Permanent dipole 1–induced dipole 2	$-\frac{(\alpha_{0,1}\mu_2^2+\alpha_{0,2}\mu_1^2)}{x^6}$	α_0 = polarizability (always negative)	Debye	6
Permanent dipole 1–permanent dipole 2	$-\frac{2}{3}\frac{\mu_1^2\mu_2^2}{kTx^6}$	Free rotation of dipoles (always negative)	Keesom	6
Induced dipole 1–induced dipole 2	$-\frac{3}{2}\frac{h}{x^6}\frac{\nu_1\nu_2}{\nu_1+\nu_2}\alpha_{0,1}\alpha_{0,2}$	ν = characteristic vibrational frequency of electrons (always negative)	London	6
Induced dipole 1–induced dipole 2 (retarded)	$-\frac{23}{8\pi^2}hc\frac{\alpha_{0,1}\alpha_{0,2}}{x^7}$	h = Planck's constant, c = speed of light. Applies if $x > c/\nu$ (always negative)	Casimmir and Polder	7
Repulsion	$+\frac{\xi}{x^{12}}$	Exponent in range of 9–15. 12 mathematically convenient (always positive)		12

$$\phi = f(x^{-12}) - g(x^{-6}) = \phi_r - \phi_a \qquad (11)$$

where $f(x^{-12})$ represents the repulsive interparticle interactions, $g(x^{-6})$ represents the attractive interparticle interactions, and x is the distance between particles. Therefore, in order to study the intermolecular interactions, we must consider in detail both attractive and repulsive interactions as well as their cause-effect.

2.2.2.1 Attractive Interactions

In order to quantify the attractive intermolecular interactions, the polyelectrolyute molecule in solution can be considered as a colloidal particle.

The main sources of attractive interactions are:

a) Interaction between dipoles, whether permanent or induced. The interactions of this type are the result of the electric field produced by one dipole acting upon a second dipole.

b) The London forces are dispersion forces between a pair of induced dipoles. The London attractions emerge from the contributions to the "bond" between a pair of particles.

The potential energy of attraction (ϕ_a), between two particles of several geometrics, is shown in Table 8; where A is the Hamaker constant calculated by the expression:

$$A = \left(\frac{\rho N_a \pi}{M}\right)^2 \beta \qquad (12)$$

where β is a constant used to represent the various constants in the Debye, Keesom and London equations, shown in Table 7; N_A is the Avogadro's number; ρ is the charge density and M is the molecular weight.

2.2.2.2 Repulsive Interactions

The repulsive interactions between colloidal particles are the result of the overlapping of their double layers. For the derivation of the equation that describes the repulsive potential energy between two infinite planes must be assumed that all the forces on any volume element of the solution must be in equilibrium. In general, two types of forces operate on this volume element: pressure and electrostatic forces (F_x and F_{el}, respectively).

TABLE 8. *Potential energy of attraction between two particles with the indicated geometries.* (Hiemenz, 1977).

Particles	Φ_A	Definitions/limitations
Two spheres	$-\frac{A}{6}\left[\frac{2R_1R_2}{s_0^2+2R_1s_0+2R_2s_0}+\frac{2R_1R_2}{s_0^2+2R_1s_0+2R_2s_0+4R_1R_2}+\ln\left(\frac{s_0^2+2R_1s_0+2R_2s_0}{s_0^2+2R_1s_0+2R_2s_0+4R_1R_2}\right)\right]$	R_1, R_2 = radii, s_0 = separation of surfaces
Two spheres of equal radius	$-\frac{A}{6}\left[\frac{2R^2}{s_0^2+4Rs_0}+\frac{2R^2}{s_0^2+4Rs_0+4R^2}+\ln\left(\frac{s_0^2+4Rs_0}{s_0^2+4Rs_0+4R^2}\right)\right]$	$R_1 = R_2 = R$
Two spheres of equal radius	$-\frac{AR}{12s_0}$	$R \gg s_0$
Two spheres of unequal radius	$-\frac{AR_1R_2}{6s_0(R_1+R_2)}$	R_1 and $R_2 \gg s_0$
Two plates of equal thickness	$-\frac{A}{12\pi}\left[\frac{1}{D^2}+\frac{1}{(D+2\delta)^2}-\frac{2}{(D+\delta)^2}\right]$	δ = thickness of plates
Identical blocks	$-\frac{A}{12\pi D^2}$	$\delta \to \infty$

Then:

$$F_x = \frac{dP}{dx} \tag{13}$$

and

$$F_{el} = \rho \frac{d\Psi}{dx} \tag{14}$$

then at equilibrium:

$$\frac{dP}{dx} - \frac{\varepsilon}{4\pi} \frac{d^2\Psi}{dx^2} \frac{d\Psi}{dx} = 0 \tag{15}$$

that under the boundary conditions:

$P = P_o$ at $\Psi = 0$

and

$P = P_d$ at $\Psi = \Psi_d$

solves to:

$$F_R = 64 n_o K T \gamma_o^2 \exp(-2dK) \tag{16}$$

where d is the distance between the plates; F_R is the total repulsion force and γ_0 is calculated by:

$$\gamma_o = \frac{\exp(Ze\Psi_o/2KT)-1}{\exp(Ze\Psi_o/2KT)+1} \quad (17)$$

and if $d\phi_R = -F_R d(2d)$ then

$$\phi_R = \frac{64n_o KT\gamma_o^2}{K} \exp\ (-2dK) \quad (18)$$

This equation for ϕ_R shows the following:

1. The potential energy of repulsion may be extended to long distances from the surfaces.

2. The range of action of the potential energy of the repulsion is restricted by increasing the electrolyte concentration in solution.

Therefore, it is clear for the above discussion that for dilute solutions of linear flexible polyelectrolytes, the macroion will extend with either an increase of the number of charged groups and contract with an increase of the concentration of macroions and simple salts. This conclusion is supported by the increase of the reduced viscosity with dilution and by the relationship between the intrinsic viscosity, the degree of ionization and the counterion concentration.

3 MATERIALS AND METHODS

3.1 Materials

Chitosan, Madera chitosan (Bio-Shell, Inc., Albany, Oregon) obtained from Tanner (snow) crab, *Chionoecetes bairdi,* with less than 1% of protein and ash, and with 80% deacetilation, will be used.

Analytical grade hydrochloric acid for solubilization, sodium hydroxide for pH adjustment, sodium chloride for obtaining different ionic strengths, and D- 6-^{3}H glucose aqueous solution (Smershan/Searle Corp. Ill. U.S.A.) will be utilized without further purification.

An estimate of the molecular weight of chitosan was obtained from the intrinsic viscosity of dilute chitosan solutions in 1% acetic acid with 2% LiCl at 25°C. The Mark-Kuhn-Houwink parameters used ($K_n \times 10^2 = 72.8$, "a" = 0.186) as well as the experimental solution conditions were selected from the literature (Berkovich *et al* ., 1980). The estimated value of molecular weight obtained for chitosan is 130,000 daltons.

Measurements of the pH and counterion activity coefficients will be obtained using an Orion pH electrode model 91-02 and an Orion combination chloride electrode model 96-17B connected to an Orion microprocessor pH/millivolt meter model 811 (Orion Research Inc., Cambridge, MA). The experimental temperature was 25°C.

3.2 Methods

3.2.1 Preparation of Stock Solutions:

Solutions of chitosan will be prepared (Muzzarelli, 1973) by using hydrochloric acid as solvent at concentrations of 0.04 M for the intrinsic viscosity experiments and 0.08 M for the potentiometric titration, the counterion activity, the glucose diffusion experiments and the rheology of concentrated chitosan solutions.

To obtain a good dispersion, chitosan powder will be sifted slowly onto the surface of the vortexing acid solution in order to minimize agglomeration. The addition of chitosan powder will be completed before thickening of the solution destroys the vortex. The solution will be stirred until total solubilization while controlling the mixing to prevent excessive aeration.

After solubilization is completed, the chitosan solutions will be allowed to rest overnight (12 hours approximately) and filtered. Then, NaCl will be added in order to adjust the ionic strength between 0.05 M and 0.50 M. Immediately, the chitosan solutions will be partially neutralized using standard solutions of NaOH containing NaCl (in order to keep the counterion concentration constant), to the desirable pH.

Once the titration is completed, the chitosan solutions will be kept in a cool room at 4 °C until further testing.

3.2.2 Intrinsic Viscosity

Viscosity data will be obtained with Cannon-Fenske capillary viscometers immersed in a water tank at 25.0 ± 0.1°C.

In order to study the effect of the pH and the ionic strength on the hydrodynamic behavior of chitosan solutions, the intrinsic viscosity at pH values between 3.0 and 6.0 and ionic strength ranging from 0.05 M to 0.50 M will be obtained.

Intrinsic viscosity at each set of experimental conditions, $[\eta]$, will be determined by fitting the measurements of both η_{sp}/C and ln η_r/C for at least five polymer concentrations, C, using the equations (Kraemer, 1938; Huggins, 1942):

$$\frac{\eta_{sp}}{C} = [\eta] + K' [\eta]^2 C \qquad (19)$$

$$\frac{\ln \eta_r}{C} = [\eta] + K'' [\eta]^2 C \qquad (20)$$

where η_{sp} = specific viscosity

η_r = relative viscosity

K' and K" = Huggins' and Kraemer's constants.

Solutions of chitosan concentrations ranging between 0.01 g/dl and 0.10 g/dl will be prepared by diluting the stock solutions with hydrochloric acid (10^{-3} M) - NaCl solutions.

During the past 30 years, great effort has been made to understand the effect of both "short-range" and "long-range" interactions between chain segments upon the conformation and dimension of polymers in solution. One of the most simple and effective source of experimental information about chain dimension is the study of the viscosity of dilute polymer solutions.

In absence of both types of interactions, a long macromolecular chain can be modeled by means of the random-flight statistics (Flory, 1953). However, the presence of either short or long range interactions introduce appreciable deviations from the statistical model.

A molecule in solution when only affected by short range interactions is in its "unperturbed" state. Therefore, the molecule radius of gyration $< S^2 >$ can be defined as (Flory, 1953):

$$\langle S^2 \rangle = sl^2n/6 \tag{21}$$

where "n" is the number of bonds, "l" is the bond length and "s" is a correction factor independent of "n." However, if both types of interactions are taken into consideration, the radius of gyration is defined as:

$$\langle S^2 \rangle = (\Xi^2 s)l^2n/6 \tag{22}$$

where " Ξ " is a correction factor attributed to the so-called "excluded volume effect" which cannot be explained by Gaussian statistics (Tamura and Kurata, 1952; Flory, 1953).

Polymer molecules in solution show that the intrinsic viscosity is proportional to the effective hydrodynamic volume (Fox and Flory, 1949):

$$[\eta] = \frac{\Phi \langle S^2 \rangle^{3/2}}{M} \tag{23}$$

where $[\eta]$ is the intrinsic viscosity, Φ is a universal constant and M is the molecular weight of the polymer. Transformation of such relationships, under the assumption of no excluded volume effects, allows the calculation of the intrinsic viscosity at theta condition where the molecule shows its unperturbed dimensions (Flory, 1953):

$$[\eta] = K\ M^{1/2} \tag{24}$$

where "K" is a constant for a given polymer, that is independent of the solvent and the molecular weight, M, defined as:

$$K = \Phi\ \left(\frac{<S^2>}{M}\right)^{3/2} \tag{25}$$

therefore, the linear expansion coefficient of the polymer coil, α_η^3 , is represented by the ratio:

$$\alpha_\eta^3 = \frac{[\eta]}{[\eta]_\theta} \tag{26}$$

where it is assumed that the Flory constant, Φ , is independent of the expansion coefficient. However, numerous experiments convincingly demonstrated that the Flory's parameter does depend on the degree of swelling of the polymer molecule (Takahashi <u>et al</u> ., 1967), suggesting that an approximation of the function, $\Phi(\varepsilon)$, must be used. A theoretical examination of the functionality of Flory's parameter with the expansion coefficient, $\Phi(\varepsilon)$, leads to the expression (Ptitsyn and Eizner, 1959):

$$\Phi(\varepsilon) = 2.86 \times 10^{21} \left(1 - 2.63\,\varepsilon + 2.86\,\varepsilon^{2} \right) \tag{27}$$

where

$$\varepsilon = \left(\alpha_{\eta}^{2} - 1 \right) \Big/ \left(5\,\alpha_{\eta}^{2} - 3 \right) \tag{28}$$

In general, a more careful treatment to the calculation of the expansion coefficient would lead to the expression:

$$\alpha_{\eta}^{3} = \frac{\Phi_{\Theta}}{\Phi(\varepsilon)} \frac{[\eta]}{[\eta]_{\Theta}} \tag{29}$$

The dynamic behavior of the polyelectrolyte in solution is much more complicated than that of the ordinary non-electrolyte polymer in solution since the dissolved ionic polymers display an electrostatic charge effect. Such electrostatic charges exert repulsive forces between the fixed charged groups on the macromolecular backbone expanding the polyion. This resulting in a larger increase of the hydrodynamic domain than that of the non-charged counterparts.

This phenomena is represented by the anomalous relationship between the reduced viscosity and polymer concentration at low ionic strengths. At a low ionic strength, the reduced viscosity at the limit of infinite dilution tends to increase instead of the expected linear decrease.

This characteristic behavior of polyelectrolyte solutions can be suppressed by the addition of simple salts. Therefore, increasing the ionic strength of the media makes the relationsihp between the reduced viscosity and the polymer concentration approaches to that of the ordinary non-electrolytic polymer solutions. This effect can be interpreted qualitatively as the result of the counterions neutralizing the charges on polyion molecules.

In order to estimate the molecular expansion caused by the electrostatic repulsive forces on polyelectrolytes in solutions, a linear electrostatic expansion factor " $\alpha_{\eta e}$ " defined as:

$$\alpha^{3}_{\eta e} = \frac{\Phi(o)}{\Phi(\varepsilon)} \frac{[\eta]}{[\eta]_{0}} \tag{30}$$

may be useful, where $[\eta]_0$ is the intrinsic viscosity of the nonionic "parent" polymer, obtained by extrapolating to infinite ionic strength the relationship between the intrinsic viscosity and the ionic strength (Nagasawa, 1975).

Currently, there are sufficient experimental results (Cox, 1960; Takahashi and Nagasawa, 1964; Ross and Scruggs, 1968; Noda *et al* ., 1970) as well as theoretical indications (Fixman, 1964; Yeh and Isihara, 1971) that show the linear relationship between the intrinsic viscosity and the reciprocal of the square root of the ionic strength. Then the application of the Stockmayer-Fixman relationship (Stockmayer and Fixman, 1963) allowed, by linear regression analyses, to obtain the intrinsic viscosity corresponding to the uncharged state of the polyion.

Several approaches relating the expansion factor with intrinsic molecular parameters have been proposed:

a) Flory approach (Flory, 1953):

$$\alpha_\eta^5 - \alpha_\eta^3 = 2.60\ Z \qquad (31)$$

Z, the excluded volume function represented by:

$$Z = \left(\frac{3}{2\pi}\right)^{3/2} B\ A^{-3}\ M^{1/2} \qquad (32)$$

where

$$A = \frac{\langle h_0^2 \rangle}{M} \qquad (33)$$

and

$$B = \frac{\beta}{M_s^2} \tag{34}$$

where $\langle h_o^2 \rangle$ is the mean end to end distance of the unperturbed polymer, M and M_s are the molecular weights of the polymer and segment respectively, and β is the excluded volume.

b) Stockmayer-Fixman approach (Stockmayer and Fixman, 1963):

$$\begin{aligned} \alpha_\eta^3 - 1 &= 2\,Z \qquad \text{for} \quad 1 < \alpha_\eta^2 < 2 \\ \alpha_\eta^2 - 0.35 &= (1.7\,Z)^{1/2} \qquad \text{for} \quad 2 < \alpha_\eta^2 < 25 \end{aligned} \tag{35}$$

For polyelectrolytes (Takahashi and Nagasawa, 1964) the expresion for B as equation (34) has been replaced by

$$B = B_o + B_e = B_o + C/C_s \tag{36}$$

where C is a numerical constant, B_o is the value of B at infinite ionic strength and B_e corresponds to excluded volume due to electrostatic interaction. Then, the Stockmayer-Fixman equation will be:

$$[\eta] = K + 0.51\,\Phi(\varepsilon)\left(B_o + \frac{C}{\sqrt{C_s}}\right)M \tag{37}$$

or

$$[\eta] = (\text{const.})_1 + (\text{const.})_2 \frac{1}{\sqrt{C_s}} \qquad (38)$$

which explains the relationship between the intrinsic viscosity and the ionic strength.

3.2.3 Potentiometric Titration:

Measurements of pH will be made using an Orion pH electrode model 91-02 connected to an Orion microprocessor pH/millivolt meter model 811 (Orion Research Inc., Cambridge, MA). The temperature of the system will be controlled at 25.0 ± 0.1°C. All titrations will be carried out under nitrogen atmosphere to eliminate CO_2 absorption from the air.

The general procedure for titration will be: 50 ml of chitosan solution placed in a beaker where the electrode will be immersed; a magnetic stirring will be used to provide stirring. After recording the initial solution pH a known volume of standardized solutions of NaOH-NaCl will be added. The new pH value will be read after an equilibrium is reached.

Before each titration the electrode will be standardized with buffers at the same temperature.

The fraction of ionizable groups dissociated, α , will be determined as follows: The components of the experimental system are: NaOH, HCl, NaCl and chitosan, "Ch." The following reactions must be considered:

$$\mathrm{NaOH} \longrightarrow \mathrm{Na^+} + \mathrm{OH^-}$$
$$\mathrm{NaCl} \longrightarrow \mathrm{Na^+} + \mathrm{Cl^-}$$
$$\mathrm{HCl} \longrightarrow \mathrm{H^+} + \mathrm{Cl^-}$$
$$\mathrm{H^+} + \mathrm{OH^-} \rightleftharpoons \mathrm{H_2O}$$
$$\mathrm{Ch} + \mathrm{H^+} \rightleftharpoons \mathrm{ChH^+}$$

By the general electroneutrality concept, we have:

$$[\mathrm{ChH^+}] = [\mathrm{Cl^-}] + [\mathrm{OH^-}] - [\mathrm{Na^+}] - [\mathrm{H^+}] \qquad (39)$$

and if "α" is defined as:

$$\alpha = \frac{[\mathrm{ChH^+}]}{[\mathrm{Ch}]} \qquad (40)$$

then:

$$\alpha = \frac{[\mathrm{Cl^-}] + [\mathrm{OH^-}] - [\mathrm{Na^+}] - [\mathrm{H^+}]}{[\mathrm{Ch}]} \qquad (41)$$

being [Ch] expressed in monomolar.

At this point, it should be noted that the [Cl^-] will be calculated by multiplying the total chloride ion concentration by the activity coefficient in order to account for any non-ideal behavior.

In the titration of a polyion in solution with a strong acid or base, the removal of a proton depends on the charge density of the polyelectrolyte (Kotin and Nagasawa, 1962). According to Lifson (1957) the pH of a polyelectrolyte solution is expressed as:

$$pH = pK_o + \log a - 0.4343 \frac{e\Psi}{KT} \tag{42}$$

where pK_o is the intrinsic dissociation constant, "a" is the ratio between the activity of the ionized to the non-ionized group, T is the temperature and ψ is the electrostatic potential of the polyelectrolyte due to long-range electrostatic interactions. When nearest-neighbor interactions are incorporated, the term "log a" in equation (42) is replaced by the common expression: $\log(\alpha/(1-\alpha))$, where "α" is the degree of ionization of the ionizable group. Therefore, the pH of the polyelectrolyte solution can be represented by (Overbeck, 1948; Katchaslky <u>et al</u>., 1954):

$$pH = pK_o + \lg\left(\frac{\alpha}{1-\alpha}\right) - 0.4343 \frac{e\Psi}{KT} \tag{43}$$

By analogy with

$$pKa = pH - lg\left(\frac{\alpha}{1-\alpha}\right) \tag{44}$$

is qualitatively expected that the apparent polymer pK values increase with increasing α. A quantitative evaluation of the apparent pK is obtained by calculating the electrostatic potential at the site of the dissociable charged point by the use of Debye-Huckel linear theory (Edsall and Wyman, 1958). This treatment combined with the modeling of polyelectrolytes as a cylinder with uniform charge density, predict a linear increase of the apparent pK with increasing values of α, however, a curvature is obtained with the experimental data (Nagasawa and Holtzer, 1964; Rippol _et_ _al_., 1971; Senior _et_ _al_., 1971; Muroga _et_ _al_., 1972; Cleland _et_ _al_., 1982).

Other theoretical approaches have dealt with the problem of calculating the electrostatic potential by the analytical solution of the Poisson-Boltzman equation (Nagasawa, 1971). However, the need of selecting an appropriate value of both the radius of the polyion and pK_o make its application difficult (Ishikawa, 1979).

In one of the most recent approaches, Manning and Holtzer (1973), the apparent pK is defined as:

$$pKa = pK_a^0 - g(\alpha) \log K^2 + W_{self}(\alpha) \tag{45}$$

where K is the Debye screening parameter, and $g(\alpha)$ is defined as

$$g(\alpha) = \begin{cases} \dfrac{\alpha}{\alpha_1} & \alpha < \alpha_1 \\ 1 & \alpha > \alpha_1 \end{cases} \qquad (46)$$

where α_1 is the value of the degree of ionization of the ionizable group, corresponding to the critical charge density and $W_{self}(\alpha)$ is a function of the self-energy of the polyion when it is ionized. If the polyion is assumed to be a charged cylinder in a uniform dielectric medium then:

$$W_{self}(\alpha) = -g(\alpha) \log a^2 \qquad (47)$$

where "a" is the radius of the cylinder.

Therefore, the potentiometric titration of the polyelectrolyte solution will give us a way to obtain an approximate model of the molecule and also information on the molecular parameters such as "$\Psi(a)$" and "a."

3.2.4 Single-Ion Activity Coefficient:

Measurement of the cell potential (in mV) of chitosan solutions will be performed with an Orion microprocessor pH/millivoltmeter model 811 (Orion Research, Cambridge, MA). An Orion combination chloride electrode model 96-17B in conjunction with an Orion double junction reference electrode model 90-02 will be used for the measurement of chloride ion activity coefficients. All measurements will be made at 25.0 ± 0.1°C.

Chitosan solutions at concentrations between 0.05 g/dl and 0.20 g/dl will be tested. The pH range as well as NaCl concentration will be set between 3.0-5.5 and 0.01 M - 0.50 M, respectively.

The electromotive force (e.m.f.), E, will be related with the activity of the chlorine ion, a_{cl^-} , by the expression:

$$E = E_o + \frac{R\ T}{F} \ln a_{cl^-} \qquad (48)$$

where E_o is the standard e.m.f. of the cell, R the gas constant, T the temperature, and F the Faraday charge.

Calibration curves will be determined before and after every series of measurements on both the polyelectrolyte and the blank solutions with standard HCl solutions in order to test for Nerstian electrode slopes.

The values of the activity coefficient ratio between polyelectrolyte and its correspondent blank solution will be calculated by the expression:

$$\frac{\gamma_{Cl^-,ch}}{\gamma_{Cl^-,b}} = \exp\left[\frac{(E_b - E_{ch})\ F}{R\ T}\right] \qquad (49)$$

where the subscripts "ch" and "b" represent the chitosan and blank solutions, respectively.

The study of the activity coefficient of counterions in complex systems, polyelectrolyte-simple electrolyte, has been used to understand the interactions between ions and polyelectrolytes in solution (Podlas and Ander, 1970; Archer et al ., 1972). Since charged polyions can bind single ions (Rice and Nagasawa, 1961; Strauss and Leung, 1965), the counterion activity coefficients are significantly lower in polyelectrolyte solutions than in simple salt solutions of similar concentrations (Lapanje and Gubunsek, 1967; Podlas and Ander, 1969).

Under the assumption that the infinite line charge theory (Manning, 1969a; Manning, 1975) describes the behavior of polyelectrolytes in solution, two modes of counterion-polyion interaction can be considered. One, if the linear charge density, ξ , is below the critical value, ξ_c, the counterion-polyion interaction by the Debye-Huckel approximation leads to a counterion activity coefficient, γ_1 of the form (Manning, 1969a).

$$\gamma_1 = \exp\left[-\frac{0.5\,\xi\,X}{X+2}\right] \tag{50}$$

where $X = n_p/n_s$, the concentration ratio of polyelectrolyte, n_p, to simple salt, n_s, in equivalents. Second, if $\xi \geq \xi_c$, counterions condense onto the polyion, and the counterion activity coefficient is represented by (Manning, 1969a):

$$\gamma_1 = \frac{\xi^{-1}\,X + 1}{X + 1}\,\exp\left[\frac{-\,0.5\,\xi^{-1}\,X}{\xi^{-1}\,X + 2}\right] \tag{51}$$

where the pre-exponential term accounts for the fraction of uncondensed counterions in solution.

More recently, other theoretical approaches have succeeded in explaining the decrease in the activity coefficient of the electrolytes in the presence of polyelectrolytes. The most successful aproach (Iwasa and Kwat, 1977; Iwasa *et al* ., 1978) accounts for a strong interaction between the counterions and the charged points onto the polyelectrolyte backbone, expressing the counter-ion activity coefficient for monovalent salt as:

for $\xi \leq \xi_c$

$$\gamma_1 = \exp\left\{ \frac{\xi X}{X + 2} \left[-0.5 + 0.39\, \xi \left(\frac{X}{X + 2} - 1 \right) \right] \right\} \qquad (52)$$

and for $\xi \geq \xi_c$

$$\gamma_1 = \frac{\xi^{-1} X + 1}{X + 1} \exp\left\{ \frac{\xi^{-1} X}{\xi^{-1} X + 2} \left[-0.5 + 0.39 \left(\frac{\xi^{-1} X}{\xi^{-1} X + 2} - 1 \right) \right] \right\} \qquad (53)$$

However, it has been shown (Gueron and Weisbach, 1979; Kowblansky and Zema, 1981) that the experimental results are underestimated in both theoretical approaches. A possible explanation of this underestimation is that the polarization induced in the counterion sheath by an ion far from the polyelectrolyte molecule can cause a strong polyion-counterion interaction, which in turn reduces significantly the activity coefficient of the counterions (Gueron and Weisbach, 1979). Therefore, it must be pointed out that the study of the activity coefficient of the counterions will give an indication of the effect of the degree of ionization upon the interaction between the counterions and polyions in solution.

3.2.5 Diffusion of Nonionic Compound: Glucose:

Self-diffusion experiments will be performed in a constant temperature bath thermostatted at 25.0 ± 0.1°C. The open-end capillary method will be employed.

Capillaries of fixed diameter and length will be filled with a known volume of chitosan solution containing 1% D- 6-^{3}H glucose and tidily capped with a dialysis membrane. After cleaning the outside surface, the capillaries will be immersed in a container which will be subsequently filled with a radioactive-inert solution of identical composition to that place inside the capillaries. The chitosan concentration, pH and ionic strength selected will be the same as those set for the activity of single ion experiment previously described.

Since both solutions, inside and outside of the capillaries are equivalent in composition, no concentration gradient will exist. Self-diffusion coefficient will be calculated by the expression:

$$D = \frac{\pi}{4}\left(1 - \frac{C}{C_0}\right)\frac{L^2}{t} \tag{54}$$

where t is the diffusion time, C_o is the radioactive content at time zero, C is the radioactive content at time "t" and L corresponds to the solution height inside the capillary. A standardized diffusion time will be set for all experimental conditions, then the capillaries will be removed from the container and the radioactive content inside each capillary will be measured by using a Beckman, Liquid Scintillation System LS-230.

The diffusion of low molecular weight compounds through both dilute and non-dilute polymer solutions has received great attention owing to its importance in polymer technology. In the diffusion of small particles in ionic solutions, the electrostatic effects are of capital importance, even under conditions where there is no net flow charge. This effect is due to the influence of the counterions surrounding the polyion which produces a cooperative effect on the diffusion coefficient once a concentration gradient is obtained in order to keep the overall neutrality (Florence and Johnson, 1975; Yoshida, 1978; Magdelenat et al ., 1979; Trifiletti and Ander, 1979; Ander et al ., 1979).

In the last 25 years, several studies have dealt with the diffusion of both small molecules such as glucose (Nystrom and Roots, 1980) and large molecules such as serum albumin (Laurent et al ., 1979) through polymer solutions.

A common result can be observed from these experiments: A decrease in the diffusion rate of the diffusant with increasing polymer concentration. This effect has been explained by the effect of the polymer concentration upon the interactions between polyion and both the counterions and the solvent molecules (Nystrom and Roots, 1980).

Self-diffusion of small molecules, such as solvent molecules, can be used to evaluate changes in the hydration layers around the polyion. It must be expected that the self-diffusion coefficient of solvent molecules in a polyion solution would be smaller than that in pure solvent for the following reasons (Wang, 1954):

1. A fraction of solvent molecules are attached on the polyion backbone and therefore, unable to contribute to the rate of self-diffusion of solvent.
2. Polyion molecules have a larger volume and a smaller self-diffusion coefficient than solvent molecules.

In order to evaluate specifically the solvent effect on the self-diffusion coefficient and under the assumption of "non-hydration" of the polyion, the following differential equation can be evaluated (Sanborn and Arleman, 1955).

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \qquad (55)$$

or

$$\frac{D\,t}{h^2} = \frac{4}{\pi^2} \ln\left[\frac{8}{\pi^2}\,\frac{C_o}{C_{av}}\right] \qquad (56)$$

where: C_o = the initial concentration of diffusant molecule.

C_{av} = the average concentration of the diffusant molecule at the end of the run.

D = diffusion coefficient of the diffusant molecule.

h = the height of the capillary tube.

t = time of the run.

Finally, the ratio of the self-diffusion coefficient of the solvent molecule, or any other small molecule, with and without polymeric content can be used in order to, evaluate the solvent effect.

Several theories have been developed in order to evaluate the obstruction effect of the polymer on the diffusion coefficient (Wang, 1954; Mackie and Meares, 1955; Prager, 1960). Among them, the most used is the Wang approach (Wang, 1954) with the following expressions:

$$\frac{D}{D_0} = 1 - \alpha\left(v_2 + \frac{H}{\rho_1}\right)C_2 \qquad (57)$$

and

$$\frac{D}{D_c} = \left\{1 - \alpha\left[\frac{v_2 + H/\rho_1}{v_2 + (1 - W_2)/\rho_1 W_2}\right]\right\}\left[1 - \frac{W_2}{1 - W_2}\right]H \qquad (58)$$

where α is a geometrical factor depending on the shape of the polymer molecule with value of 3/2 for spheres and 5/3 for rods; v_2 is the partial specific volume of the polymer ; ρ_1 is the density of the solvent; C_2 is the polymer concentration; w_2 is the mass fraction of polymer and H is the solvent bound per unit mass of polymer.

Therefore, the obstruction effect of the polyion molecules will be strongly affected by the size and shape of the diffundant molecule. Thus, experiments where water is used as diffundant molecule are difficult to perform.

In this sense other bigger molecules such as glucose, glucose derivatives or dyes can and are being used giving the same information respect to changes in the solvatation around the polyion in solution (Farag et al ., 1976a,b; Nystrom and Roots, 1980).

Finally, the self-diffusion coefficient of both the solvent molecules and non-interacting particles can be used to calculate the internal viscosity of polymer solutions. In this sense the diffusion coefficient D in the liquid phase can be related with the internal viscosity, μ_I , by the well known Stokes-Einstein equation:

$$D = \frac{R\,T}{6\,\pi\,r\,\mu_I} \tag{59}$$

where "r" is the radius of the diffundant molecules.

This approach can be used in order to understand the effect of charge density on the microenvironment surrounding the polyelectrolyte backbone as a result of changes in hydration of the polyion.

3.2.6 Rheological Measurements of Concentrated Solutions

The viscosity of concentrated chitosan solutions, (chitosan concentrations between 0.50 g/dl and 1.50 g/dl) at pH's values between 3.0 and 5.0 and counterion concentrations between 0.1M and 0.4M), as a function of the shear rate (shear rates up to 10^3 reciprocal seconds) will be determined with a cone-plate arrengement of a Rheometric Mechanical Spectrometer (Rheometrics, Inc., Union, NJ) at 25.0°C.

The Rheometric Mechanical Spectrometer (R.M.S.) is a rotational shear rheometer with interchangeable test fixtures. It measures torque and normal forces over a wide range of both steady and dynamic shear. Table 9 shows the performance specification of the instrument.

For the determination of the viscosity a sample of the chitosan solution will be placed in the gap between the cone and plate of the R.M.S.. The equipment fixtures selected in this study are:

Plate diameter = 50 mm
Cone angle = 0.019 radians
Strain = 20%

Once the chitosan solution is placed in the gap of the R.M.S., the values of the torque are recorded as the cone rotates at a specified angular velocity (ω).

The shear rate will be obtained by the expression:

$$\dot{\gamma} = \omega / \tan \beta \tag{60}$$

where $\overset{o}{\gamma}$ = shear rate
β = cone angle

Table 9

Performance Specification of Rheometrics Mechanical Spectrometer

Rotational Speed	10 - 10^2 rad/s.
Speed Control	Analog
ERD Frequency	10 - 200 rad/s
Temperature Measurement	Thermocuple in contact with plate/cone
Oscillatory Shear Frequency	10 - 500 rad/s
Oscillatory Shear Strain	0.1 - 99.9%
Torque	10 Nm (full scale)
Shear Stress	10 - 10^3 Pa
Normal Force	1N
Normal Stress	1 - 10^3 Pa

and the shear stress (Γ) is calculated from the torque (M) by the expression:

$$\Gamma = 3\ M\ (\ 2\pi R^3 \omega\ )^{-1} \tag{61}$$

where R = the cone radius

Therefore, the viscosity at any shear rate will be obtained by:

$$\eta_{app} = \Gamma/\dot{\gamma} = 3M\beta\ (2\pi R^3 \omega)^{-1} \tag{62}$$

At this point it is important to notice that the cone and plate geometry is selected for this study because the shear rate across the gap is constant at difference of other geometrics where the shear rate varies along the gap.

4. RESULTS AND DISCUSSION

4.1 Rheology of Dilute Chitosan Solutions

4.1.1 Intrinsic Viscosity of Chitosan at Low Ionic Strenth of the Media

It is a well known phenomenon that the reduced viscosity of uncharged polymers decreases linearly with decreasing the polymer concentration following the Huggins equation (Huggins, 1942). However, this is not the case for charged polyelectrolytes where, at low ionic strength of the media, the reduced viscosity increases with further dilution (Wolff, 1978).

Figure 8 shows the dependence of the reduced viscosity, η_{sp}/c, on chitosan concentration. The reduced viscosity increases with decreasing the polyelectrolyte concentration below 10^{-3} g/dl. Figure 8 also shows the reduced viscosity-concentration relationship as affected by the acid concentration. The increase in reduced viscosity with dilution is progressive at lower acid concentration. The increase in the reduced viscosity starts to occur at high chitosan concentration as the acid concentration decreases. This phenomenon is due to the expansion of the polyelectrolyte backbone caused by greater Coulombic re-

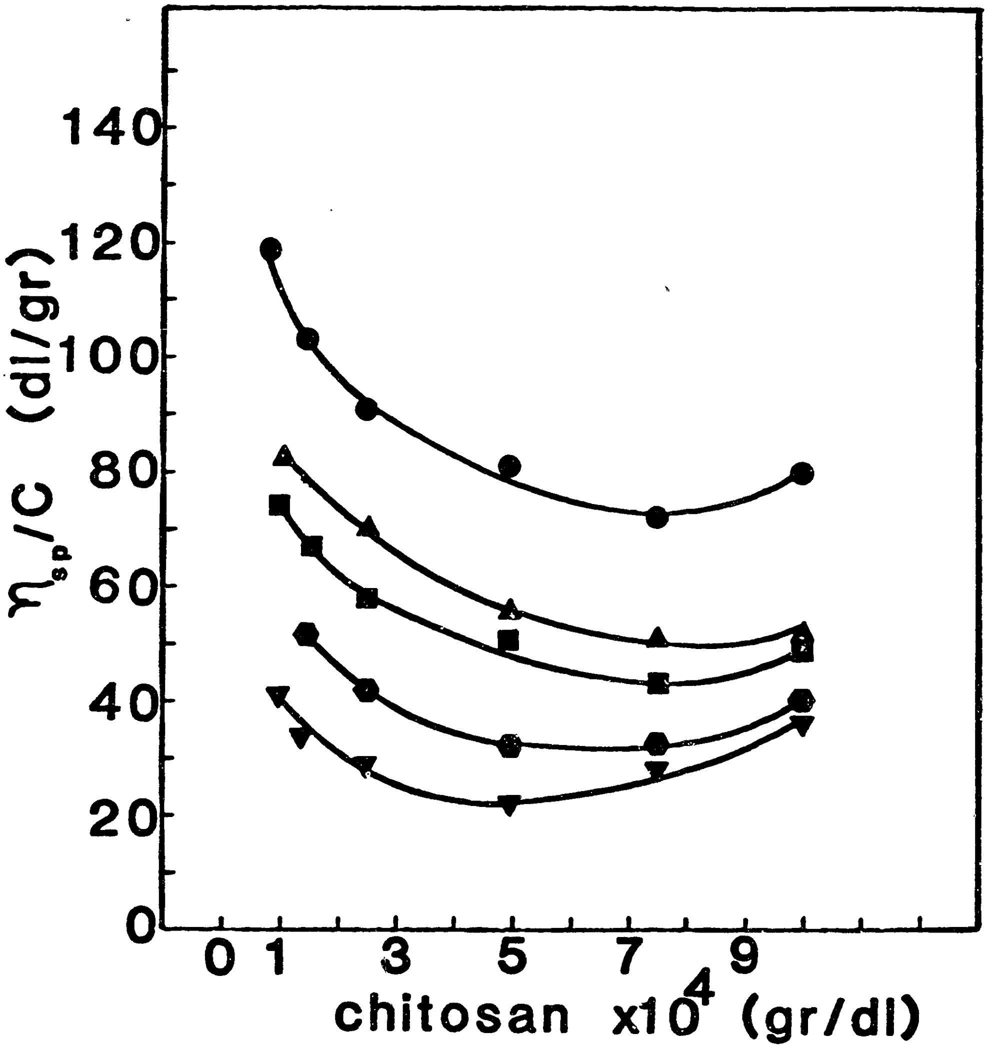


Figure 8. Reduced viscosity (dl/gr) versus chitosan concentration (gr/dl) at different acetic acid concentration: ● 1.7 x 10^{-3}M; ▲ 8.3 x 10^{-3}M; ■ 1.7 x 10^{-2}; ⬢ 8.3 x 10^{-2}M; ▼ 1.7 x 10^{-1} M.

pulsive forces between the charged points (Chien et al., 1976).

The increase in the reduced viscosity at near zero polymer concentration makes the extrapolation to zero concentration no longer possible. Therefore, the intrinsic viscosity of chitosan in solutions at low counterion concentration in the media cannot be obtained by the usual methods of extrapolation to zero concentration (Huggins, 1942; Kraemer, 1938).

In order to describe this dependence of the reduced viscosity on polyion concentration, several semi-empirical equations have been proposed (Fouss, 1948; Liberti and Stivala, 1966; Schaefgen and Trivisonno, 1952; Yuan et al., 1972). The following equation is used to represent our experimental data (Yuan et al., 1972):

$$\frac{\eta_{sp}}{C} = [\eta]_{\infty}(1 + KC^{-1/2}) \qquad (63)$$

where: η_{sp} = specific viscosity

C = chitosan concentration

K = constant

The parameter $[\eta]_{\infty}$ has been related to the intrinsic viscosity, $[\eta]$, at infinite salt solution, and K has been related to the electrostatic effects between the polyion and the solvent. The relationship between $[\eta]_\infty$ and $[\eta]$ in salt solutions has been shown to apply to other polysaccharides such as heparin solutions (Yuan et al., 1972) and sodium carboxymethylcellulose solutions (Trivedi et al., 1981).

A straight line is obtained when the reduced viscosity is plotted against the reciprocal of the square root of the chitosan concentration according to the Yuan's expression (Figure 9). The values of $[\eta]_\infty$, K and the linear correlation coefficient for the experimental values are shown in Table 10. The decrease in $[\eta]_\infty$ at high acetic acid concentration suggests that some of the charges on the chitosan backbone have been neutralized by the acetate ions, leading to an increase in the flexibility of the polyion chain which in turn causes the macroion domain to shrink (Chien et al., 1976). The evidence of an increase in the polyelectrolyte-counterions interactions, as acetate concentration increases, is shown by the higher K values at increased acid concentration in the media (Table 10).

Given that the acetic acid acts both as a solvent and as a simple electrolyte when added to the chitosan solution, a linear relationship is expected between $[\eta]_\infty$ and the reciprocal of the square root of the acetic acid concentration (Takahasi and Nagasawa, 1964). However, when

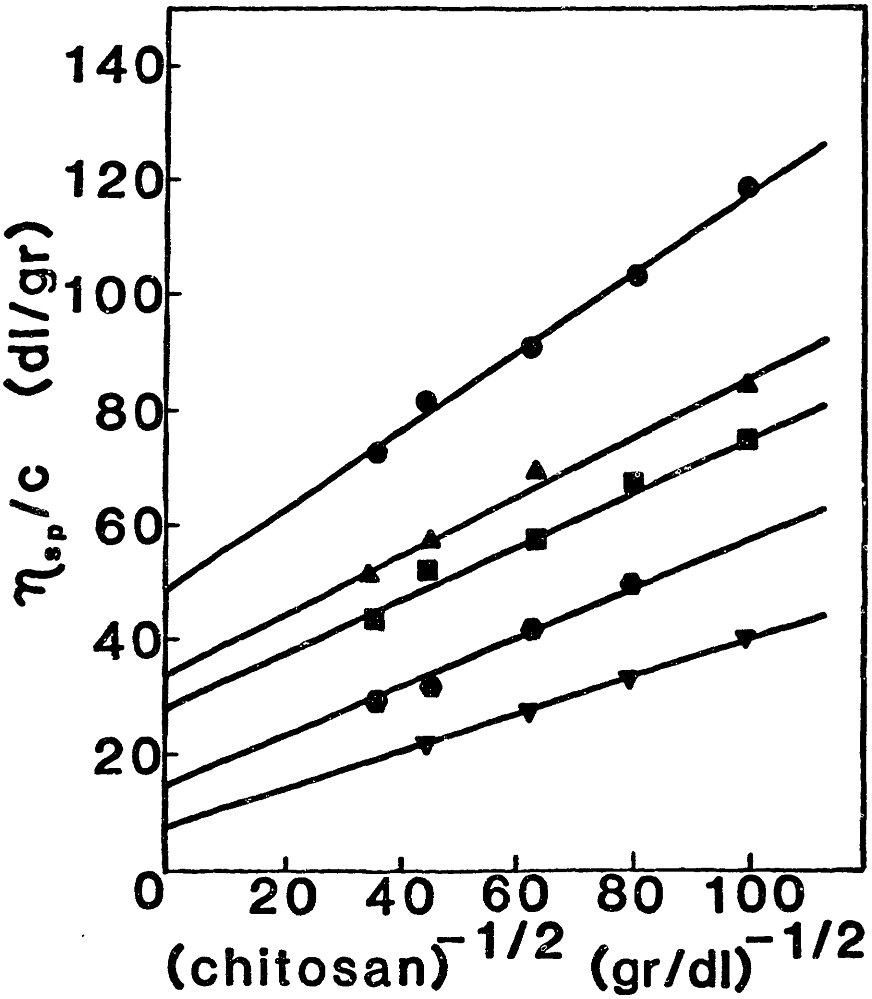


Figure 9. Reduced viscosity (dl/gr) versus the reciprocal of the square root of chitosan concentration (gr/dl)$^{-1/2}$ at different acetic acid concentration: ● 1.7 x 10^{-3}M; ▲ 8.3 x 10^{-3}M; ■ 1.7 x 10^{-2}M; ● 8.3 x 10^{-2}M; ▼ 1.7 x 10^{-1}M.

Table 10

Acetic Acid Concentration (M)	$[\eta]_\infty$ (dl/gm)	K $(gr/dl)^2$	Corresponding Coefficient
1.7×10^{-3}	48.60	1.43×10^{-2}	0.9935
8.3×10^{-3}	32.56	1.51×10^{-2}	0.9921
1.7×10^{-2}	27.22	1.70×10^{-2}	0.9917
8.3×10^{-2}	12.57	3.46×10^{-2}	0.9933
1.7×10^{-1}	6.46	4.71×10^{-2}	0.9952

$[\eta]_\infty$ is plotted against the reciprocal of the square root of the acetic acid concentration (Figure 10), a deviation from linearity is observed. This effect can be attributed to an increase in the intramolecular hydrogen bonding caused by the shielding of the electrostatic repulsive forces as the acetic acid concentration increases, thus favoring the additional shrinking process (Noda et al., 1970).

Therefore, this set of experiments shows the polyelectrolytic nature of chitosan in dilute solution, as well as the importance of polyion-counterion interaction on the shrinking process of chitosan.

4.1.2 Intrinsic Viscosity of Chitosan at High Ionic Strength of the Media

It has been previously shown that dilute chitosan solutions at low ionic strength of the media demonstrate a typical polyelectrolyte behavior, as represented by the increase in the reduced viscosity upon dilution. However, this effect has been suppressed by increasing the ionic strength of the media (Rodriguez-Sanchez et al., 1982). Therefore, for further study of the polyelectrolyte, the effect of the charge density and the counterion concentration upon the rheological response of chitosan in solution is examined.

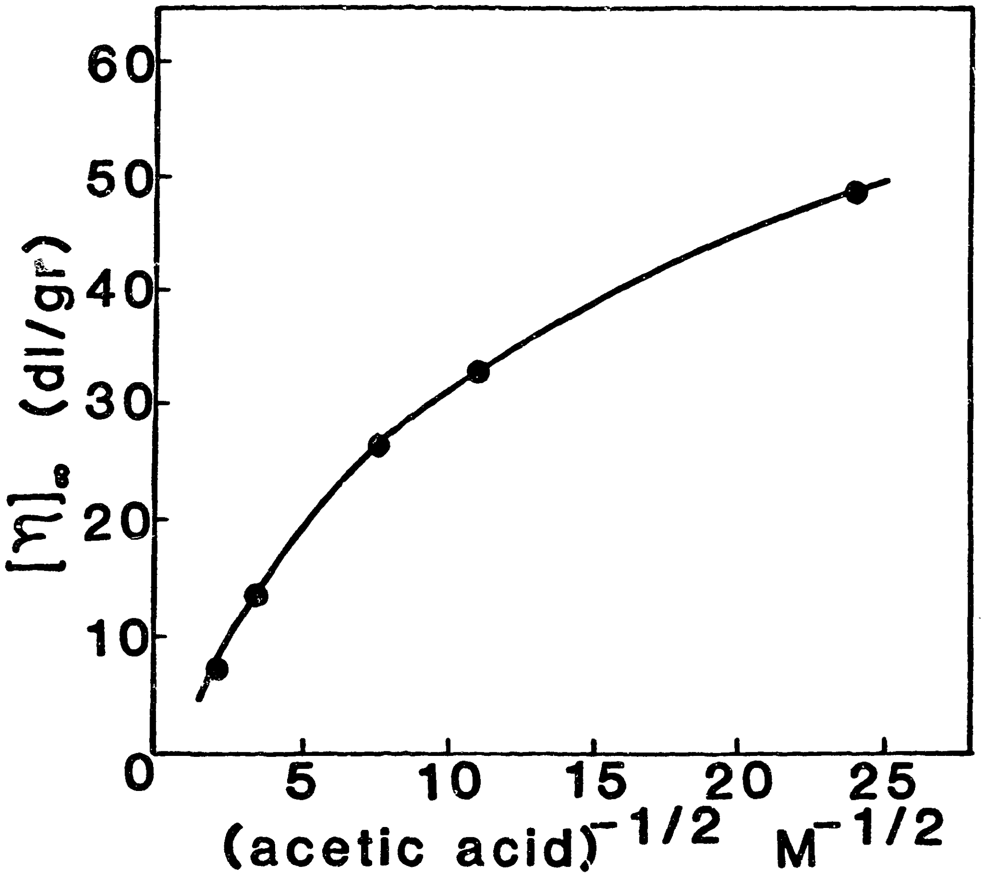


Figure 10. Intrinsic viscosity $[\eta]_\infty$ (dl/gr) versus the reciprocal of the square root of the acetic acid concentration ($M^{-1/2}$).

In this study the effect of the degree of ionization and/or the ionic strength (0.05 ml/L - 0.50 mol/L) are evaluated for pH values between 3.0 and 6.0 which represent ionization degrees between 1.00 and 0.50, respectively (Figure 11). The range of pH values used was limited because of the chitosan insolubility at pH's values higher than 6.0 (Muzzarelli, 1973).

Intrinsic viscosity is obtained by extrapolation to zero chitosan concentration of both $\eta sp/c$ and $(\ln \eta_r)/c$, for at least five chitosan concentrations, using the equations (Huggins, 1942; Kraemer, 1938):

$$\frac{\eta_{sp}}{c} = [\eta] + K' [\eta]^2 c \qquad (64)$$

$$\frac{Ln\eta_r}{c} = [\eta] + K''[\eta]^2 c \qquad (65)$$

where: η_{sp} = specific viscosity

η_r = relative viscosity

$[\eta]$ = intrinsic viscosity

c = chitosan concentration

k'& k" = Huggins' and Kraemer's constants, respectively.

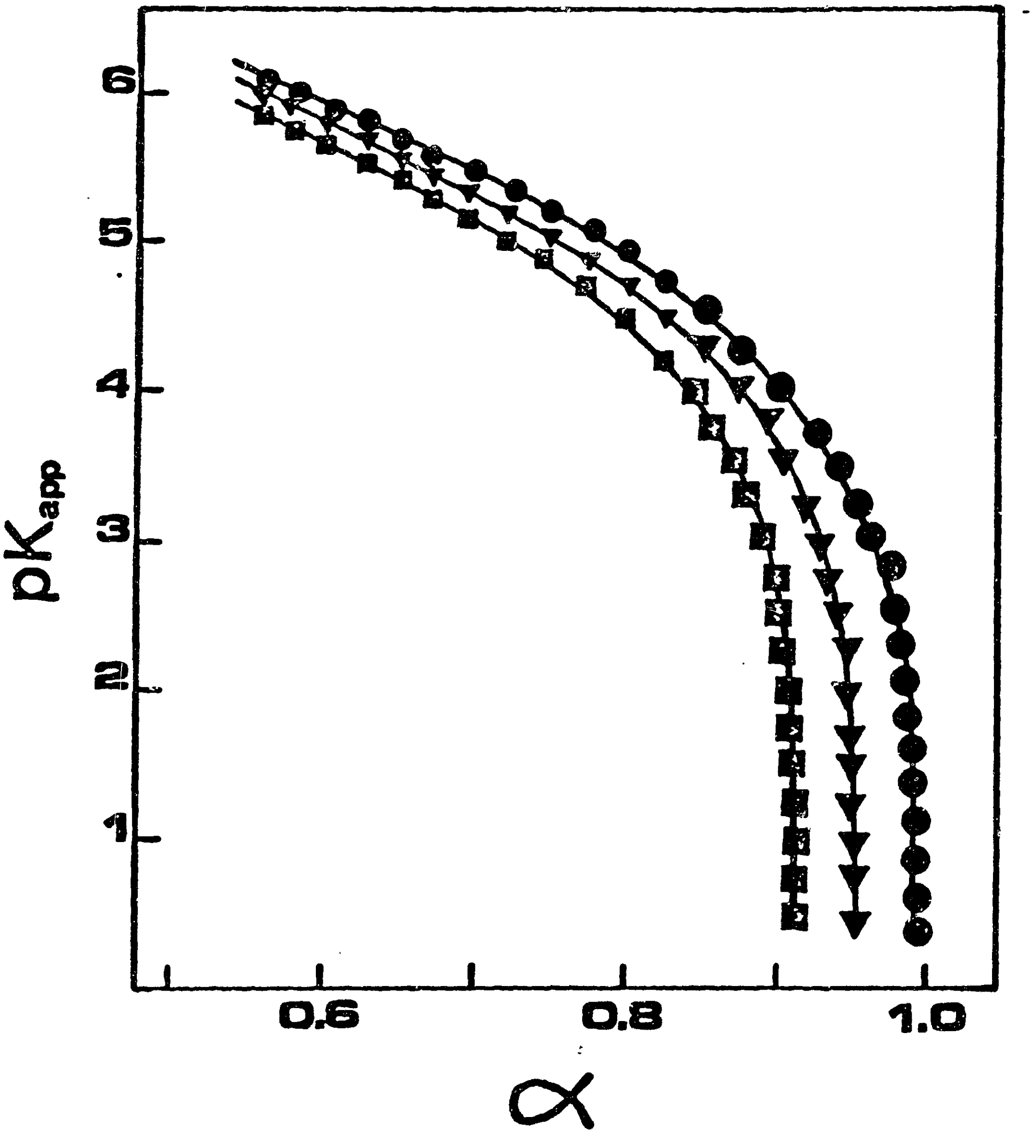


Figure 11. Potentiometric tritration for dilute chitosan solutions: pK_{app} versus degree of ionization (α): counterion concentration: ■, 0.05 mol/L; ▲, 0.10 mol/L; ●, 0.50 mol/L.

A typical concentration dependence of the viscosity of chitosan solutions is shown in Figure 12.

Intrinsic viscosity values, thus obtained, are plotted against the degree of ionization on Figure 13. Here it is indicated that, at constant counterion concentration, the intrinsic viscosity increases with increasing the degree of ionization. This behavior is the result of an increase in the electrostatic repulsion between charged neighboring groups which induces the chain expansion. Figure 13 also shows that for a given degree of ionization, the intrinsic viscosity decreases with increasing the counterion concentration in the media. The effect of the counterion shielding upon the intrinsic viscosity of polyelectrolyte solutions is highlighted. Therefore, from these results it can be stated that chitosan molecules in solution expand because of either an increase in the repulsive electrostatic forces between charged points, or a decrease in the counterion shielding.

The dependency between the intrinsic viscosity of chitosan and the counterion concentration is shown by the plot of the intrinsic viscosity versus the reciprocal of the square root of the ionic strength at constant degree of ionization (Figure 14). The resulting linearity indicates that the relationship between the intrinsic viscosity and the counterion concentration can be represented

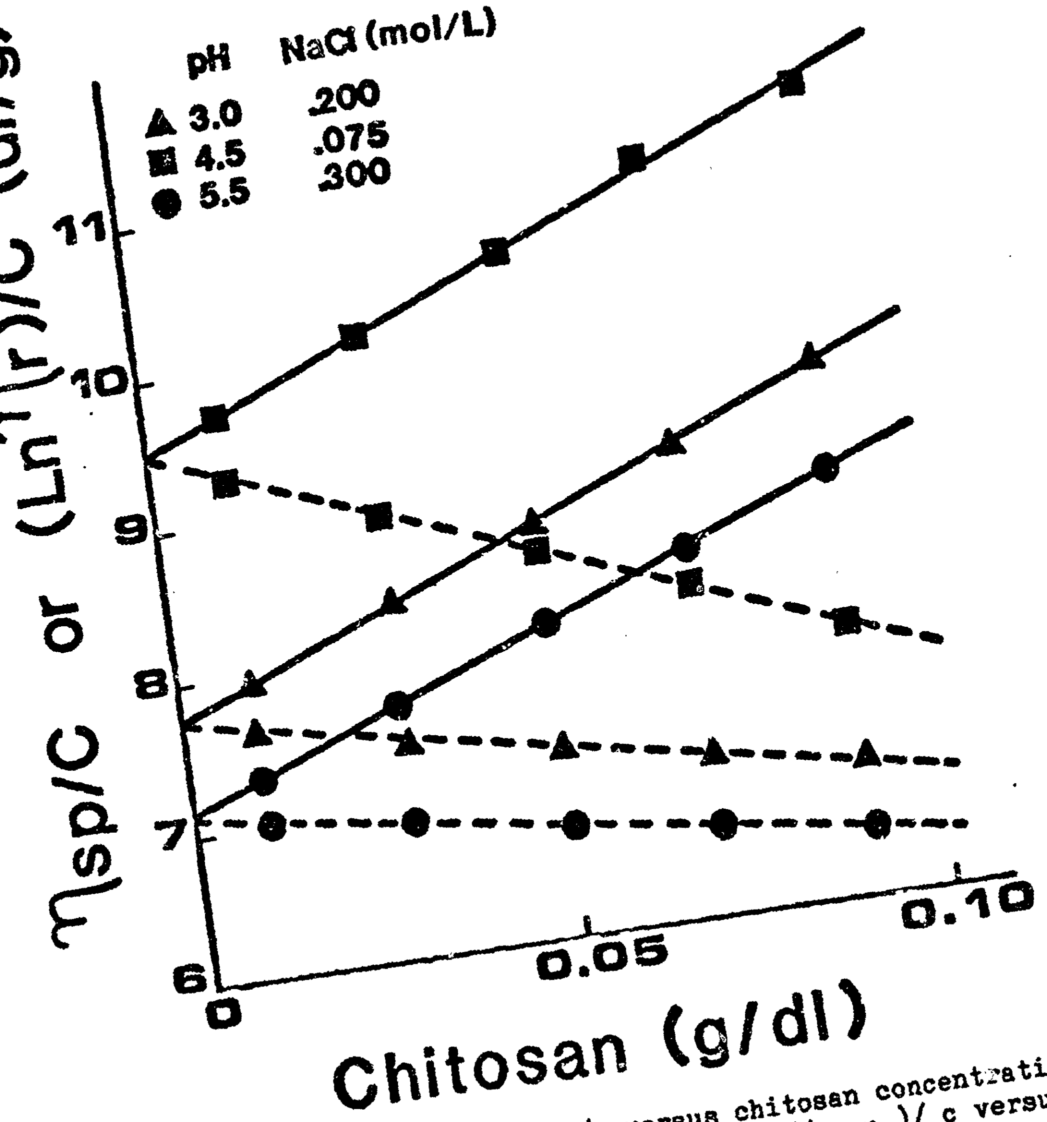


Figure 12. Huggins' type plot (η_{sp}/c versus chitosan concentration, solid line) and Kraemer's type plot ($(\ln \eta_r)/c$ versus chitosan concentration, dashed line) for dilute chitosan solution.

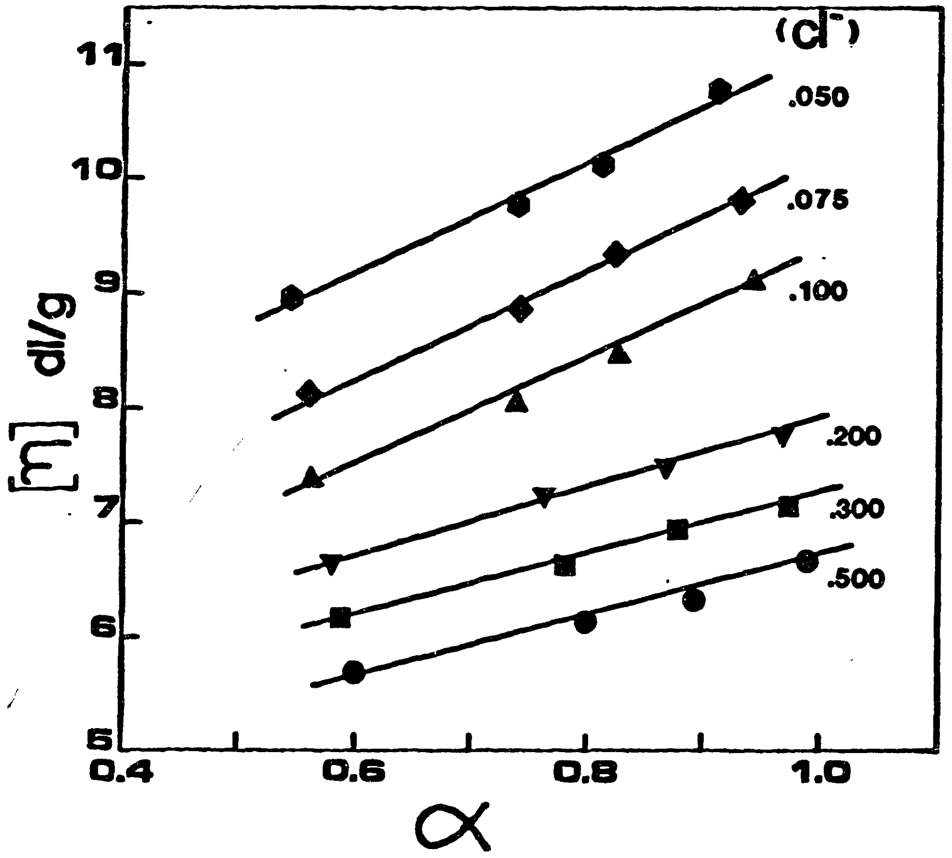


Figure 13. Intrinsic viscosity of chitosan [η] as affected by degree of ionization (α) and counterion concentration (CL⁻).

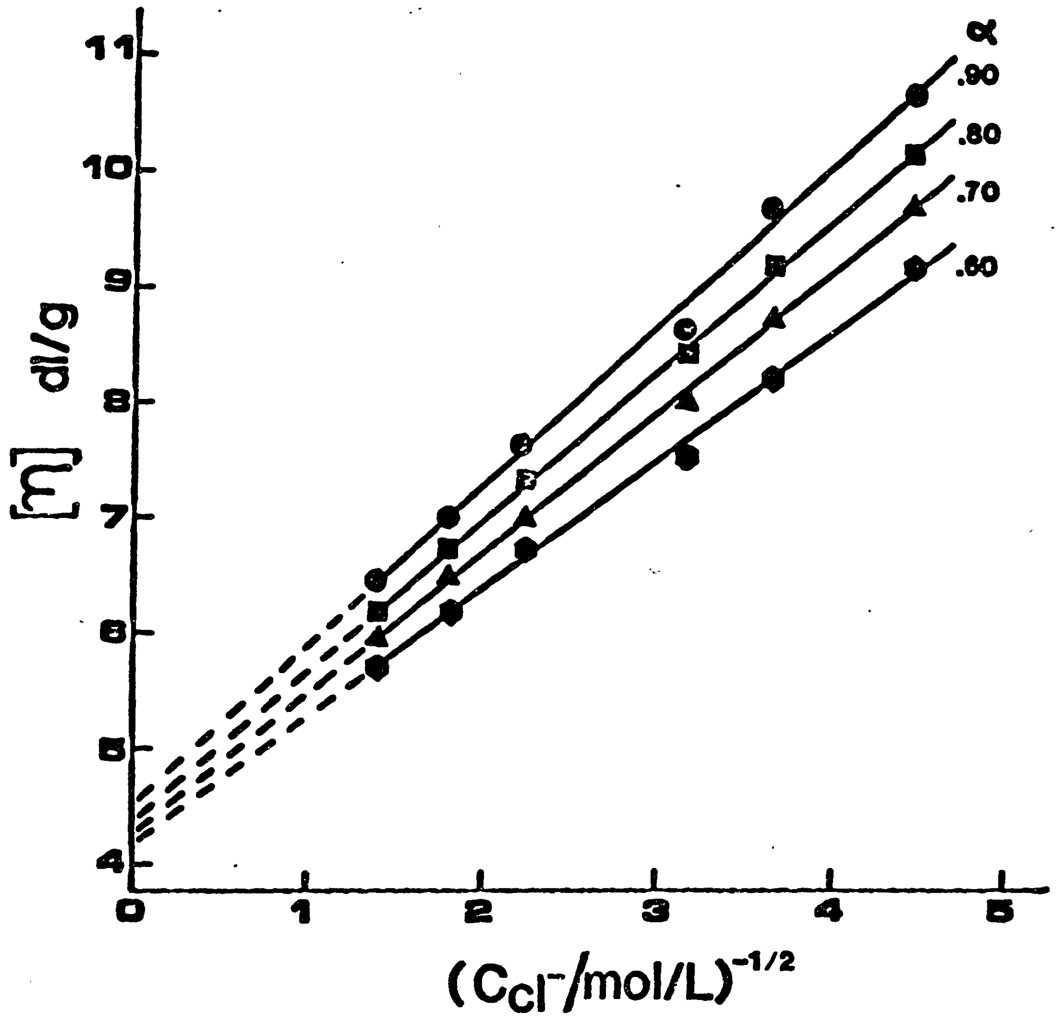


Figure 14. Intrinsic viscosity, [η], versus the reciprocal of the square root of counterion concentration at different degrees of ionization.

by the expression (Takahashi and Nagasawa, 1964):

$$\frac{[\eta]}{M^{1/2}} = K + 0.51\phi(B_o + A\ C_S^{-1/2})M^{1/2} \qquad (66)$$

where: C_s = counterion concentration

M = polymer molecular weight

Φ = Flory's constant

B_0 = mutual excluded volume due to nonelectrostatic interactions

K & A = constants

Expression (66) reduces to:

$$[\eta] = A_1 + A_2\ C_S^{-1/2} \qquad (67)$$

for constant molecular weight; where both A_1 and A_2 can be directly determined from Figure 14. Values for both A_1 and A_2 are given in Table 11 as a function of the degree of ionization.

The linear expansion coefficient of chitosan in dilute solution, α_η^3, can be calculated by the expression (Flory, 1953):

Table 11

α	A_1	A_2 (dl/g)	B (dl/g)/(mol/L)$^{1/2}$
0.90	4.40	1.418	0.083
0.80	4.35	1.309	0.082
0.70	4.25	1.209	0.082
0.60	4.17	1.104	0.080

Slope and intercept from the relationship between $[\eta]$ and the reciprocal of the square root of the counterion concentration, and chain flexibility parameter, "B", for dilute chitosan solutions as affected by the degree of ionization, α.

$$\alpha_{\eta}^{3} = \frac{[\eta]}{[\eta]_{\theta}} \tag{68}$$

where $[\eta]_{\Theta}$ is the intrinsic viscosity at Θ condition equal to 4.03 dl/g. The value of $[\eta]_{\Theta}$ is calculated from the extrapolation at infinite molecular weight of chitosan solution using the Ptytsyn and Eizner expression (Ptytsyn and Eizner, 1962; Berkovich et al., 1980). Table 12 shows the expansion coefficient of chitosan in dilute solutions as affected by both the degree of ionization and the ionic strength. These results indicate that the hydrodynamic volume of chitosan expands as either the degree of ionization increases or the ionic strength decreases; indicating the importance of the repulsive forces between charged points on the rheology of dilute chitosan solutions.

The swelling of polyelectrolytes in solution can be described by the electrostatic expansion factor ($\alpha_{\eta e}^{3}$) obtained by (Nagasawa, 1975; Kowblansky and Zema, 1981):

$$\alpha_{\eta e}^{3} = \frac{[\eta]}{[\eta]_{o}} \tag{69}$$

where $[\eta]_{o}$ represents the intrinsic viscosity of the non-ionic parent polymer. The value of $[\eta]_{o}$ is obtained by extrapolating to infinite counterion concentration the $[\eta]$ (A_1 in Table 11). The obtained electrostatic expan-

Table 12

Expansion Coefficient (α_η^3) of Dilute Chitosan Solutions as Function of the Counterion Concentration and the Degree of Ionization

$[Cl^-]$(mol/L)	0.500	0.300	0.200	0.100	0.075	0.050
α						
0.90	1.56	1.70	1.85	2.16	2.36	2.59
0.80	1.54	1.67	1.81	2.09	2.28	2.52
0.70	1.48	1.62	1.73	1.99	2.16	2.41
0.60	1.42	1.54	1.67	1.88	2.07	2.28

sion factor, ($\alpha_{\eta e}^{3}$), as a function of the degree of ionization and the ionic strength of the media, are given in Table 13. Here, it is observed the sensitivity of the chitosan chain to changes in the ionic strength and in a lesser extent to changes in the degree of ionization.

It has been shown (Smidsrod and Huag, 1971) that the dependency of the intrinsic viscosity of polyelectrolytes to the ionic strength of the media can be used, successfully, to evaluate the macroion chain flexibility. Following the procedure indicated by Smidsrod and Haug (1971) I calculated the chain flexibility parameter "B" as follows:

$$B = S/([\eta]_{0.1})^{\gamma} \tag{70}$$

where: S = slope from the $[\eta]$ versus the square root of the counterion concentration plots (A_2 in Table 11)

$[\eta]_{0.1}$ = the intrinsic viscosity at 0.1 mol/L NaCl

γ = constant = 1.3 (Robinson-Lang _et_ _al_., 1982)

The calculated chain flexibility parameter, "B", as a function of the degree of ionization is presented in Table 11. A comparison between the chain flexibility parameter of chitosan molecules in dilute solution with other polyelectrolytes can be observed in Table 14, where the chain

Table 13

Electrostatic Expansion ($\alpha_{n}^{3}{}_{e}$) of dilute chitosan solutions as function of counterion concentration and degree of ionization

$[Cl^-]$ (mol/L)	0.500	0.300	0.200	0.100	0.075	0.050
α						
.90	1.43	1.56	1.69	1.98	2.16	2.37
.80	1.43	1.55	1.68	1.94	2.11	2.33
.70	1.40	1.53	1.64	1.88	2.04	2.28
.60	1.37	1.49	1.61	1.81	2.00	2.20

Table 14*

Polymer	B	Reference
Polyphosphate	0.44	Cox, 1960
Sodium Polyacrylate	0.27	Smidrod & Huag, 1971
Polyacrylate	0.23	Takahashi & Nagasawa, 1964
Amylose xanthate	0.22	Pramnik & Choudhari, 1968
Carboxymethylamylose	0.20	Patel *et al.*, 1967
Carboxymethylcellulose	0.078- 0.065	Triverdi & Patel, 1982
Hyaluronic Acid	0.07	Robinson Lang, 1982
Sodium Pectinate	0.044	Smidrod & Huag, 1971
DNA	0.006	Sharp & Bloomfield, 1968

[*] "Stiffness parameter", B, for well known polymer as reported in the literature.

flexibility parameter varies from 0.006 for a rigid rod like DNA to 0.44 for a flexible polyphosphate. Therefore, chitosan with a chain flexibility parameter of near 0.08, can be considered stiffer than amylose derivative, with a chain flexibility parameter of 0.22, but more flexible that sodium pectinate with a chain flexibility parameter of 0.044. Moreover, it is observed that chitosan is as flexible as sodium carboxymethylcellulose of similar molecular weight and degree of substitution equal to one (Trivedi and Patel, 1982).

The local conformational freedom of polysaccharides when there is a minimum polyion-solvent interaction has been successfully determined by calculating the characteristic ratio C_∞ by the expression (Yamakawa, 1971):

$$C_\infty = \frac{6(R_G)_\theta^2}{N_w \, \ell^2} \qquad (71)$$

where K = 1.02 cm^3/g is obtained from the slope of the linear relationship between $M_w/[\eta]$ and $M_w^{1/2}$ (Rice and Nagasawa, 1961). The characteristic ratio thus calculated has a value of 14.79. This value is consistent with the behavior of chitosan in solution when compared with other C_∞ values such as 14.2 for carboxymethyl cellulose (Brown and Henley, 1964) and semiflexible β (1,4) glucan derivative and 5.0 for amylose and pullulan (Brant and Boeble, 1975; Kato et al., 1982) as flexible α(1-4) glucans.

Similarly, I have calculated the ratio, C, between the radius of gyration of the noncharged chitosan molecules in solution, and the theoretical radius of gyration by the expression:

$$C = \frac{6(R_G)_o^2}{N_w \ell^2} \quad (72)$$

where $(R_G)_o^2$ = radius of gyration of the non-charged chitosan molecule

N_w = weight average degree of polymerization, which is 767 in this case.

Values of the intrinsic viscosity and radius of gyration of the non-charged chitosan molecule, as well as, C are shown in Table 15 as a function of the degree of ionization. It must be pointed out that the radius of gyration of the non-charged chitosan molecule used for the calculation of C is obtained from the extrapolation of the linear relationship between $[\eta]$ and the reciprocal of the square root of the ionic strength to infinite ionic strength indicated by the expression (Stockmayer and Fixman, 1963; Cox, 1960):

Table 15
Intrinsic Viscosity of the Non-Charged Chitosan Molecules in Solution (A); Non-Charged Radius of Gyration $(R_G)_o$ and Ratio between $(R_G)_o$ and the Theoretical Radius of Gyration (C) as affected by the Degree of Ionization (α).

α	A (dl/g)	$(R_G)_o \times 10^6$ (cm)	C	C∞
0.90	4.40	2.42	16.15	14.79
0.80	4.35	2.39	15.76	
0.70	4.25	2.38	15.57	
0.60	4.17	2.36	15.35	

$$\frac{[\eta]}{M^{1/2}} = K + 0.51\phi(B_o + AC_s^{-1/2})M^{1/2} \qquad (73)$$

where K,A = constants

B_o = mutual excluded volume due to nonelectrostatic interactions.

Therefore, the ratio "C" obtained by the equation (72) included both the unperturbed and nonelectrostatic contribution to the excluded volume on the local conformational freedom of chitosan in solution. Thus, since the values of C_∞ and C are similar, it is concluded that the non-electrostatic contribution to the excluded volume is negligible for the interpretation of the hydrodynamic behavior of chitosan molecules in dilute solutions.

Therefore, it can be concluded that chitosan molecules in solution behave as a worm-like nondraining polymer with a flexibility that can be controlled by both changing the degree of ionization and the counterion concentration. Also, it is shown that the non-electrostatic contribution to the excluded volume can be ignored when evaluating the hydrodynamic behavior of chitosan in dilute regime.

4.1.3 Rheological Model of Chitosan

One of the most simple and effective sources of experimental information about the chain dimension and the chain conformation is the analysis of the rheological behavior of dilute polymer solutions as affected by the degree of ionization and the molecular weight of the polyelectrolyte.

In order to model the chitosan molecule in solution I will analyze the effect of the degree of ionization, and the ionic strength of the media on the intrinsic viscosity of chitosan molecules.

Dilute chitosan solutions have been shown to demonstrate a characteristic polyelectrolyte behavior at low ionic strength, as represented by an increase in the specific viscosity upon dilution (Kienzle-Sterzer et al., 1982); and that this effect is suppressed by increasing salt concentration (Rodriguez-Sanchez et al., 1982; Kienzle-Sterzer et al., 1983). These studies demonstrated the effect of the solution conditions on the hydrodynamic behavior of chitosan in solution.

In order to explain the polyelectrolyte phenomena in solution, the Manning theoretical approach is commonly used (Manning, 1969a,b). Recently, an extension of this approach has been made in order to take the polyelectrolyte coil extension into consideration (Manning, 1978). In this approach the polyelectrolyte chain is considered to behave like a rigid rod with elastic bending capability. Then the persistance length is related to the change on the free energy (ΔG) associated with the bend-straight process by (Bloomfield et al., 1974):

$$\Delta G = \theta^2 \left(\frac{RTP_L}{2\ell} \right) \qquad (74)$$

where: θ = characteristic angle
R = gas constant
T = absolute temperature
P_L = persistance length
ℓ = segment length

and if the electrostatic contribution to the chain free energy is considered, then the electrostatic contribution to the persistance length will be represented by:

$$P_L = P_o + \zeta^{-1} \left(\frac{\ell^2}{12b} \right) \left[2\zeta - 1 + \ln \left(\frac{I_Z^{loc}}{I_Z} \right) + \ln Kb \right] \qquad (75)$$

where: P_o = non-electrostatic contribution to the persistance length

b = axial charge spacing

I^{loc} = local concentration of condensed counterions

I_z = bulk concentration of counterions

ξ = charge density

K = Debye-Huckel screening parameter.

Therefore, if this term is introduced together with the relationship between persistance length and the contour length given by the wormlike theory (Kratky and Porod, 1949):

$$\langle h^2\rangle = 2P_L\left\langle L - P_L\left[1 - \exp\left(-\frac{L}{P_L}\right)\right]\right\rangle \tag{76}$$

where L = contour length

$$[\eta] = \phi\,\frac{\langle h^2\rangle^{3/2}}{M_w} \tag{77}$$

$\langle h^2\rangle$ = mean-square end to end distance given (Fox and Flory, 1949)

Φ = Flory's constant

M_w = molecular weight

that for high molecular weight (where $L >> P_L$) reduces to:

$$\langle h^2 \rangle = 2P_L L \tag{73}$$

it is expected that $[\eta]^{2/3}$ be a linear function of the reciprocal of the logarithm of the counterion bulk concentration.

Thus, the relationship between the intrinsic viscosity and the ionic strength of the media will provide a test for both the Manning counterion condensation theory and the conceptual approach and approximation implicit in the previous development.

Figure 15, shows the relationship between $[\eta]^{2/3}$ of dilute chitosan solutions and the logarithm of the reciprocal of the salt concentration for degrees of ionization equal to 0.9 and 0.6. The linear relationship obtained indicates that chitosan molecule in solution behaves as a nonfree draining system composed of a series of rigid rods with elastic bonding joints. This suggested model can be explained by both the stiff nature of the chain configuration of the glucosamine ring and the limited rotation of

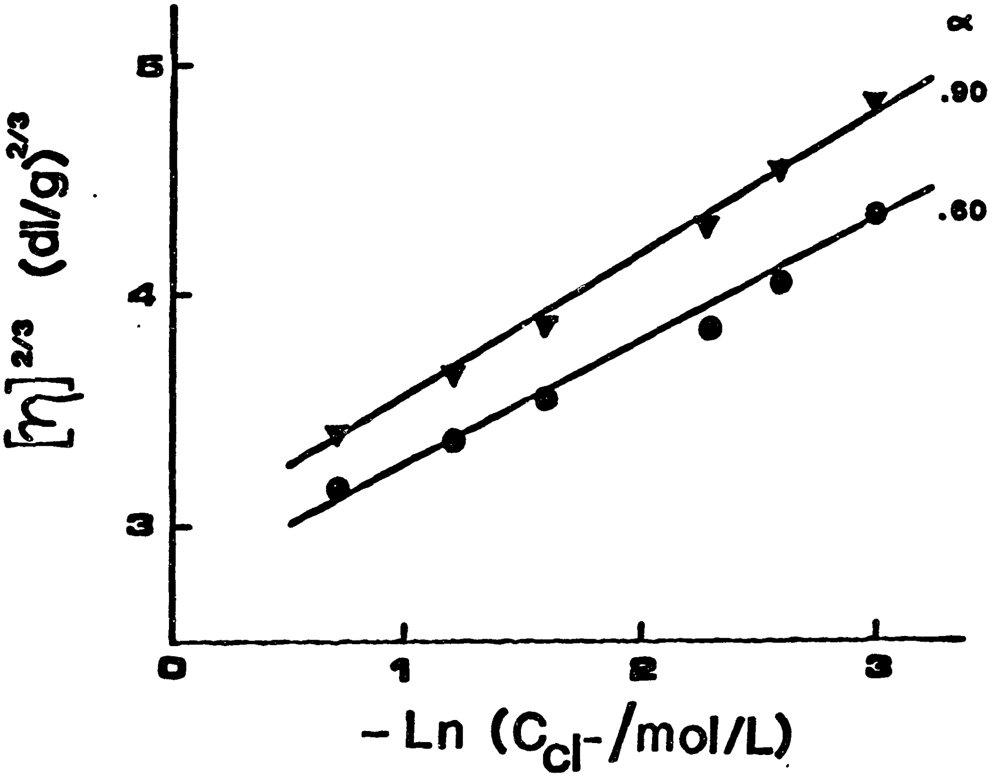


Figure 15. Relationship between $[\eta]^{2/3}$ and the logarithm of the reciprocal of the counterion concentration.

the $\beta(1-4)$ glucosidic link related to the chemical structure of the chitosan molecule. This is also supported by the obtained values of the characteristic ratio ($C_\infty = 15$) and the stiffness parameter ($B = 0.08$) previously reported (Kienzle-Sterzer et al., 1983).

In order to quantify the effect of the degree of ionization and ionic strength on the local conformation of chitosan molecules in solution, I calculated the persistance length of chitosan in solution, which would give us an estimate on both the number of stastical chain segments and the relative stiffness of the polyion (Kamide and Saito, 1983; Miyamoto, 1981; Dayan et al., 1982; Kay and Treloar, 1974; Tan and Gasper, 1974).

The overall persistance length of chitosan in dilute solution as affected by the degree of ionization and the ionic strength is calculated from the intrinsic viscosity values by using the Yamakawa and Fujii equation (Yamakawa and Fujii, 1974):

$$[\eta] = \phi\left(\frac{2P_L}{M_L}\right)^{3/2} \cdot M_w^{1/2} \qquad (79)$$

where: $M_L = \frac{M_w}{L}$ and $L = \left(\frac{M_w}{m_o}\right) \ell$

and m_o = monomer molecular weight = 168.9 daltons

M_w = molecular weight = 1.3×10^5 daltons

ℓ = 5.33 Å (Berkovich et al., 1980) for the glucosamine monomer.

$\Phi = 2.6 \times 10^{23}$ when $[\eta]$ is in cm^3/g.

Table 16 shows the value of P_L for dilute chitosan solutions for degree of ionization, α , between 0.60 and 0.90 and counterion concentration between 0.50 M and 0.05 M. The results showed in this table indicate that the persistance length increases with decreasing the counterion concentration and the degree of ionization. These tendencies demonstrate the effect of the repulsive forces on the chitosan backbone on the molecular expansion of chitosan in solution. In order to represent this effect, the persistance length of chitosan in dilute solution is plotted against the electrostatic expansion term ($\alpha^3_{\eta e}$)(Figure 16). The electrostatic expansion term has been previously discussed and reported (Kienzle-Sterzer et al., 1983). Figure 16 shows that the linear relationship between P_L and $\alpha^3_{\eta e}$ isindependent of the degree of ionization, thus indicating that in dilute chitosan solutions the changes in the persistance length are due to electrostatic interactions. In order to quantify the effect of the electrostatic contribution to the persistance length, I would

Table 16

End to End Distance and the Persistance Length (Total, P_L; Electrostatic Contribution, P_{eL}; and Non-Electrostatic Contribution, P_o) of Chitosan in Solution as Affected by Degree of Ionization (α) and Counterion Concentration

	$[CL^-]$ (M)	0.500	0.300	0.200	0.100	0.075	0.050
	K^{-1} (Å)	4.30	5.55	6.80	9.61	11.10	13.60
α							
	$\langle h^2\rangle \times 10^{11}$ (cm^2)	4.63	4.90	5.17	5.75	6.05	6.53
0.90	P_L (Å)	56.40	59.70	63.00	70.05	73.70	79.55
	P_{eL} (Å)	0.65	3.95	7.25	14.30	17.95	23.80
	P_o (Å)	55.75	(K^{-1} = 0)				
	$\langle h^2\rangle \times 10^{11}$ (cm^2)	4.53	4.79	5.03	5.58	5.86	6.31
0.80	P_L (Å)	55.20	58.40	61.30	68.00	71.40	76.90
	P_{eL} (Å)	0.65	3.85	6.75	13.45	16.85	22.35
	P_o (Å)	54.55	(K^{-1} = 0)				
	$\langle h^2\rangle \times 10^{11}$ (cm^2)	4.41	4.65	4.88	5.40	5.66	6.08
0.70	P_L (Å)	53.75	56.65	59.45	65.80	68.95	74.05
	P_{eL} (Å)	0.60	3.50	6.30	12.65	15.80	20.90
	P_o (Å)	53.15	(K^{-1} = 0)				
	$\langle h^2\rangle \times 10^{11}$ (cm^2)	4.29	4.52	4.73	5.21	5.45	5.85
0.60	P_L (Å)	52.25	55.05	57.65	63.45	66.40	71.25
	P_{eL} (Å)	0.50	3.30	5.90	11.70	14.65	19.50
	P_o (Å)	51.75	(K^{-1} = 0)				

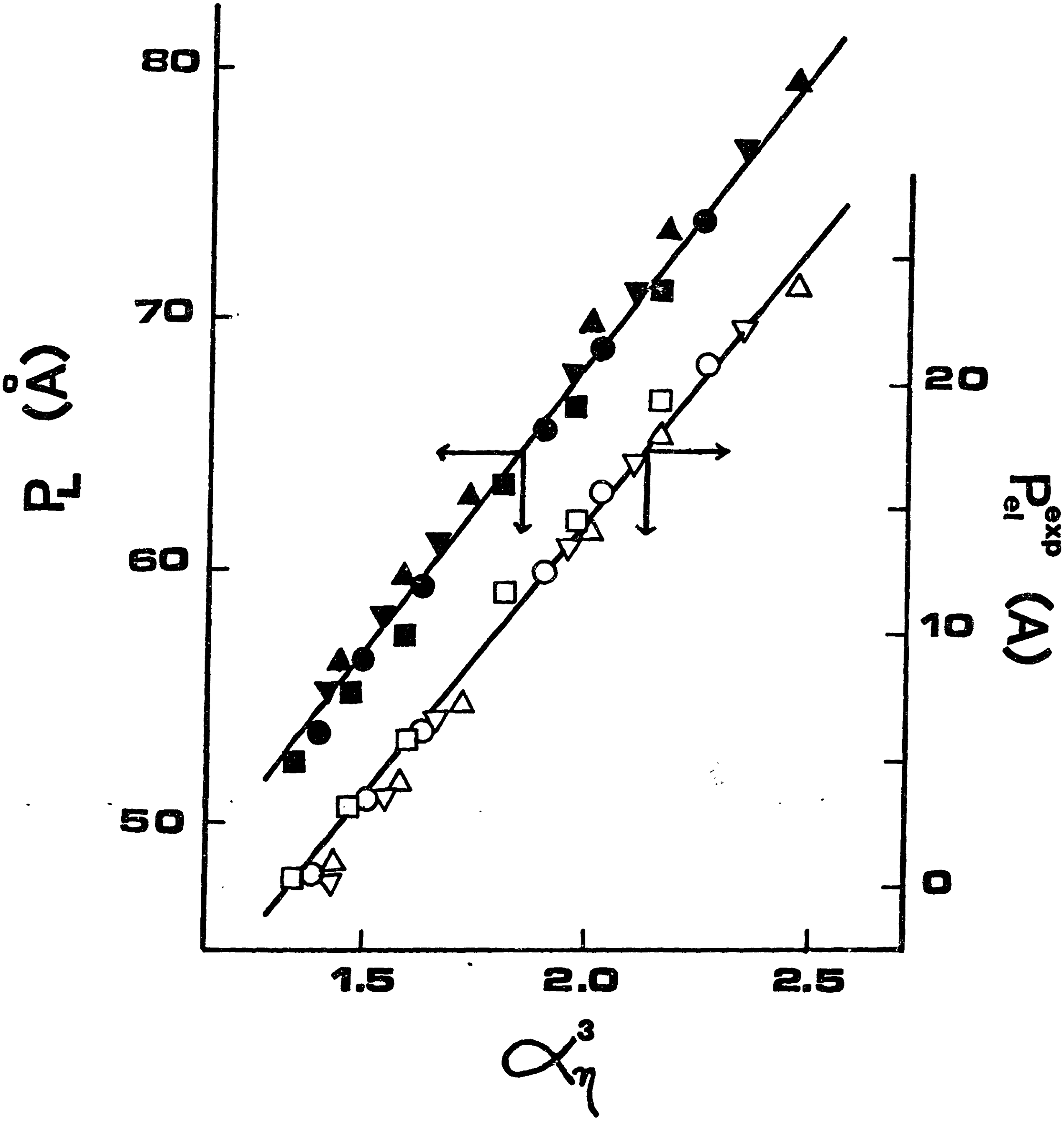


Figure 16. Overall persistance length (P_L) and the electrostatic persistance length (P_{eL}^{exp}) as a function of the electrostatic expansion term (α_η^3) for different degrees of ionization and counterion concentration.

calculate the electrostatic persistance length (P_{eL}) by the expression (Skolnick and Fixman, 1977):

$$<h_2> = 2P_L L = 2(P_o + P_{eL})L \tag{80}$$

similarly to equation (75), it is appropriated because $L >> P_L$ for all cases ($L = 4.11 \times 10^3$ Å), and P_o, (Table 16), is the persistance length in the absence of electrostatic forces ($K^{-1} = 0$). Values of P_{eL} as affected by and the ionic strength are shown in Table 16, and their relationship with the electrostatic expansion, $\alpha^3_{\eta e}$, on Figure 16. These results clearly indicate that as the number of charges on the chitosan backbone are increased, represented by an increase in α , the chain stiffens as indicated by the increase in the electrostatic persistance length. Moreover, as the ionic strength is decreased the chain stiffness is greater indicating the significance of the chain-counterion interaction on the molecular stiffness, and that, this effect is the most important.

Also, the values of P_L at infinite ionic strength for chitosan (P_o) shown in Table 16 are similar to those reported values for sodium carboxymethylcellulose, Ds= 0.88, P_o = 50 Å (Kamide et al., 1983), but larger than that of typical flexible vinyl type polymers (P_o= 10 Å) (Kamide et al., 1983), and smaller than those of more stiff cellulose derivative polymers such as hydroxyethyl cellulose (P_o = 82 Å) (Brown *et al.*, 1963) and ethyl hydroxyethyl cellulose (P_o = 92 Å) (Manley, 1956), therefore I can conclude that chitosan has a flexibility similar to carboxymethylcellulose, which is in perfect agreement with the previous values of the extension ratio C_∞ and the stiffness parameter B (Kienzle-Sterzer *et al.*, 1983).

The electrostatic contribution to the excluded volume indicates the expansion of the polyelectrolyte chains due to the repulsion between the backbone charges. The electrostatic contribution to the excluded volume (B_e^{exp}) can be calculated from the relationship between [η] and the counterion concentration by the expression obtained from the Stockmayer-Fixman relationship (Stockmayer and Fixman, 1963; Noda *et al.*, 1970):

$$B_e^{exp} = \frac{mC_s^{-1/2}}{0.51\phi M_w} \tag{81}$$

where m = slope from [η] vs. $C^{-1/2}$ curves previously reported (Kienzle-Sterzer *et al.*, 1983).

Values of B_e^{exp} for dilute chitosan solutions as affected by the degree of ionization and the ionic strength are shown in Table 17. Table 17 indicates that the elec-

Table 17

Experimental and Theoretical Values of the Electrostatic Contribution to the Excluded Volume as a Function of Degree of Ionization (α) and Counterion Concentration

α	[CL$^-$] (M)	0.500	0.300	0.200	0.100	0.075	0.050
0.90	$Be^{exp} \times 10^{26}$ (cm^3)	1.16	1.50	1.84	2.60	3.00	3.68
	$Be^{th} \times 10^{26}$ (cm^3)	0.83	1.12	1.54	2.42	2.91	3.77
0.80	$Be^{exp} \times 10^{26}$ (cm^3)	1.07	1.39	1.70	2.40	2.77	3.40
	$Be^{th} \times 10^{26}$ (cm^3)	0.75	1.04	1.35	2.26	2.72	3.54
0.70	$Be^{exp} \times 10^{26}$ (cm^3)	0.97	1.25	1.53	2.17	2.50	3.07
	$Be^{th} \times 10^{26}$ (cm^3)	0.69	0.97	1.27	1.99	2.37	3.09
0.60	$Be^{exp} \times 10^{26}$ (cm^3)	0.89	1.14	1.40	2.00	2.29	2.80
	$Be^{th} \times 10^{26}$ (cm^3)	0.60	0.89	1.18	1.85	2.18	2.96

Be^{exp}: experimental electrostatic contribution to the excluded volume.

Be^{th}: theoretical electrostatic contribution to the excluded volume calculated using the Fixman-Skolnick approach (Fixman and Skolnick, 1978).

trostatic contribution to the excluded volume increases with both increasing the degree of ionization and decreasing the ionic strength of the media. In order to compare the values of the experimental electrostatic contribution to the excluded volume (B_e^{exp}) with the theoretical values, I calculated the electrostatic contribution to the excluded volume (B_e^{th}) by the expression (Fixman and Skolnick, 1978):

$$B_e^{th} = \left(\frac{\ell^2}{Km_o}\right) R(w) \tag{82}$$

where

$$w = \frac{136.4\alpha^2}{\ell^2 \; C_s^{1/2}} \tag{83}$$

and

$$R(w) = \int_o^{\pi/2} d\theta \; \sin^2\theta \int_o^{w/\sin\theta} x^{-1} \left(1-e^{-x}\right) dx \tag{84}$$

This expression for B_e^{th} is obtained by assuming that the

i) Debye-Huckel approximation is applicable to study excluded volume effects as indicated from the linear relationship between P_{el}^{exp} and K^{-1}, Figure 17;

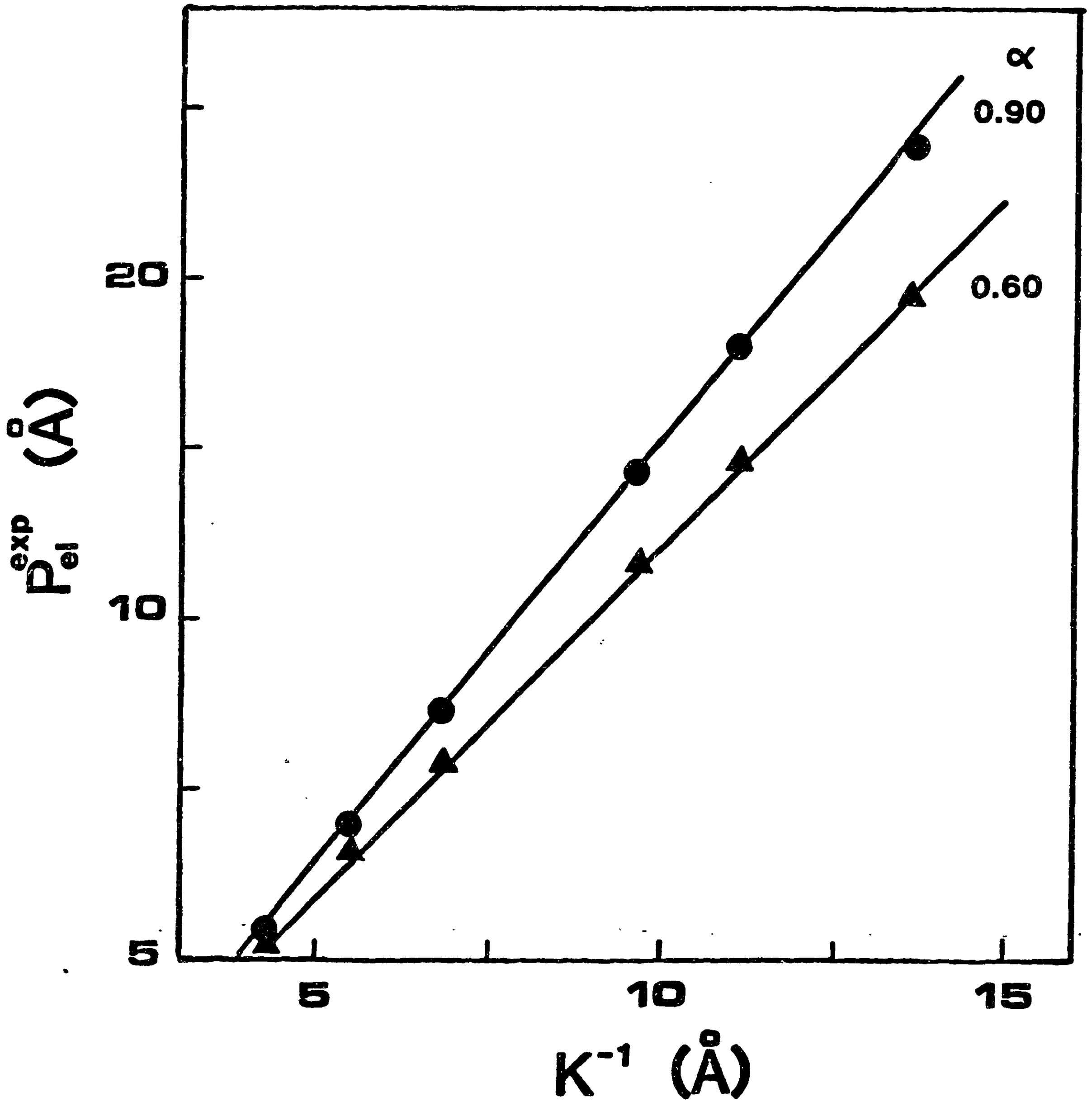


Figure 17. Electrostatic persistance length (experimental "p_{eL}^{exp}") as affected by the Deby-Huckel screening parameter (K^{-1}) for chitosan with different degrees of ionization in dilute regime.

ii) The molecule can be considered as a wormlike polymer with a continuous charge distribution as expected by the linearity between $[\eta]^{2/3}$ and $-\lg C_{CL}$ shown previously (Figure 15) and;

iii) Neglectible interactions between neighbor segments.

Table 17 presents the values of B_e^{th} for chitosan solutions for the experimental range of α and C_{CL}. The values of B_e^{th} increases as the ionic strength of the solution decreases and/or the degree of ionization increases. These findings are in agreement with the tendency shown for B_e^{exp}. However, a closer examination of this values shows that $B_e^{exp} = B_e^{th}$ for low values of ionic strength but that $B_e^{exp} > B_e^{th}$ for high values of ionic strength.

The decrease in the electrostatic contribution to the excluded volume of chitosan molecules in solution is related to the decrease in the electrostatic repulsive forces between charged points on the chitosan backbone due to either a decrease in the number of charged monomers or an increase in the shielding of charges by counterions. However, for all α the values of Be^{th} are significantly higher than the values of Be^{th} for counterion concentrations higher than 0.20 M. This tendency can be considered as the result of the collapse of the chitosan molecules with increasing ionic strength (Table 12). As the result of this collapse, the counterion cloud associated to the chitosan molecule starts to interact thus increasing the repulsive forces between segments.

Therefore, it can be concluded that chitosan in dilute solution can be considered as nondraining wormlike polymer with a flexibility similar to sodium carboxymethylcellulose which can be readily controlled by both changes in the degree of ionization and the counterion concentration. Also, it can be concluded that the changes in flexibility as represented by the changes in P_{eL} are due to electrostatic interactions which can be represented by the Debye-Huckel approximation eventhough neighboring interactions must be considered at high ionic strength.

4.1.4. Intrinsic Viscosity - Molecular Weight

It has been shown in the preceding section, that the flow behavior of chitosan is strongly influenced by the electrostatic interactions between neighboring segments, as well as between chitosan-counterions. It is also known that the intrinsic viscosity of a linear polymer is related with the molecular weight by the Mark-Houwink equation (Flory, 1953):

$$[\eta] = KM_w^a \qquad (85)$$

where "K" and "a" are characteristic constants of the polymer-solvent system. In general, for a random coil polymer the Mark-Houwink exponent is between 0.5 and 0.8 such as in the case of pullulan, sodium carboxymethyl amylose among others (See Table 18), meanwhile Mark-Houwink exponents higher than 0.8 as for hyaluronic acid, sodium alginate (Table 18) are associated to highly expanded conformation, whereas Mark-Houwink exponents lower than 0.5, as for levan hydrolysates, which is a polysaccharide composed of D-fructofuranose units joined by β(2-6)bonds obtained in the fermentation of _Streptococcus salivaricus_ (Table 18), reflect a globular compact conformation.

Therefore, from the values reported in Table 18, it is clear that chitosan conformation in solution changes from a compact spherical structure (a = 0.147) when dissolved in acetic acid-NaCl (1%-2.8% respectively) to a more random coil conformation (a = 0.71) when urea is present. The increase in the value of "a" to the region approaching the random coil clearly shows the significant of neighboring interactions on the molecular configuration of chitosan molecules in solution. This phenomena agrees

Table 18

Mark-Houwink Exponent of Selected Polysaccharides in Solution

Polysaccharide	Solvent	a	References
Levan hydrolysates	water	0.05	Stivala *et al.*, 1981
Chitosan	acetic acid 1%	0.147	Berkovich *et al.*, 1980
Chitosan	acetic acid 0.2M, NaCl 0.1M; urea 4M	0.71	Muzzarelli, 1977
Pullulan	water	0.50	Kato *et al.*, 1982
Curdlan	cardoxen-H_2O	0.53	Hirano *et al.*, 1978
Sodium carboxymethyl amylose	water	0.61	Goebel and Brant, 1970
Cellulose	cardoxen-H_2O	0.65	Hirano *et al.*, 1978
DNA	water	0.695	Triebel *et al.*, 1971
Guar galactomannan	water	0.723	Robinson *et al.*, 1982
Hyaluronic acid	water pH = 6; 0.2M NaCl	0.82	Cleland and Wang, 1970
Sodium alginate	water	1.00	Smidrod, 1970

with the results reported in the intrinsic viscosity-degree of ionization-ionic strength section.

In order to study the effect of molecular weight on the hydrodynamic properties of chitosan solutions, the persistance length of chitosan molecules for different molecular weights will be calculated using the following expressions:

1) The Benoit-Doty's equation for the unperturbed radius of gyration $<S^2>^{1/2}$ generalized to the case of polydisperse wormlike chain in the form (Benoit and Doty, 1953; Kamide *et al.*, 1983):

$$<S^2>^{1/2} = P_L \left\langle \left(\frac{h+2}{H+1}\right)\frac{M_w}{3P_LM_L} - 1 + \frac{2P_LM_L}{M_w} - \left[\frac{2(h+1)\ P_L^2M_L^2}{hM_w^2}\right]\left[1 - \left(1 + \frac{(h+1)\ P_LM_L}{M_w}\right) h\right] \right\rangle \qquad (86)$$

with $h = (\frac{Mw}{Mn} - 1)^{-1}$ and M_n the number average molecular weight.

In order to apply this equation, the following values were used:

m_o = 168.9 daltons

$\frac{Mw}{Mn} = 1.8 \pm 0.3$ (Domard and Rinaudo, 1983)

$\ell = 5.33\ \mathring{A}$ (Berkovich et al., 1980)

$$<S^2> = \frac{<h^2>^{1/2}}{6}$$

obtained in each case from the intrinsic viscosity data using equation (76).

2. The Yamakawa and Fujii equation from the intrinsic viscosity for unperturbed wormlike chain (Yamakawa and Fujii, 1974) equation (78).

Table 19 shows the values of intrinsic viscosity as well as the persistance length for chitosan in dilute solutions calculated by both methods as a function of the chitosan molecular weight. From the persistance length values reported, it can be observed that for chitosan solutions with added urea, (Muzzarelli, 1977; Skylyar et al., 1981) where hydrogen bonding is disrupted and chitosan molecules adopt an extended random coil configuration, the persistance length increased with increasing molecular weight, independently of the method used to calculate the persistance length. Whereas, when urea is not present, therefore, when hydrogen bonding is present (Berkovich et al., 1980; Ganzazade et al., 1981), the persistance length decreased as molecular weight is increased. This behavior

Table 19

Persistance Length; Intrinsic Viscosity of Dilute Chitosan Solution as a Function of the Molecular Weight

Mw	$2.5x10^{4}$	$5.0x10^{4}$	$1.0x10^{5}$	$2.5x10^{5}$	$5.0x10^{5}$	$1.0x10^{6}$
$[\eta]$ (cm^3/g)	118	193	317	607	993	1625
P_L* (Å)	30.10	31.72	34.94	39.12	41.91	46.25
P_L** (Å)	31.76	33.00	36.51	41.42	45.70	50.38
P_L (Å)	1.66	1.28	1.57	2.30	3.79	4.13
$[\eta]$ (cm^3/g)	480	546	621	737	838	954
P_L* (Å)	71.66	58.80	50.91	43.22	38.64	33.72
P_L** (Å)	76.30	66.00	57.17	47.11	40.73	35.29
P_L (Å)	4.64	7.20	6.26	3.89	2.09	1.59

(a) From: Muzzarelli, 1977; Domard and Rinaudo, 1983

(b) From: Berkovich *et al.*, 1980

P_L* : Persistance length calculated using Benoit-Doty's equation (Benoit and Doty, 1953).

P_L**: Persistance length calculated using Yamakawa-Fujii's equation (Yamakawa and Fujii, 1974).

is explained by the restrictive effect of intra-chain H bonds, represented by an increase in the local stiffness, on the hydrodynamic volume of chitosan solutions, producing a more compact spherical conformation as represented by the Mark-Houwink exponents (0.147 in absence of urea versus 0.710 in the presence of urea - Table 18).

As I pointed out previously, in the calculation of the persistance length using the Yamakawa and Fujii equation (equation (78)) the draining effect is not taken into consideration if in the wormlike cylinder model the flow rate on the surface of the cylinder is taken as zero (Yamakawa and Fujii, 1974). Therefore, the difference between the persistance length calculated by the Yamakawa and Fujii's equation and the persistance length calculated by the Denoit-Doty's equation (ΔP_L) can be used to evaluate the draining effect.

Table 19 shows the values of ΔP_L for both extended random coil chitosan and compact globular chitosan as a function of the molecular weight. Moreover, Table 19 shows that ΔP_L has a finite value that remains almost constant, for chitosan of molecular weight in the range between 2.5×10^4 daltons and 1.0×10^6 daltons. These findings indicate that eventhough chitosan molecules in solution are highly solvated as shown by the results on glucose diffusion, there is a non-draining effect, as previously stated from the linear relationship between $[\eta]^{2/3}$ and the reciprocal of the logarithm of the ionic strength of the media.

To represent the relationship between $[\eta]$ and molecular weight of chitosan solutions the following equation is selected (Ptitsyn and Eizner, 1962; Leon <u>et</u> <u>al</u>., 1982):

$$\frac{M_w}{[\eta]} = 0.4537\left(\frac{m_o}{\ell}\right)^2 \frac{1}{\phi r_o \lambda} + \frac{1}{\phi \ell^3}\left(\frac{m_o}{2\lambda}\right)^{3/2} M_w^{1/2} \tag{87}$$

where r_o is the monomeric hydrodynamic radius $\lambda = a/\ell$ where "a" is the Kratky and Parod persistance length which is just half of the Kuhn statistical segment (K_1).

Using the equation (87) on the values of $[\eta]$ of chitosan solutions for molecular weights between 2.5×10^4 daltons and 1.0×10^6 daltons, it is found that the unperturbed Kratky-Parod persistance length and the unperturbed Kuhn statistical segment are:

a) For extended random coil chitosan molecules in solution

$a = 60.8$ Å; $K_1 = 121.6$ Å

b) For compact spherical chitosan molecules in solution

$a = 26.4$ Å; $K_1 = 72.8$ Å

These values for chitosan solutions can be compared with values for the Kuhn statistical segments of other polyelectrolytes. Thus, it can be seen that for stiff polymers such as DNA, K_1 is 454 Å (Triebel *et al.*, 1971) and nitrocellulose-acetate, $K_1 = 158$ Å (Dondos and Staikos, 1980) or for semiflexible polymers such as (x-500) poly(terephtaloyl p-amino benzhydrazide) in DMSO K_1 is 80 Å (Staikos and Dondos, 1983). Therefore, it is observed that the values of the Kuhn statistical segment of chitosan reflects the role of the intramolecular H-bonding in the structure-properties of chitosan in solution.

4.2 Degree of Ionization of Dilute and Semiconcentrated Polyelectrolyte Solutions

It is well established that the properties of polyelectrolytes in solution are governed in addition to the molecular weight, by the nature of the linkages, constituent monomer and by the nature and the degree of ionization of the ionic groups (Katchalsky *et al.*, 1957; Manning, 1981). This section presents the potentiometric titration of chitosan in solution both in dilute and semiconcentrated regime as affected by polymer concentration and counterion concentration. Because the complexity of the system under study, chitosan-counterion-solvent, as well as the limit solubility of the chitosan (Muzzarelli, 1977), in order to isolate the effect of polymer-polymer interactions, the polymer concentrations (0.01 g/dl - 0.20 g/dl) are selected such that, for given counterion concentration, the polymer contribution to the ionic strength would be negligible.

Figure 18 shows the potentiometric titration curve, represented by the plot of pKapp against degree of ionization (α), for dilute chitosan solutions (chitosan concentrations between 0.01 g/dl and 0.06 g/dl) for several counterion concentrations (0.05 M Cl^- - 0.50 M Cl^-). Some features of these results are noteworthy:

1. The shape of pKapp versus α curves shows two distinct regions. One region for pKapp values between 6.0 and nearly 3.5 where the degree of ionization α changes as a function of the pKapp; and a second region (pKapp $\leq$ 3.5) where changes in pKapp do not affect the value of α. This behavior can be associated with the existence of a conformational change on the chitosan chain at pKapp values about 3.5. This result together with the relationship earlier discussed between the intrinsic viscosity of chitosan solutions and pH (Kienzle-Sterzer et al., 1983), indicates that at pKapp values higher than 3.5 the chitosan chain collapses as α decreases. Moreover, the de-

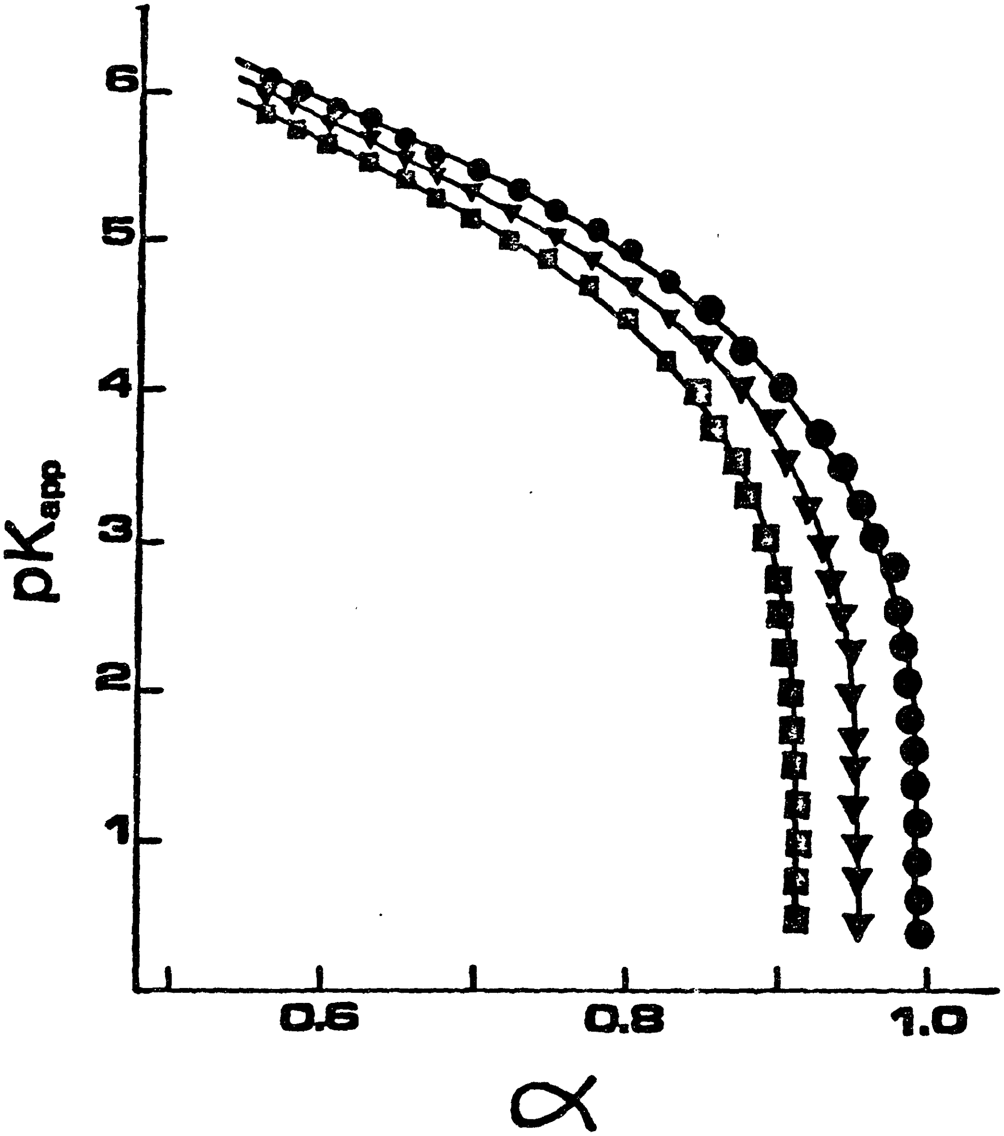


Figure 18. Potentiometric titration for dilute chitosan solutions: pKapp versus the degree of ionization (α): counterion concentration: ■,0.05 M; ▼,0.10 M; ●,0.50 M.

crease in intrinsic viscosity values as pH is increased above 4.0 supports the fact that the molecular domain collapses, on the other hand, the independency of α values as pKapp is decreased below 3.5 supports the insensitivity of the hydrodynamic volume of chitosan molecule for low pH regimes as represented by the constancy in intrinsic viscosity for pH values below 4.0 (Figure 19). This close relationship between the hydrodynamic properties and the degree of ionization for polyelectrolyte molecules has been previously reported theoretically (Knokhlof, 1980), as well as experimentally for other modeled systems as poly[thio-1-(N,N-dimethylaminomethyl)ethylene] (Vallin et al., 1980) and poly-L-lysine (Grourke and Gibbs, 1971) among others. The relationship between pKapp-degree of ionization-intrinsic viscosity highlights the controlling role of the nearest neighboring interactions, not only on the thermodynamic properties of the polyelectrolyte solutions but also on its rheological behavior. This controlling influence of the nearest neighbor interactions has been shown to be of paramount importance in the phenomenological behavior of other amino like polymers such as poly(ethylenimine) (Bloys van Treslong and Staverman, 1974; Bloys van Treslong, 1978) as well as other acid-like polyelectrolytes (Katchalsky et al., 1957; Sasaki and Minakata, 1977).

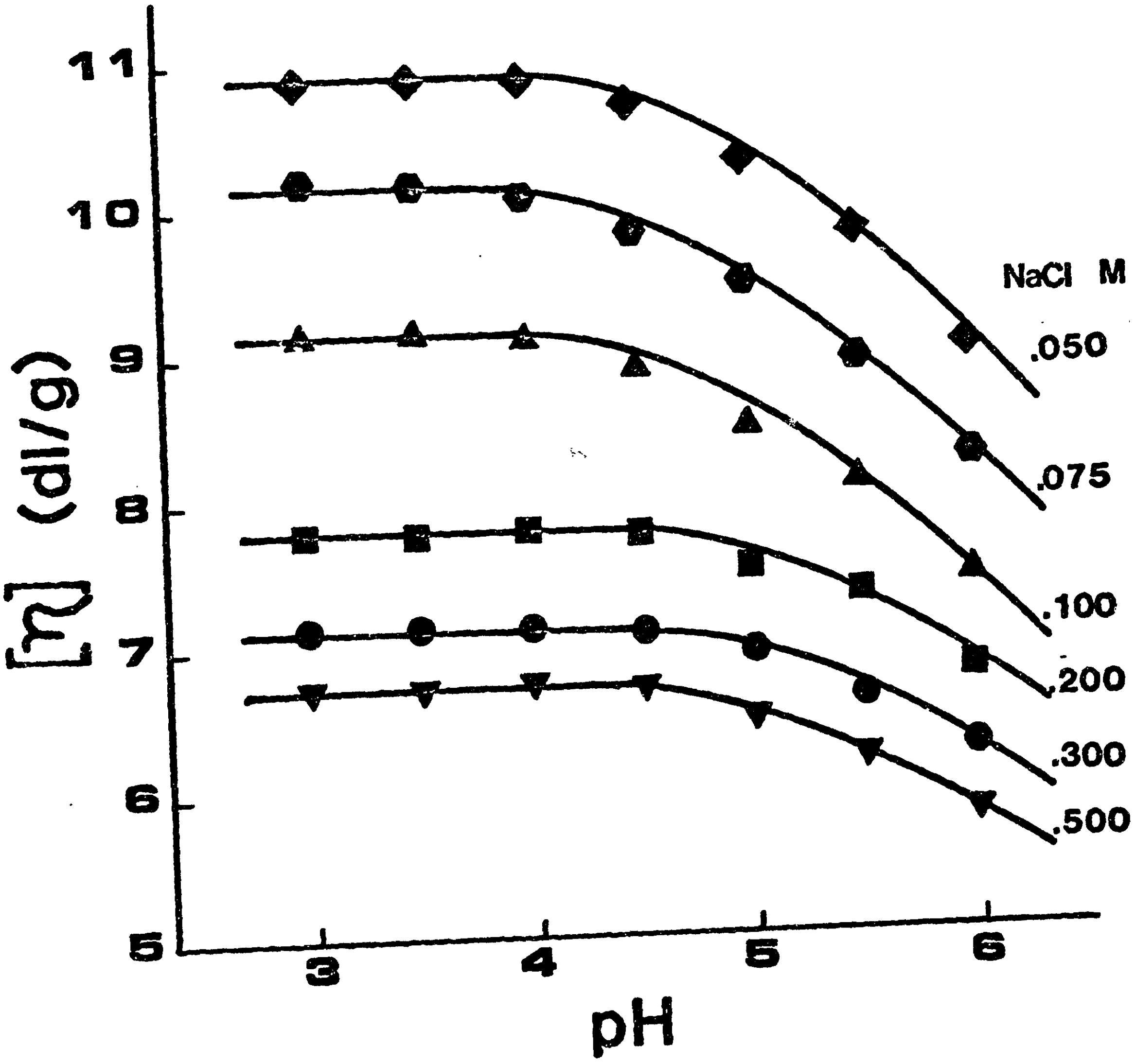


Figure 19. Intrinsic viscosity of chitosan in solution as affected by pH and ionic strength of the media.

2. The relationship between the pKapp values and the degree of ionization " α " is independent of chitosan concentration for dilute chitosan solutions. This indicates that at high ionic strength in the dilute regime, the polymer concentration does not play any controlling role, suggesting that the potentiometric properties of dilute chitosan solutions are only a function of the ionic strength of the media. This fact demonstrates and supports the concept of isolated molecules used in the calculation of intrinsic viscosity earlier reported. Also, it must be pointed out that the independency of the potentiometric behavior on the polyelectrolyte concentration for dilute polymer solution, here reported, unlikely contradicts the strong effect of the polymer concentration reported for carboxymethyl-cellulose in salt free solutions (Trivedi *et al.*, 1981a,b). According to the solution conditions selected for the carboxymethylcellulose study, any changes in the polymer concentration produces notable changes in the ionic strength of the media, which in turn controls the potentiometric behavior of polyelectrolytes in dilute solution (Manning and Holtzer, 1973; Manning, 1981).

3. A shift to higher values of the degree of ionization for a given pKapp value when the ionic strength is increased. This indicates that the increase of the coun-

terion concentration reduces the electrostatic repulsion between neighboring segments, therefore a higher electrical charge density is required in order to maintain the pKapp value constant. Similar behavior has been reported for other dilute polyelectrolyte solution systems (Nagasawa et al., 1979; Dubin and Strauss, 1970; Nagasawa, 1971; Ishikawa, 1979; Bloys van Treslong and Jansen, 1983).

Potentiometric titration studies of dilute polyelectrolyte solutions have been successfully used to calculate the additional work or energy required to remove a H^+ from the strong electrostatic forces of the charges present in the macromolecules (Nagasawa et al., 1965; Nagasawa, 1971). The difference from simple electrolytes where the relationship between pH of the solution and the degree of ionization has been expressed:

$$pH = pK_o - \log\left(\frac{1-\alpha}{\alpha}\right) \qquad (88)$$

is that in the polyelectrolyte solutions an additional term must be added in order to account for this additional work (Katchalsky ans Apitnik, 1947). Therefore:

$$pH = pK_o - \log\left(\frac{1-\alpha}{\alpha}\right) + 0.434\,\frac{\Delta G_{eL}}{RT} \qquad (89)$$

where: pK_o is the dissociation constant of the charged groups; for chitosan $pK_o = 6.2$ (Muzzarelli, 1977; Tsuka and Inoue, 1981; Park et al., 1983) and ΔG_{el} can be expressed as

$$\Delta G_{eL} = e\Psi_b \tag{92}$$

in terms of the electrostatic potential at the place where H^+ originally exists, Ψ_b. Therefore, Ψ_b can be assumed to be the electrostatic potential on the rod surface (Kotin and Nagasawa, 1962). Values for the electrostatic potential on the rod surface for dilute chitosan solutions in mV are reported in Table 20 for pKapp values between 1.0 and 5.0.

From the data of intrinsic viscosity of chitosan solutions and its relationship with pH and ionic strength, chitosan molecules have been modeled in dilute solution as a free non-draining equivalent sphere, composed of a series of rigid rods with elastic bonding joints. Recently, equation (88) has been modified (Manning and Holtzer, 1973) taking into consideration the phenomenon of counterion condensation using the basic principles of the Manning's approach of polyelectrolytes in solution (Manning, 1969a,b) as follows:

Table 20

Surface Electrostatic Potential (Ψ_b) and Self-Energy Term (W_{self}) for Dilute Chitosan Solutions at Different Ionic Strength of the Media

pKapp Ψ_b(mv)		1 307.92	2 248.71	3 189.49	4 130.28	5 71.06
[Cl^-] (M)	$K\times10^6$ (cm^{-1})			$-W_{self}$		
0.050	7.36	18.93	17.93	16.93	14.88	12.37
0.075	9.01	19.11	18.11	17.11	15.20	12.46
0.100	10.40	19.23	18.23	17.23	15.50	12.57
0.200	14.71	19.54	18.54	17.54	15.62	12.80
0.300	18.02	19.71	18.71	17.71	15.76	12.92
0.500	23.26	19.93	18.93	17.93	15.89	13.11

$$pK_{app} = pK_o - g(\alpha) \log K^2 + W_{self} \tag{93}$$

where K is the Debye screening parameter equal to 3.29 x $10^7 M^{1/2}$ for monovalent salt at 25°C in water; and g(α) is defined when the salt concentration is present in excess over the polyelectrolyte concentration as:

$$\begin{aligned} g(\alpha) &= \frac{\alpha}{\alpha_c} \qquad \alpha < \alpha_c \\ &= 1 \qquad \alpha \geq \alpha_c \end{aligned} \tag{94}$$

where α_c is the critical degree of ionization corresponding to a charge density parameter equal to 1 for monovalent counterions.

Using this corrected relationship (equation (90)) for dilute chitosan solutions, with a α_c = 0.908 calculated from the critical distance between charged points (7.33 Å), which corresponds to a charge density equal to 1 at 25°C, we obtain the values of W_{self}. Table 20 presents the values of W_{self} for dilute chitosan solutions as affected by pKapp and ionic strength of the media. The value of W_{self}, which is a measure of the change in self-energy of the polyion when an amino group is protonized, is defined as (Manning and Holtzer, 1973):

$$W_{self} = 0.434 \frac{\Omega self}{KT} \qquad (90)$$

Ω_{self} = energy of self ionization

K = Boltzman's constant

T = absolute temperature

indicating that the energy for protonation decreases as surface charge potential increases and as the ionic strength of the media decreases, albeit, the decrease is higher for charges in Ψ_b (Table 20). Thus, the critical role of surface charge potential on the electrical field around the polyion is established.

In order to examine the effect of the overlapping of the solvation layer around polyions on their thermodynamic properties, the potentiometric titration response for chitosan solutions on the semiconcentrated regime (0.01 g/dl to 0.20 g/dl) are evaluated. Figures 20 to 24 report the potentiometric titration curves (pKapp vs α) for a given chitosan concentration and ionic strength between 0.05 M Cl^- and 0.40 M Cl^-. The most important features from these figures are:

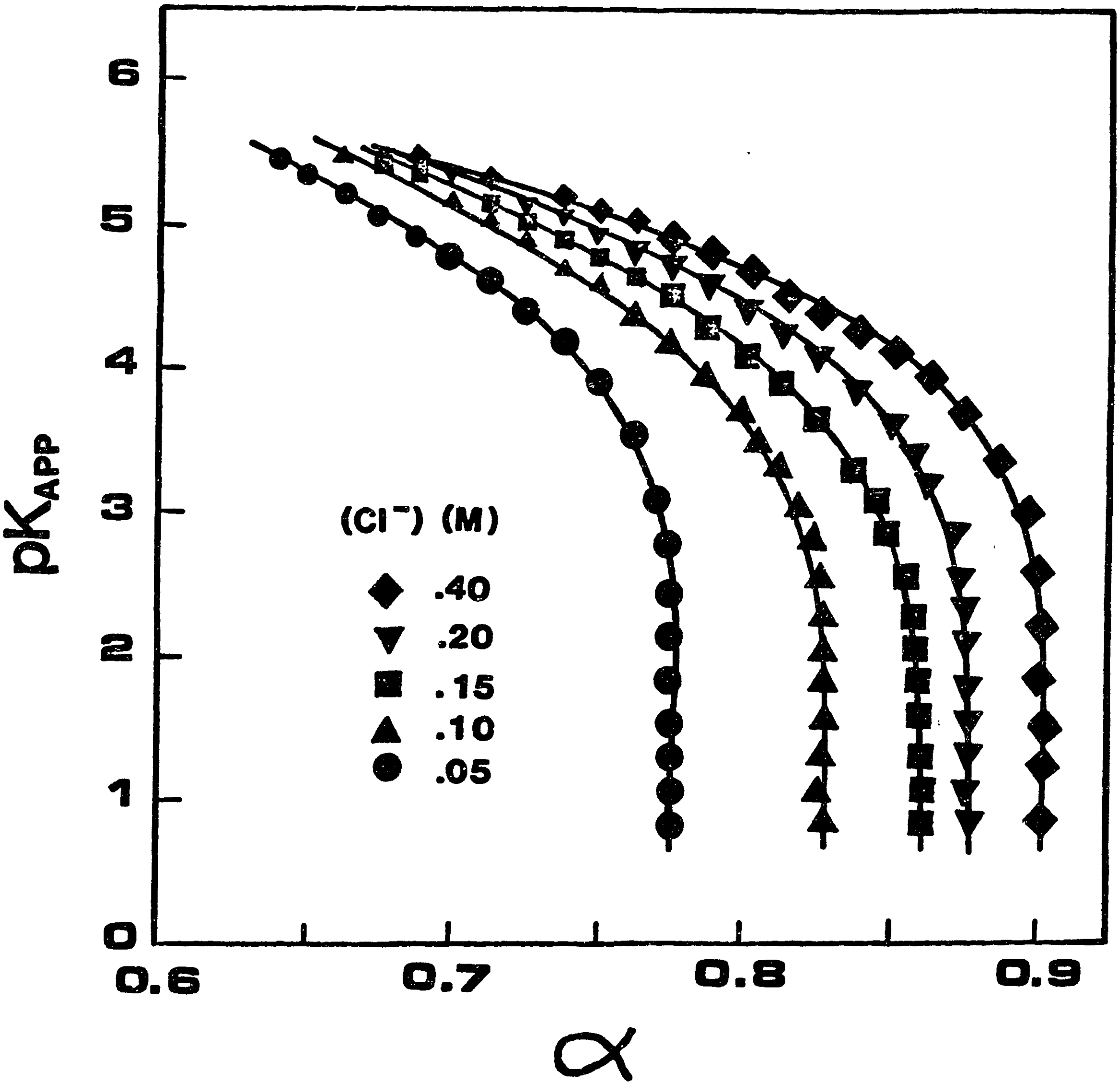


Figure 20. Potentiometric titration for semiconcentrated chitosan solutions (polymer concentration 0.10 g/dl): pKapp versus degree of ionization (α) for different counterion concentrations.

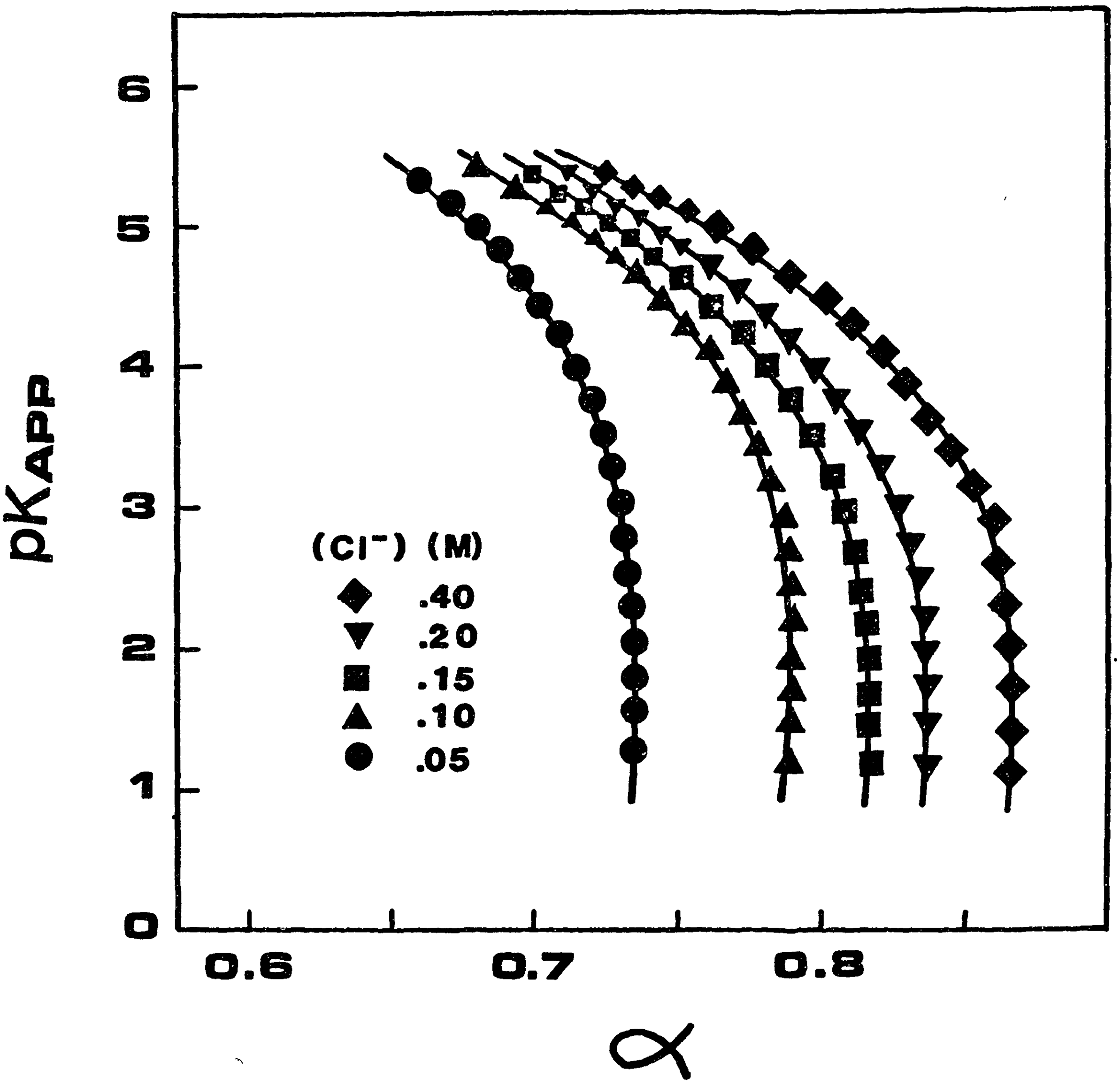


Figure 21. Potentiometric titration for semiconcentrated chitosan solutions (polymer concentration 0.12 g/dl): pKapp versus degree of ionization (α) for different counterion concentrations.

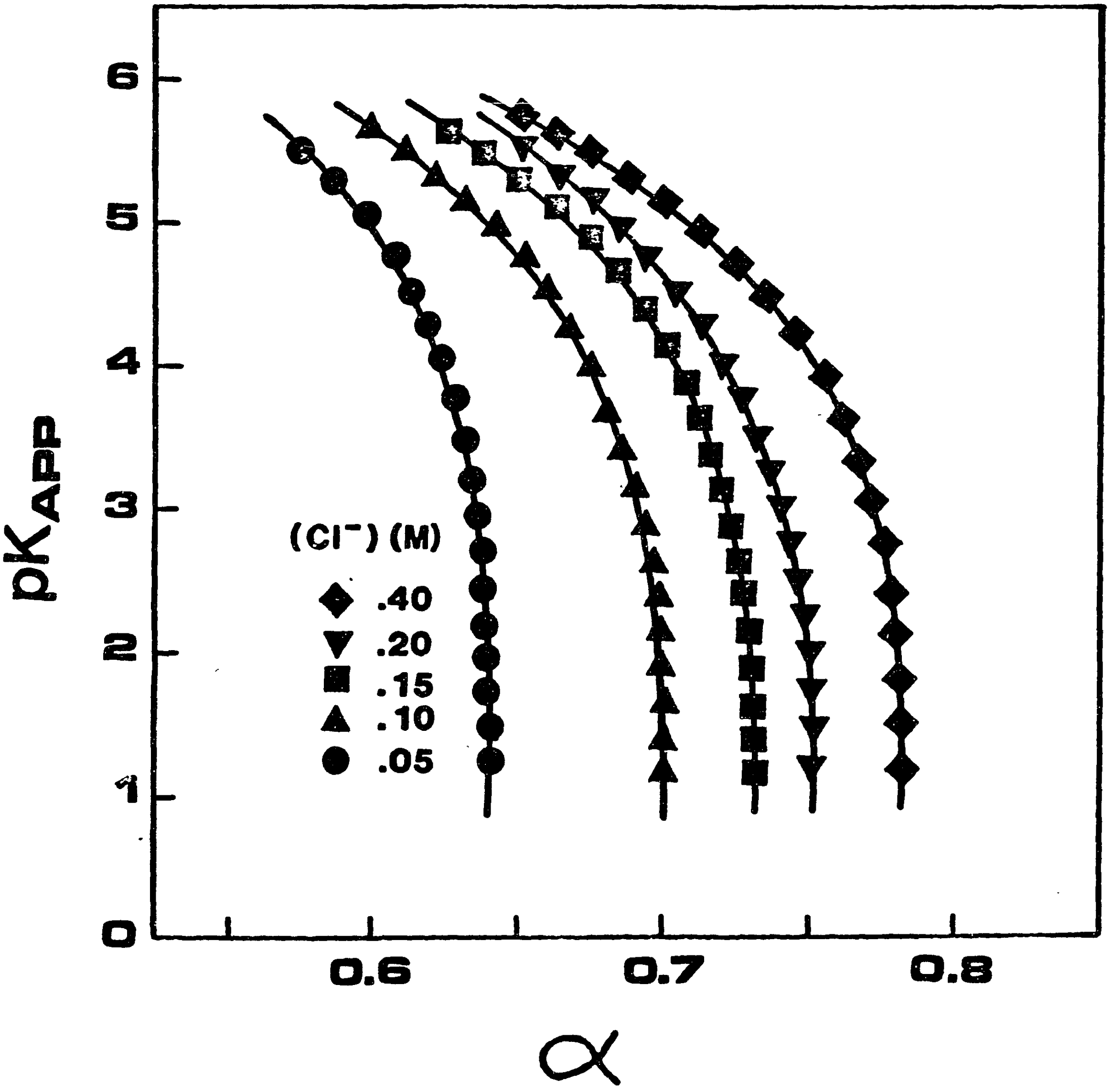


Figure 22. Potentiometric titration for semiconcentrated chitosan solutions (polymer concentration 0.15 g/dl): pKapp versus degree of ionization (α) for different counterion concentrations.

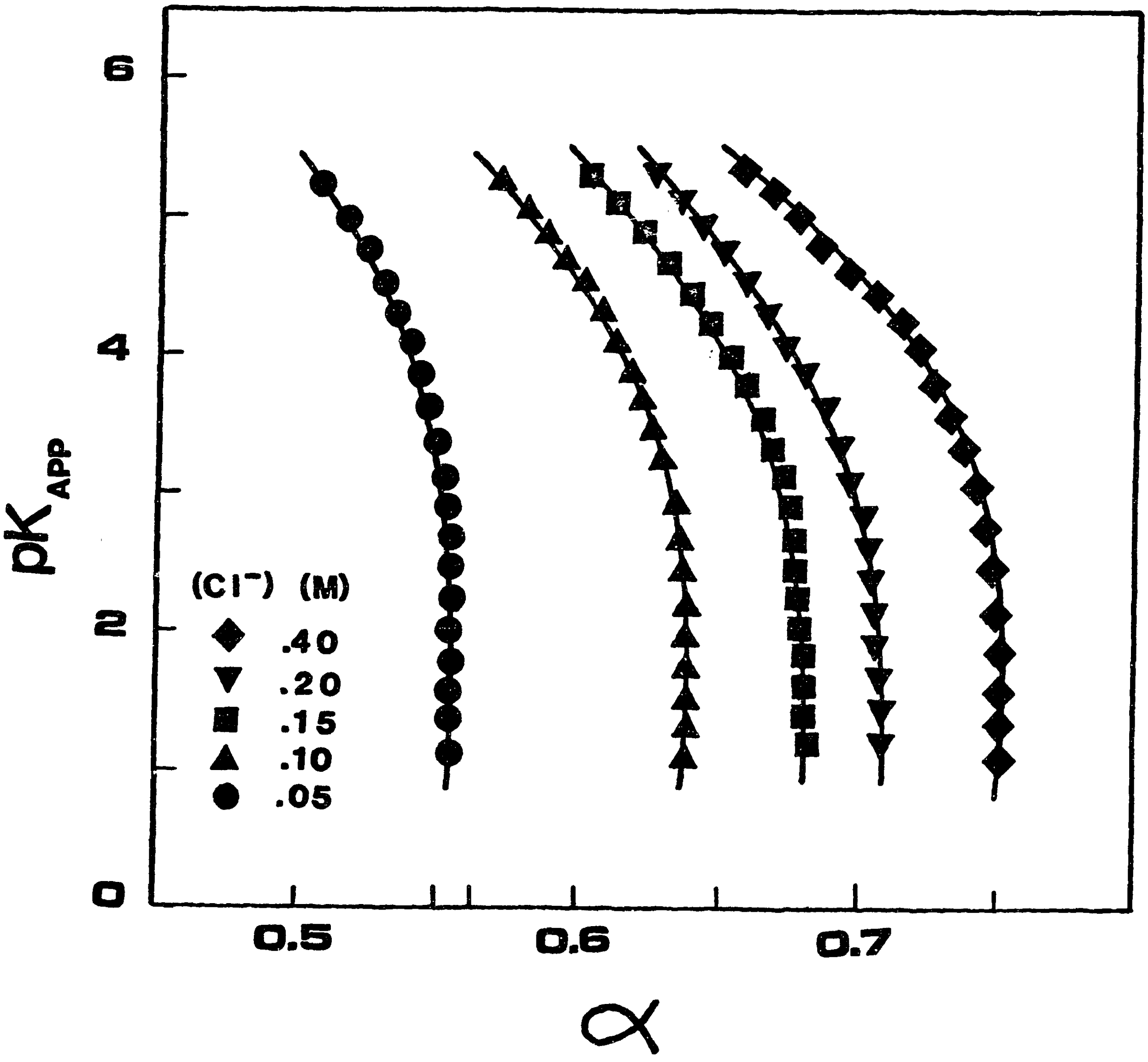
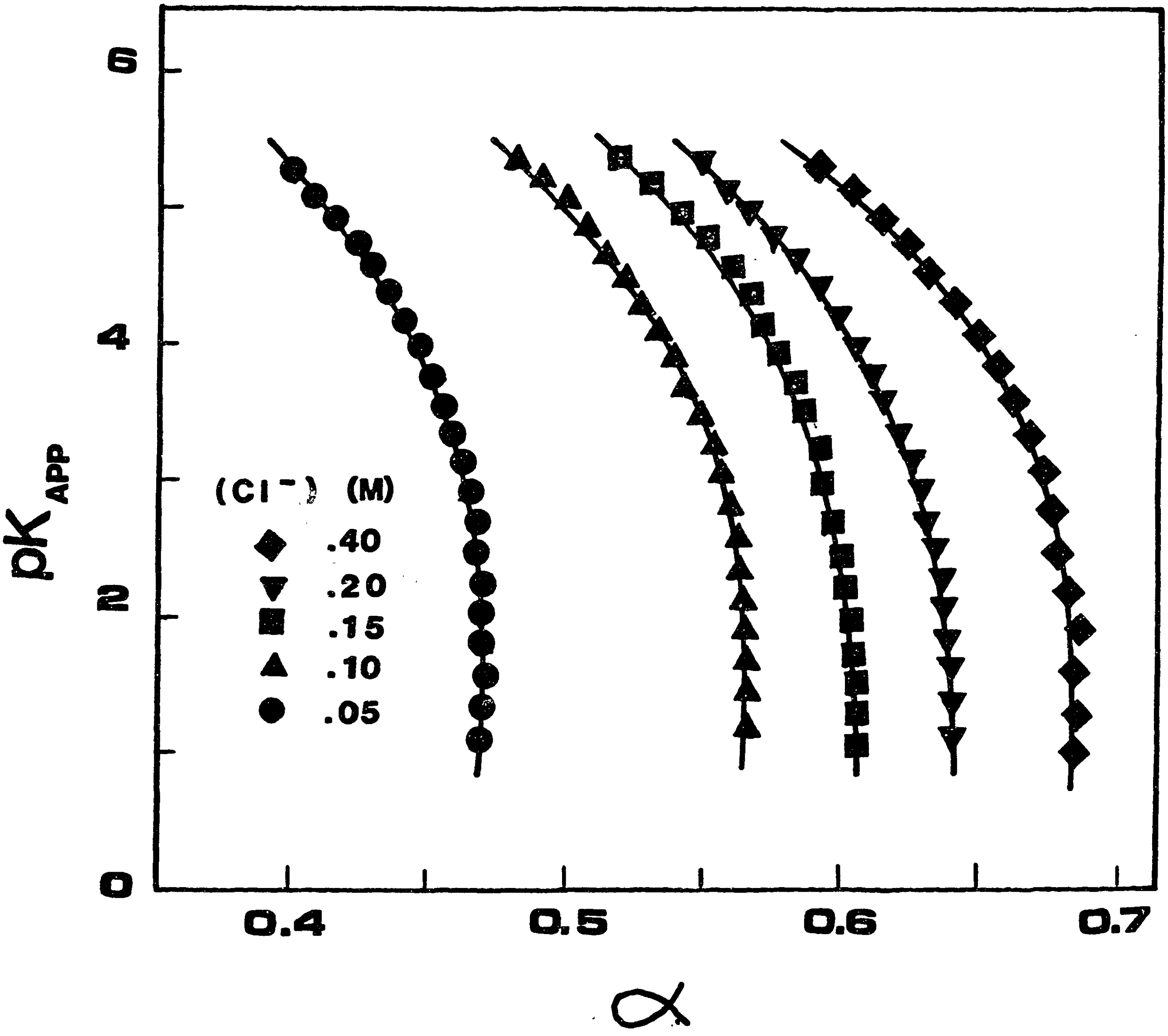


Figure 23. Potentiometric titration for semiconcentrated chitosan solutions (polymer concentration 0.17 g/dl): pKapp versus degree of ionization (α) for different counterion concentrations.



Figue 24. Potentiometric titration for semiconcentrated chitosan solutions (polymer concentration 0.20 g/dl): pKapp versus degree of ionization (α) for different counterion concentrations.

1. The shape of the relationship between pKapp and the degree of ionization remain unchanged and alike to those shown for dilute chitosan concentrations. Moreover, here is indicated that the conformational transition occurring on the chitosan molecules at pKapp around 3.5 remain unaltered even with the presence of intermolecular interactions.

2. The pKapp versus α relationship shifts toward higher values when salt concentration is increased for a given polymer concentration, and to lower values of α when the polymer concentration is increased, at constant ionic strength of the media. This behavior indicates that intermolecular interactions increase the electrostatic repulsive forces, due to an increased overlapping between the solvation sheat around the polyions, therefore a lower electrical charge is needed to overcome the increase in the electrostatic interaction, on the other hand, similar to dilute concentration behavior, an increase in salt concentration reduces the electrostatic repulsive force, thus an increase in the electrical charge is required.

In order to visualize the effect of the ionic strength on the electrostatic nature of the system, I plot the relationship between degree of ionization and the reciprocal of the square root of the salt concentration for pKapp values equal to 5.0 and 2.0 (Figures 25 and 26).

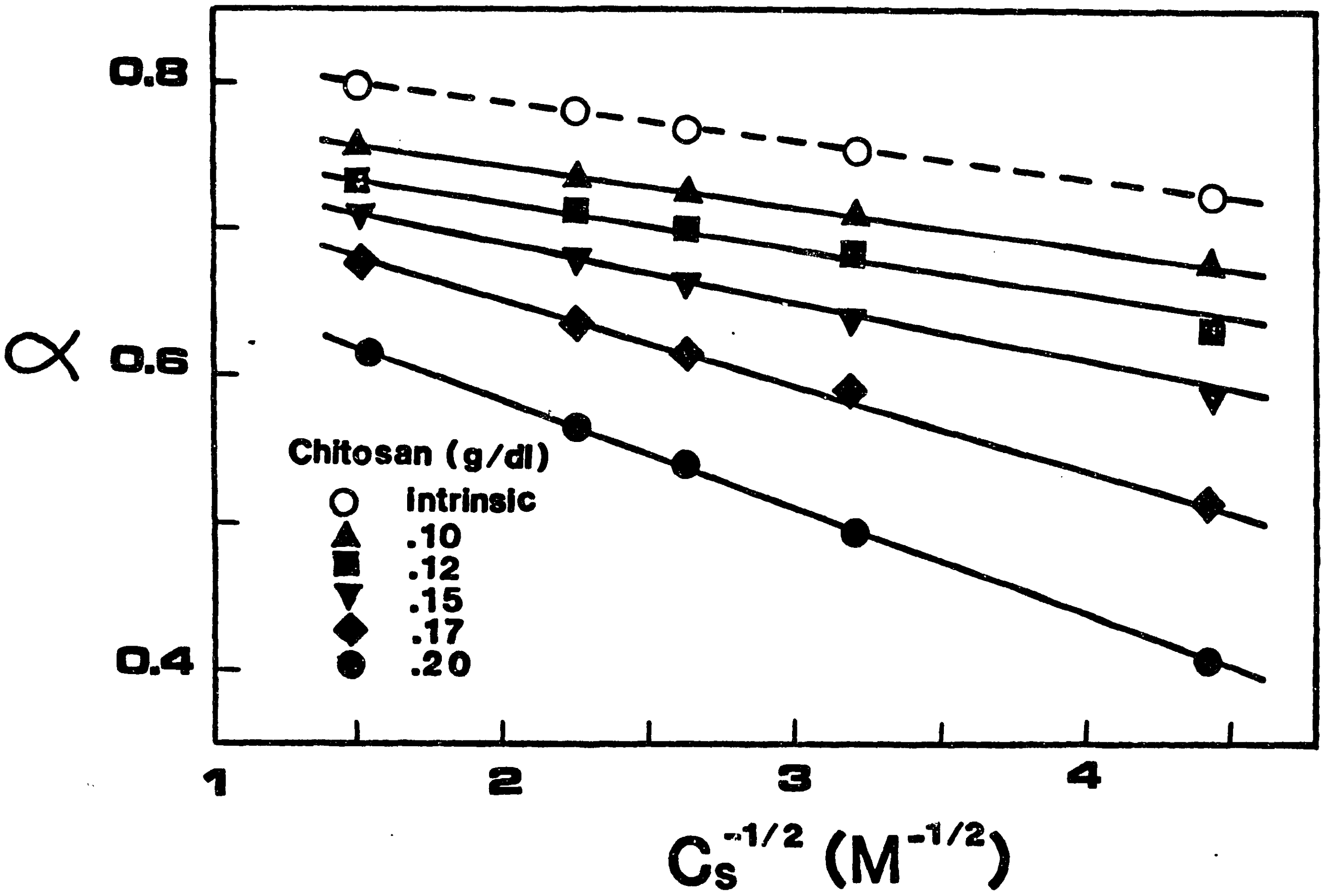


Figure 25. Relationship of the degree of ionization and the reciprocal of the squared root of the counterion concentration for various chitosan solutions. Chitosan pKapp = 5.0 .

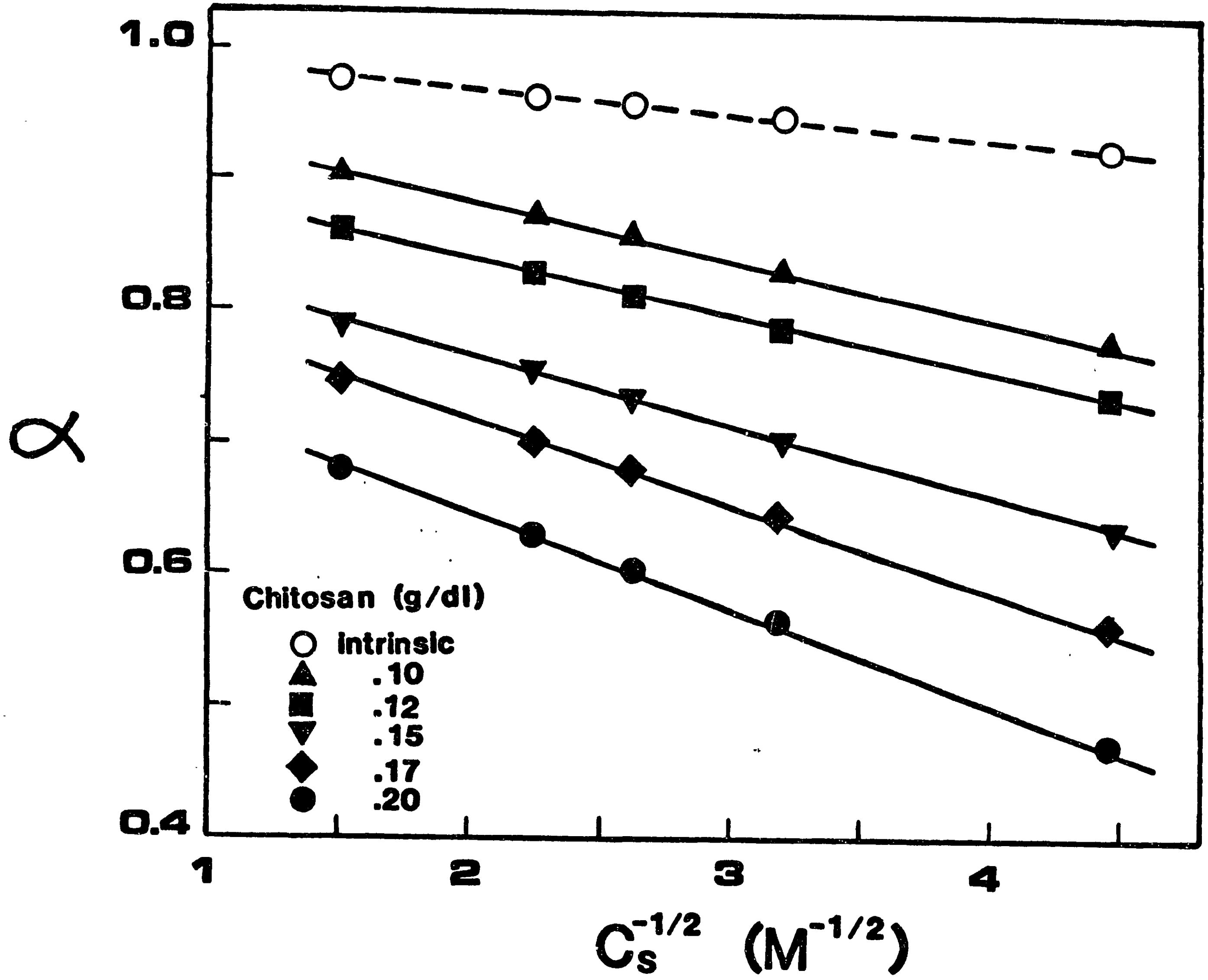


Figure 26. Relationship of the degree of ionization and the reciprocal of the squared root of the counterion concentration for various chitosan solutions.

As expected, these figures show linear relationships between α and $C_s^{-1/2}$ for fixed polymer concentration. However, as the polymer concentration increases, as well as the pKapp values decreases, the slope of the relationship between α and $C_s^{-1/2}$ increases. This tendency indicates the role of increasing the electrostatic repulsive interactions in the overall counterion solvent layer around the chitosan backbone.

Figures 27-30 represent the influence of surface charge on the relationship between degree of ionization and the chitosan concentration as function of the ionic strength. These figures display in all cases, two distinct zones: one zone, at low polyelectrolyte concentration where α is independent of the polyelectrolyte concentration, and a second one where α decreases linearly with increasing chitosan concentration. These results clearly show not only the effect of the overlapping, but the role of pKapp or surface change, on the values of α . In order to evaluate this relationship, α is plotted versus the overlapping parameter $C[\eta]$ for pKapp values equal to 5.0 and 2.0 in Figure 31. The resultant master curve for each of the constant pKapp value indicates that, for a constant surface charge, the overlapping of the solvation layer does control the degree of ionization of the chitosan molecules. However, the two different rela-

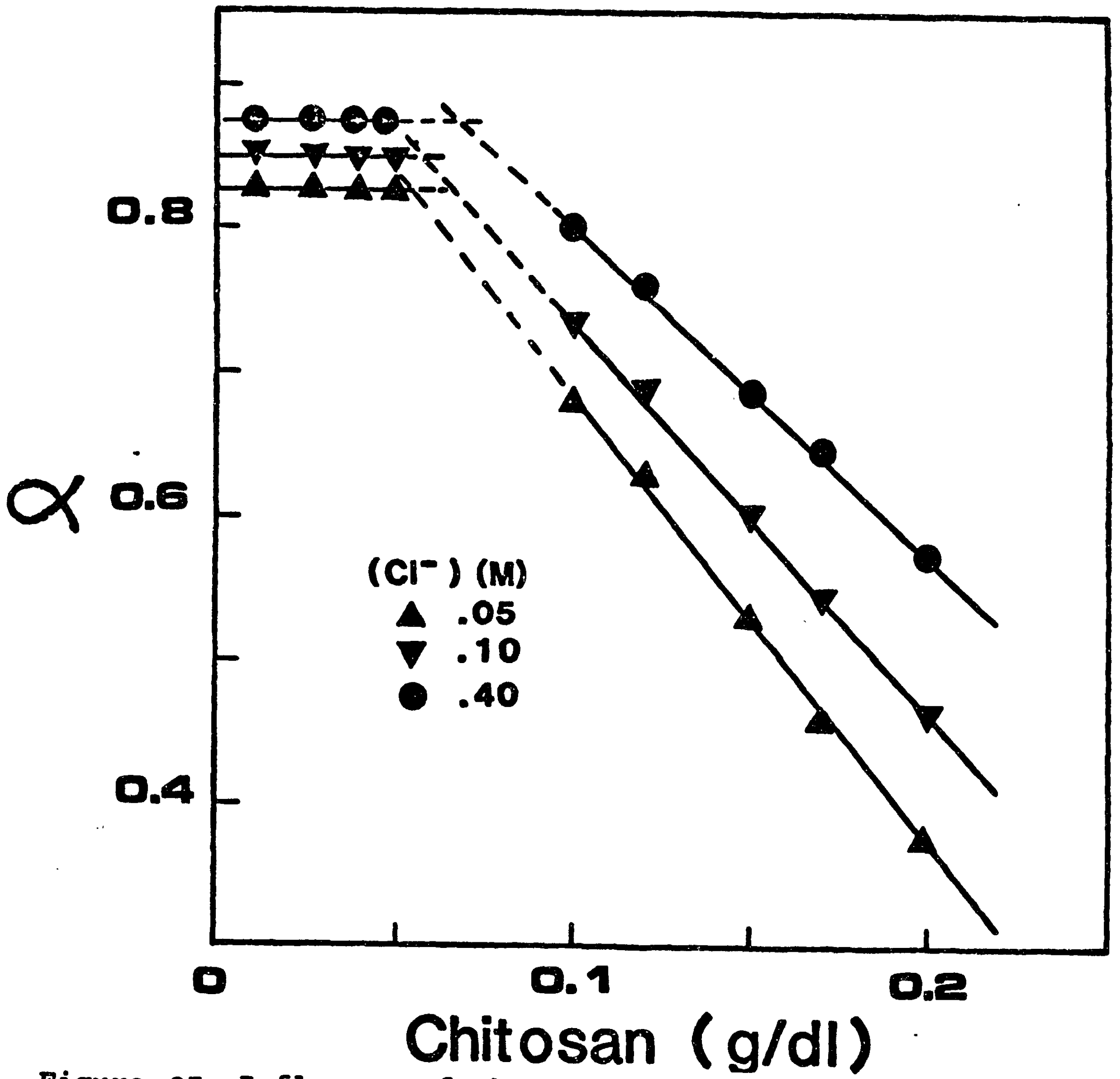


Figure 27. Influence of the counterion concentration on the relationship between the degree of ionization and the chitosan concentration for a constant pKapp value of 2.0 .

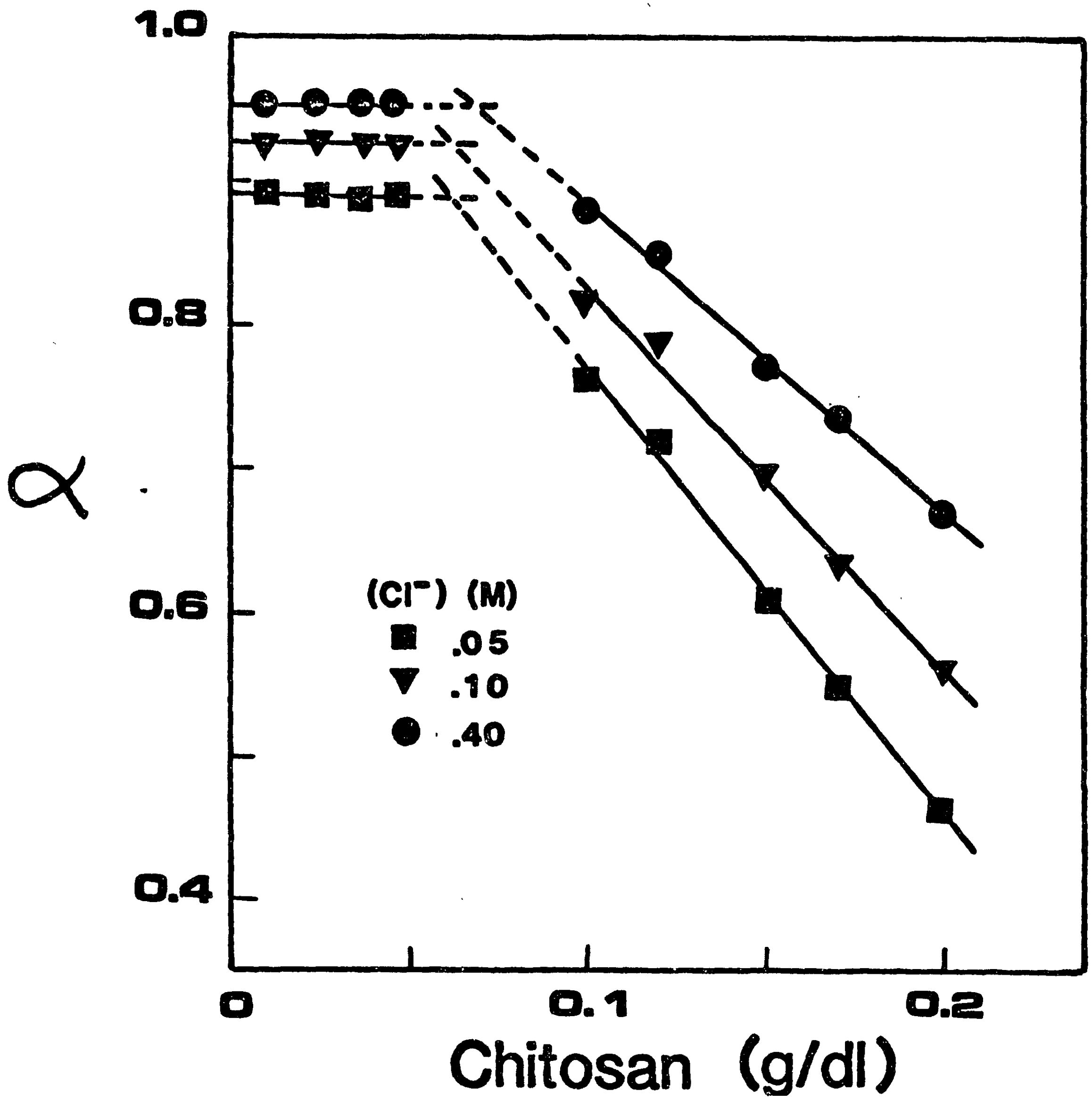


Figure 28. Influence of the counterion concentration on the relationship between the degree of ionization and the chitosan concentration for a constant pKapp value of 3.o .

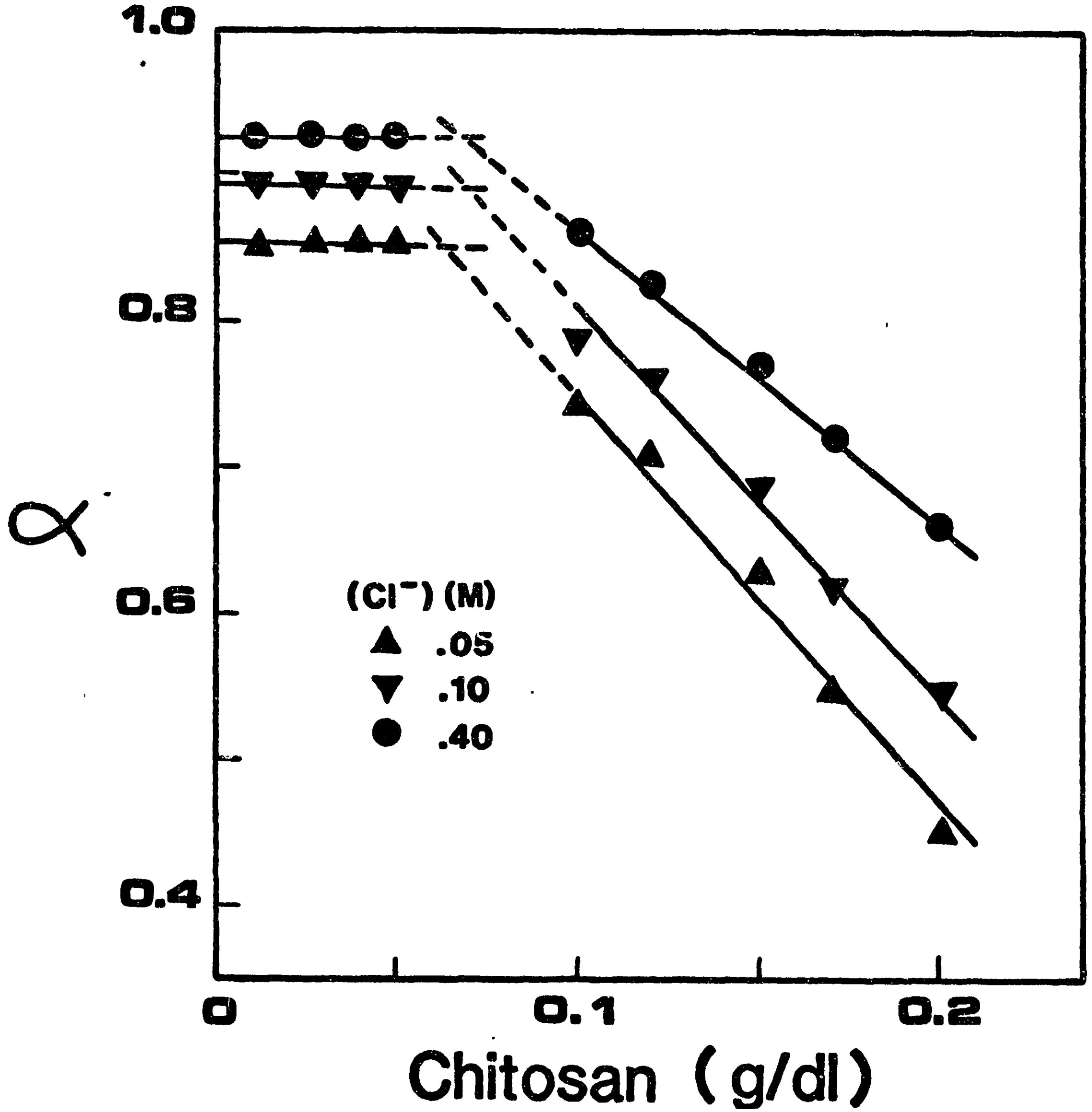


Figure 29. Influence of the counterion concentration on the relationship between the degree of ionization and the chitosan concentration for a constant pKapp value of 4.0 .

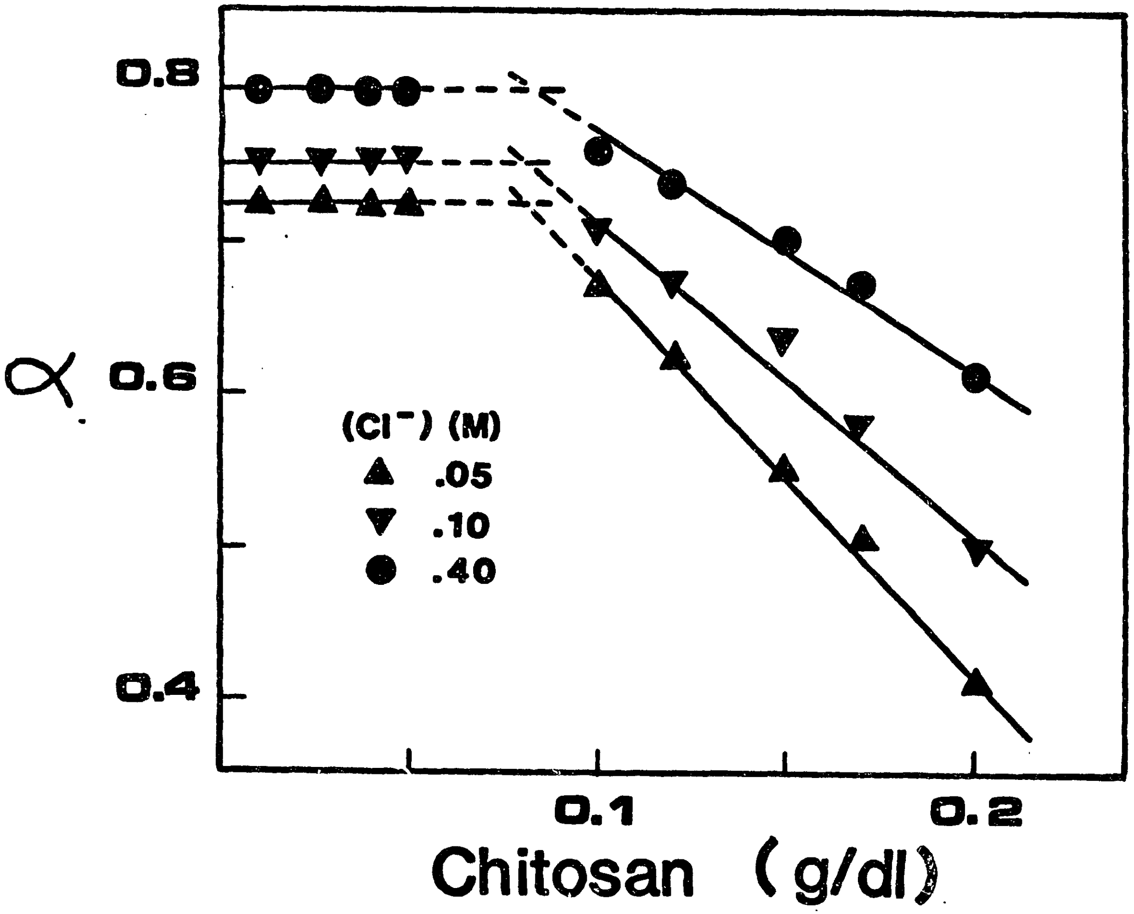


Figure 30. Influence of the counterion concentration on the relationship between the degree of ionization and the chitosan concentration for a constan pKapp value of 5.0 .

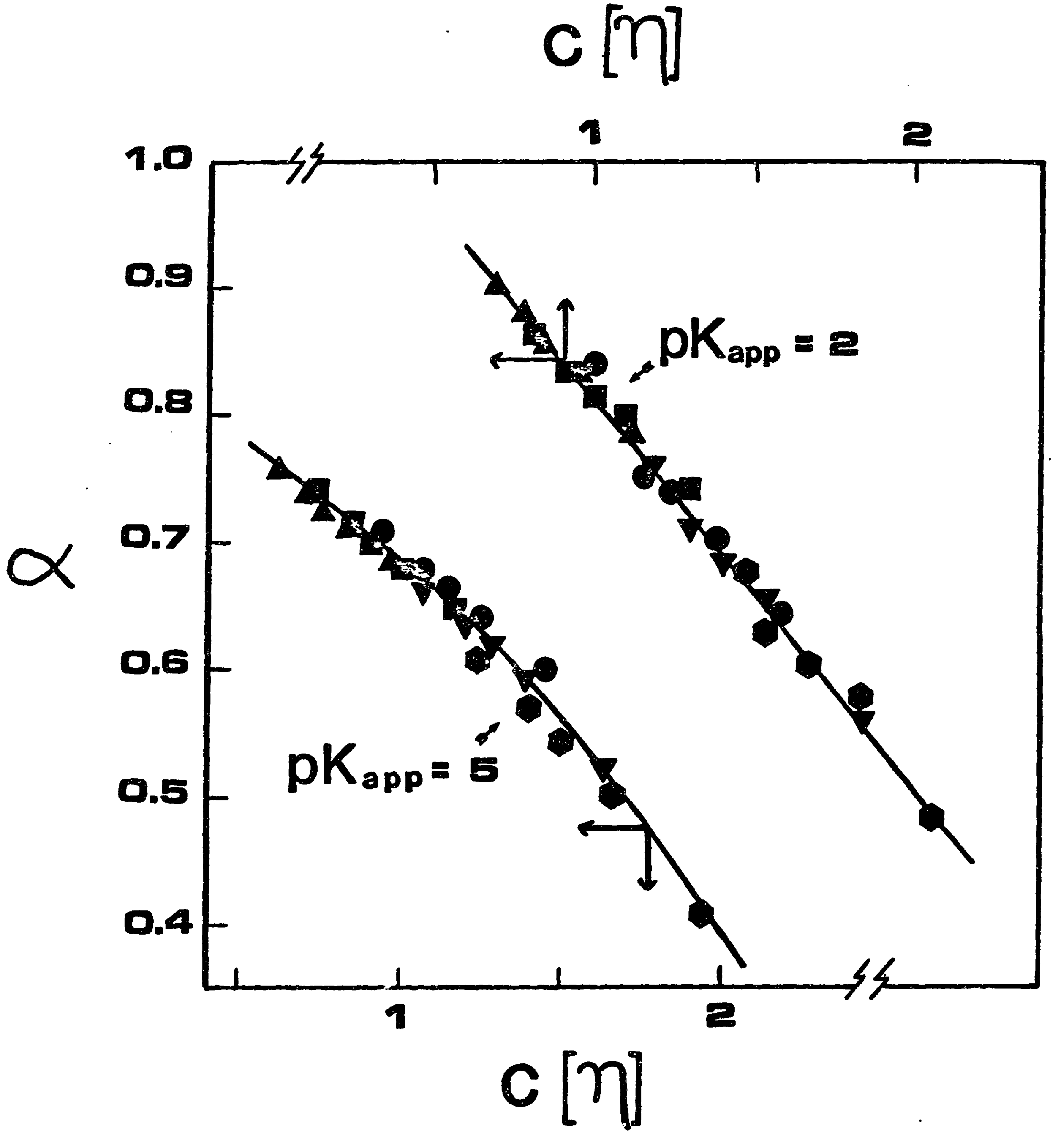


Figure 31. Degree of ionization (α) as affected by the overlapping parameter (C [η]) for constant pKapp value. Chitosan concentration: ▲,0.10 g/dl; ■,0.12 g/dl; ●,0.15 g/dl; ▼,0.17 g/dl; ◆,0.20 g/dl.

tionships obtained when the pKapp is changed indicates the complex relationship between surface charge - degree of ionization - intermolecular interactions in the semiconcentrate regime.

Figures 27 to 30 are useful to estimate the critical chitosan concentration, at the breaking point, which represents the concentration where the overlapping starts to occur. Figure 32 shows the value of the critical overlapping parameter, thus obtained, versus the degree of ionization as affected by surface charge (represented by pKapp) and salt concentration. Therefore, here I present the use of potentiometric titrations as a feasible tool to determine the value of the critical overlapping concentration which is of paramount importance in the rheological behavior of polymer in solutions (Oosawa, 1971; Odijk, 1979; Adler and Freed, 1980; Koene _et al_., 1973).

4.3 Counterion Activity Via Cationic Polyelectrolyte Solution

Chitosan is a hydrophilic polyelectrolyte that is insoluble in pure water, but soluble in dilute acid conditions (Muzzarelli, 1973). The polyelectrolytic behavior of chitosan has been demonstrated in solution, as well as in films (Kienzle-Sterzer _et al_., 1982a,b,c). Also, the

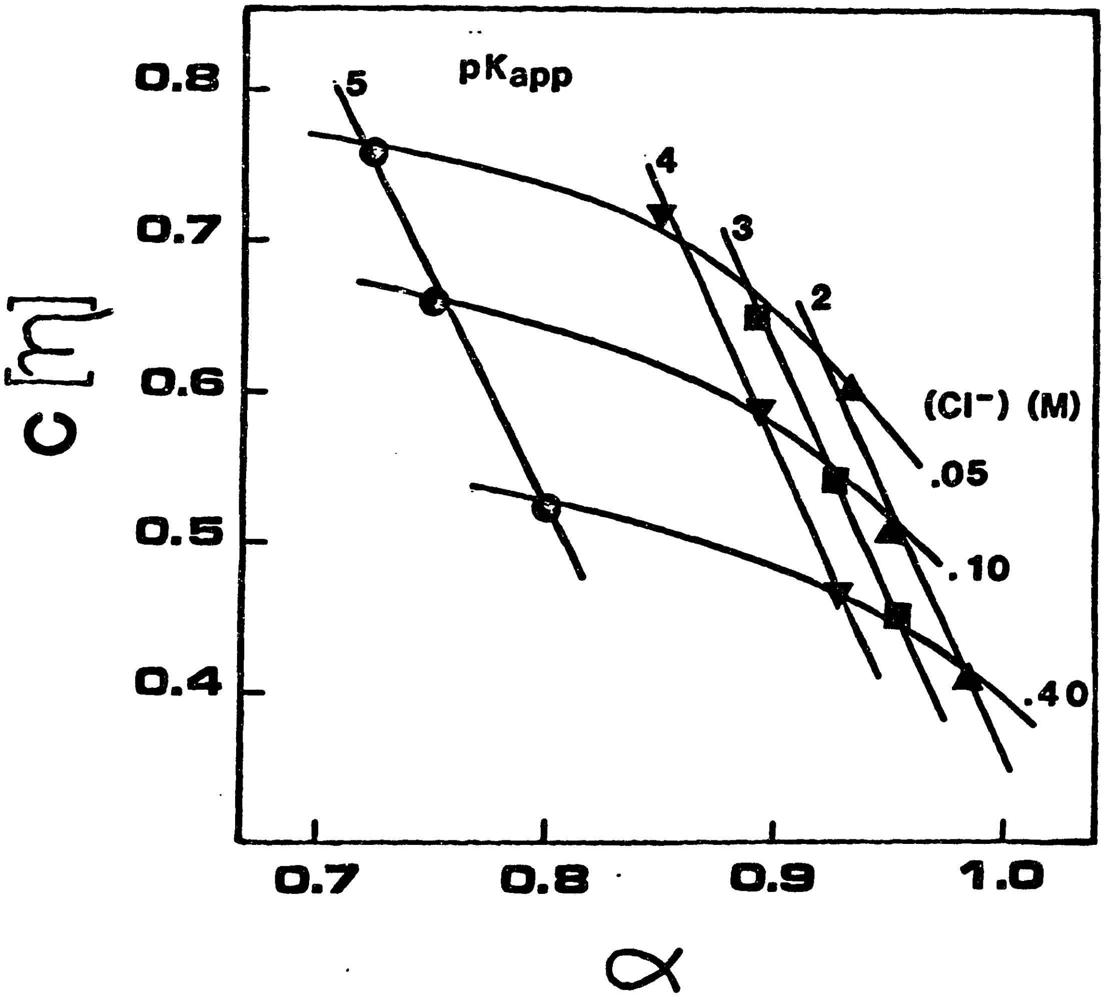


Figure 32. Effect of the degree of ionization, counterion concentration and surface charge density (represented by pKapp) on the value of the critical overlapping parameter of chitosan in solution.

effect of solution conditions, pH and ionic strength on the hydrodynamic behavior and charge density of chitosan molecules has been examined (Kienzle-Sterzer *et al.*, 1983). The results of these studies indicated that both the intrinsic viscosity and the degree of ionization remain constant below pH 4.5 independently of the ionic strength. In this study, in order to analyze the effect of polymer and counterion concentrations, counterion activity coefficients in chitosan solutions (5×10^{-3} mono M to 11×10^{-3} mono M) are studied at ionic strengths between 0.05 M and 0.40 M, and pH values below 4.5. Relative chloride ion activity coefficient, represented as the ratio of the chloride ion activity coefficient in the polyion solution (γ_{Cl^-}) to the correspondent chloride ion activity coefficient in polyelectrolyte free solution ($\gamma^o_{Cl^-}$), is shown in Figure 33. Figure 33 shows that the relative counterion activity coefficient ($\gamma_{Cl^-}/\gamma^o_{Cl^-}$) decreases with decreasing the ionic strength, but is unaffected by the polymer concentration.

The high value of the relative counterion activity coefficients ($\gamma_{Cl^-}/ \gamma^o_{Cl^-}$) indicates enhanced shielding of the charges on the chitosan backbone due to the relative high counterion concentration. Meanwhile, the fact that the counterion activity coefficient is independent of the

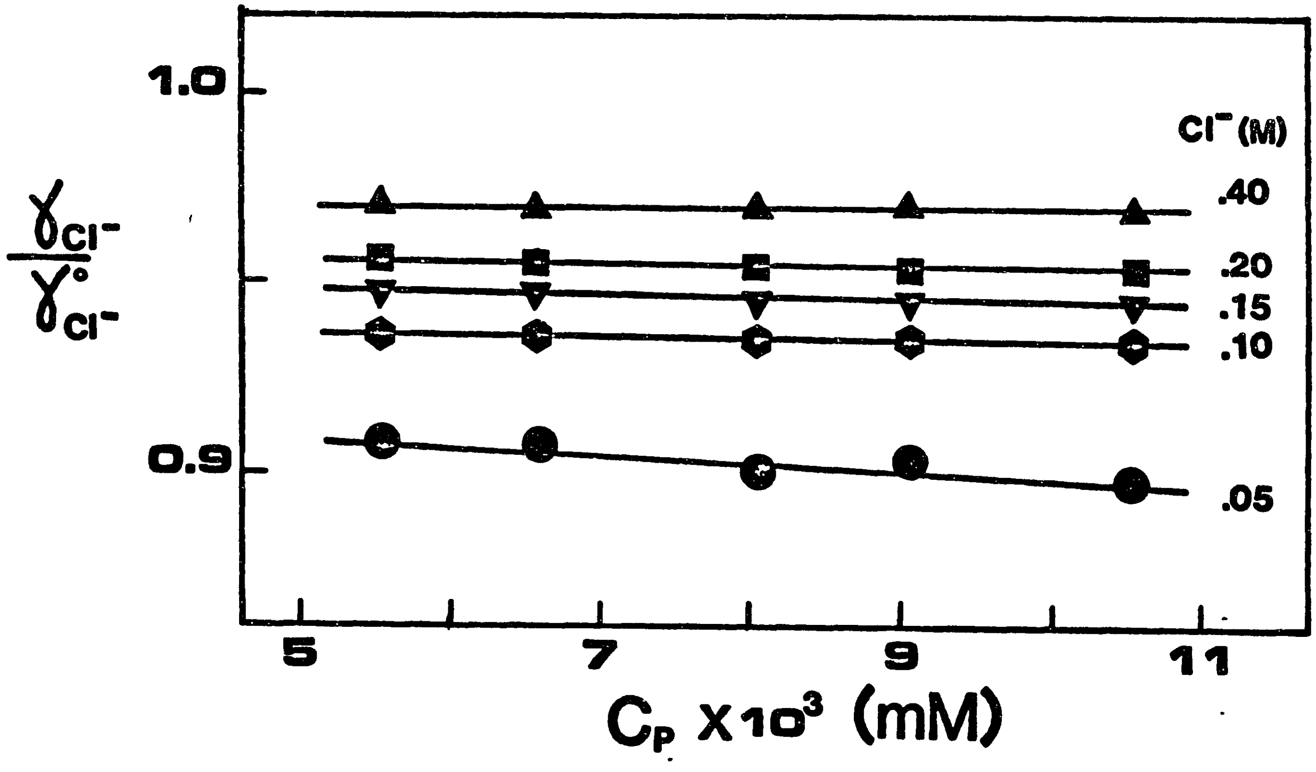


Figure 33. Relative counterion activity coefficient in semi-concentrated chitosan solutions as affected by both polyelectrolyte concentration and counterion concentration.

polymer concentration indicates that the shielding effect is sufficiently extensive. However, with ionic strength of the media significantly decreased, the relative counterion activity coefficient decreases. This confirms that at constant polymer concentration, an increase in the counterions results in an increase in the interactions between counterion-polyion. Similar behavior in the counterion activity coefficient has been observed for charged heparin (Ascoli et al., 1961), dextran phosphate (Gekko and Noguchi, 1978) and for λ- and κ-carraginan at high salt concentrations (Podlas and Ander, 1969).

The counterion activity coefficient approximates the fraction of free counterions in polymer solutions (Hayakawa and Kwak, 1982; Tan and Marans, 1976). Therefore, I calculated "n," which is defined as the bound chloride ion concentration divided by the monomolar chitosan concentration, by the expression:

$$n = \frac{\Delta C_{cl^-}}{C_p} \qquad (91)$$

C_p is the polymer concentration, and ΔC_{Cl-} represents the bound chloride concentration obtained by:

$$\Delta C_{cl^-} = C_t - C_t\, \gamma_{cl^-} / \gamma^o_{cl^-} \qquad (95)$$

where C_t is the total counterion concentration.

Figure 34 shows the relationship between "n" counterions and chitosan concentration. These straight lines have an inflection point at approximately 0.13 M chloride concentration. The change in the slope of the straight lines may be due to the transition of chitosan molecules from an extended configuration at low ionic strength to a globular compact shape at high ionic strength. This transition effect was also indicated in the earlier evaluation of the critical counterion concentration (0.10 - 0.15 M Cl^- conc.) in the relationship between the theoretical and experimental electrostatic contribution to the excluded volume.

The similarity in the shape of the relationship between "n" and the counterion concentration for different chitosan concentrations suggests that the ion binding mechanism remains unaltered with the polyelectrolyte concentration.

Figure 34 shows that the number of bound counterions per monomer increases with increasing counterion concentration and decreases with increasing polymer concentration. This indicates the repulsive effect on the bound counterion sheath around the polyion molecules as intermolecular interactions start to appear.

In order to study the effect of both the degree of ionization (α) and the chain overlapping on the number of

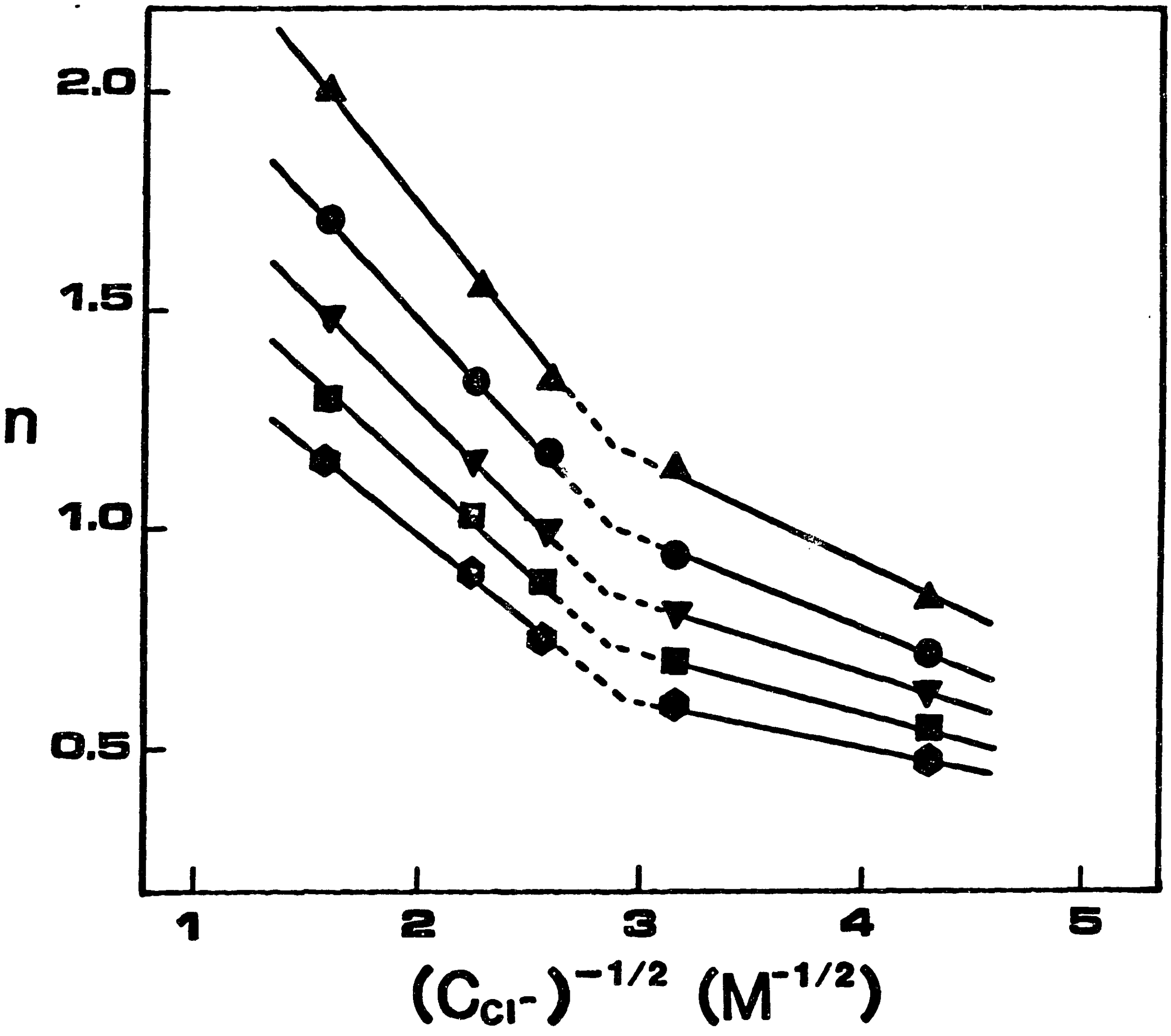


Figure 34. Effect of counterion concentration on the number of counterions bound per glucosamine residue "n" for a given chitosan concentration. Chitosan concentration: ⬢,10.58 x 10^{-3} mM; ■,9.00 x 10^{-3} mM; ▼,8.00 x 10^{-3} mM; ●,6.50 x 10^{-3} mM; ▲,5.60 x 10^{-3} mM.

bound counterions, "n" is plotted against α , and the overlapping parameter, represented by the term $C[\eta]$, in Figure 35. "n" increases with α , independent of the chitosan and salt concentration, due to an increase in the electrostatic interaction between polyion and counterions when the number of charged points are increased. On the other hand, a reduction in "n" is observed when the overlapping parameter is increased. This effect is due to an increase in the interaction between counterions bound to the polyion molecules, which in turn allows a distribution of the counterions around the charged points on the polymer backbone.

In recent years, attention has been given to the development of theoretical treatments in order to predict the activity coefficients of ions in mixtures of polyelectrolytes and simple electrolytes (Manning, 1969, 1977, 1978; Wells, 1973; Rinaudo and Milas, 1976; Iwasa and Kwak, 1977; Bloys and Moonen, 1978; Kowblansky and Zema, 1981a,b; Yaryer _et al_., 1983). It has been found that counterion condensation occurs, when the charge density of the polyion (ξ) is greater than that of the critical charge density ($\xi_c = 1$ for monovalent counterion), until the polyion charge density is equal to the critical one.

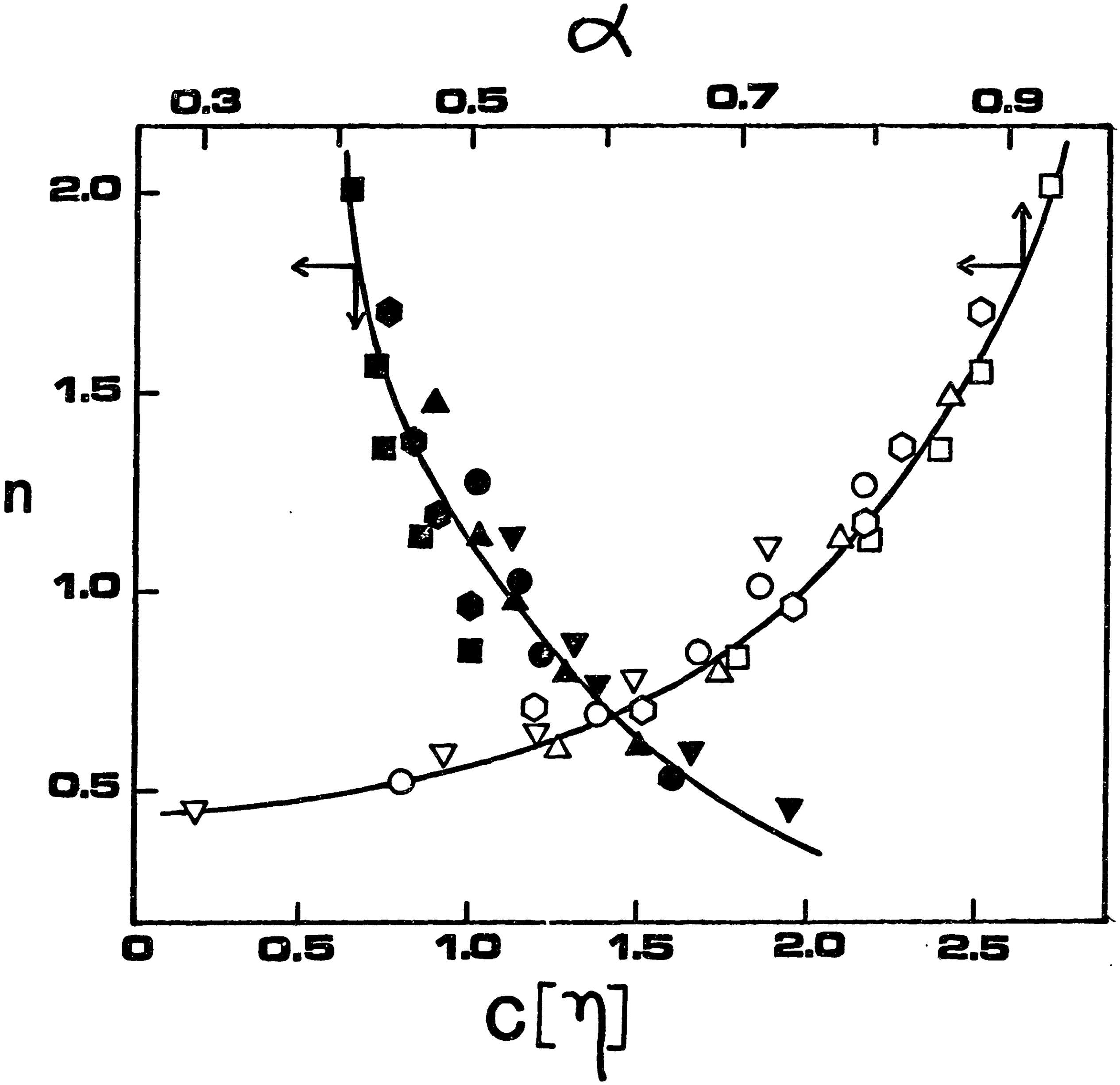


Figure 35. Number of counterions bound per monomer residue, "n", as affected by both the overlapping parameter (C[η]) and the degree of ionization (α). Simbols represent as Figure 33.

According to the Manning theory of polyelectrolyte solution, the counterion activity coefficient can be represented by (Manning, 1969):

$$\gamma_1 = \exp\left[-\frac{0.5\ \xi\ X}{X + 2}\right] \tag{96}$$

where X is the concentration ratio of polyelectrolyte to simple salt.

Similarly, Iwasa et al. (1977) predicts a counterion activity coefficient for monovalent counterions, by the equation:

$$\gamma_1 = \frac{\xi^{-1}\ X + 1}{X + 1}\ \exp\left[\frac{-\ 0.5\ \xi^{-1}\ X}{\xi^{-1}\ X + 2}\right] \tag{97}$$

for $\xi \geq 1$.

Figure 36 shows the relationship between $\gamma_{Cl-}/\gamma^{0}_{Cl}$ and X for the experimental results which are compared with the theoretical curves obtained from both Manning's and Iwasa's approach with $\xi = \xi_c = 1$. The values obtained from both that of Manning's and Iwasa's theories are

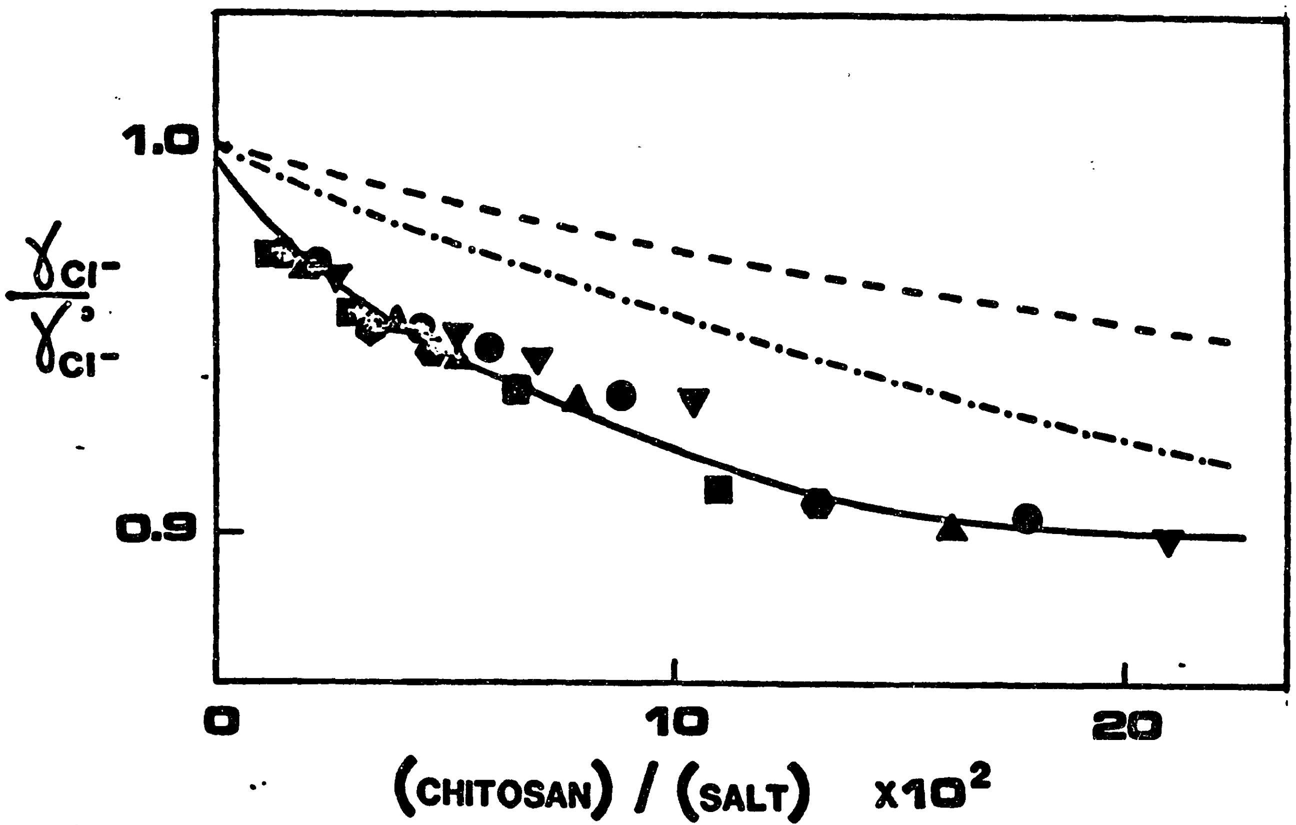


Figure 36. Relative counterion activity coefficient in semi-concentrated chitosan solution against the dimensionless concentration: (Chitosan)/(salt).
---- : Theoretical behaviour according to Manning's theory (Manning,1969); -.-.-.- : Theoretical behaviour accoerding to Iwasa et el.'s approach (Iwasa et al.,1977), both were calculated for $\xi = \xi_c = 1$.
Simbols represent as Figure 33.

higher than the experimental values of the relative counterion activity.

Since both theories deal with dilute polymer solutions, the difference between these and the experimental values should represent the intermolecular interactions since the experiment employed overlapping parameters in the range 0.5 to 2.0. In this region, the increase in intermolecular interaction will perturb the counterion sheath around polyion molecules, therefore decrease the number of counterions bound per monomer.

4.4 Diffusion of Small Molecules in Polyelectrolyte Solutions

The self-diffusion coefficient of glucose in semi-concentrated polymer solutions (chitosan concentrations between 0.05 g/dl and 0.20 g/dl) as affected by both the pH and the ionic strength of the media has been evaluated. Figures 37-39 show the effect of chitosan concentration on the relative self-diffusion coefficient of glucose (D/D_o) for counterion concentrations ranging from 0.10 M Cl^- to 1.0 M Cl^- and pH values of 3,4,5 and 6 respectively. The following features are shown in these figures:

1. A linear relationship between D/D_o and the polyelectrolyte concentration for all the selec-

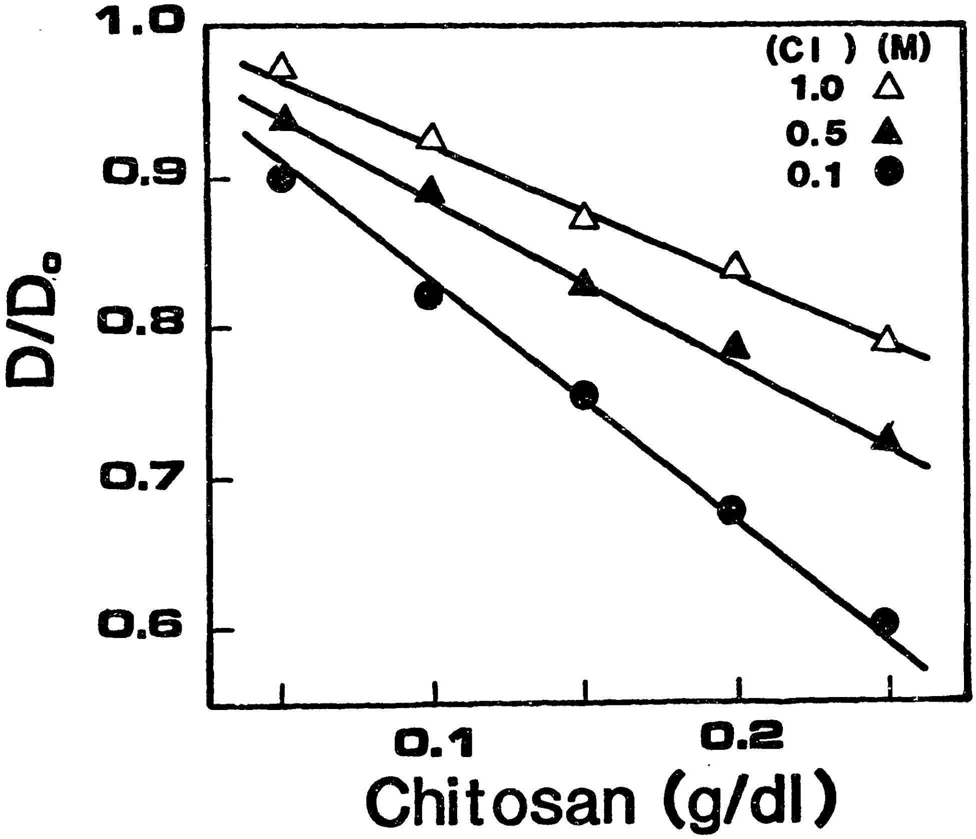


Figure 37. Relative diffusion coefficient of glucose in semi-concentrated chitosan solutions as affected by chitosan concentration and ionic strength of the media for pH values of 3.0 and 4.0 .

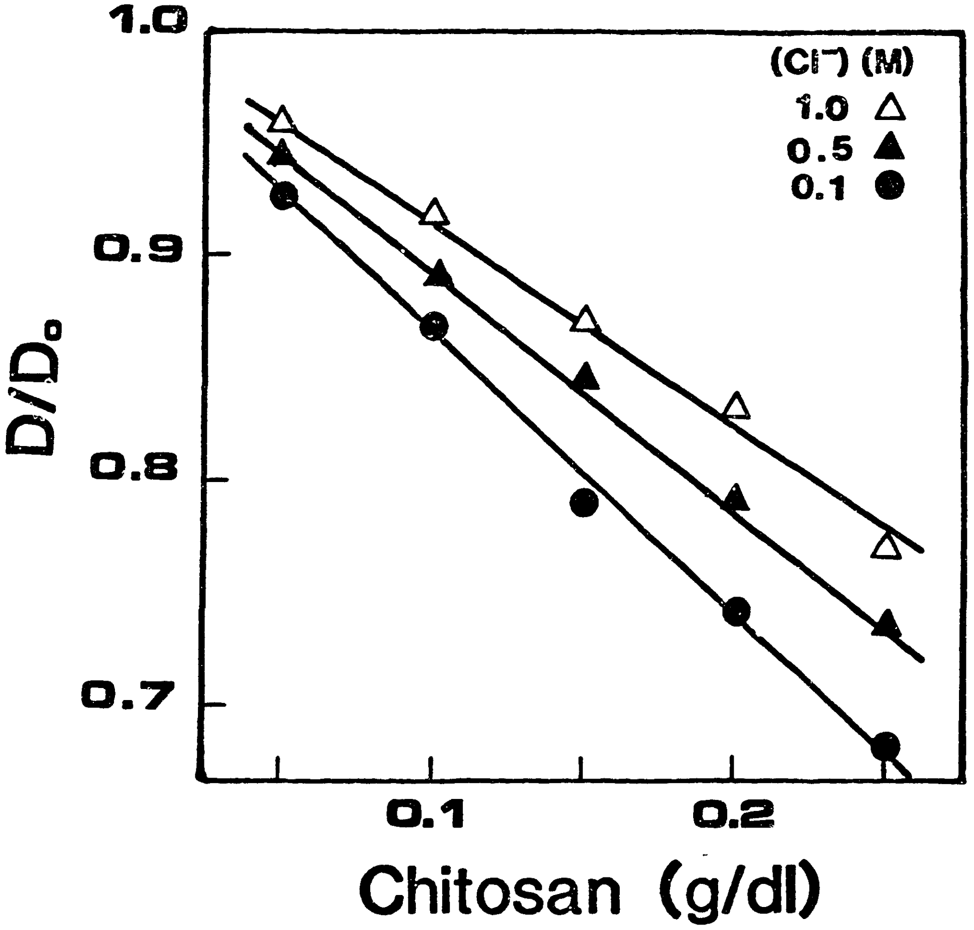


Figure 38. Relative diffusion coefficient of glucose in semi-concentrated chitosan solutions as affected by chitosan concentration and ionic strength of the media for pH value of 5.0 .

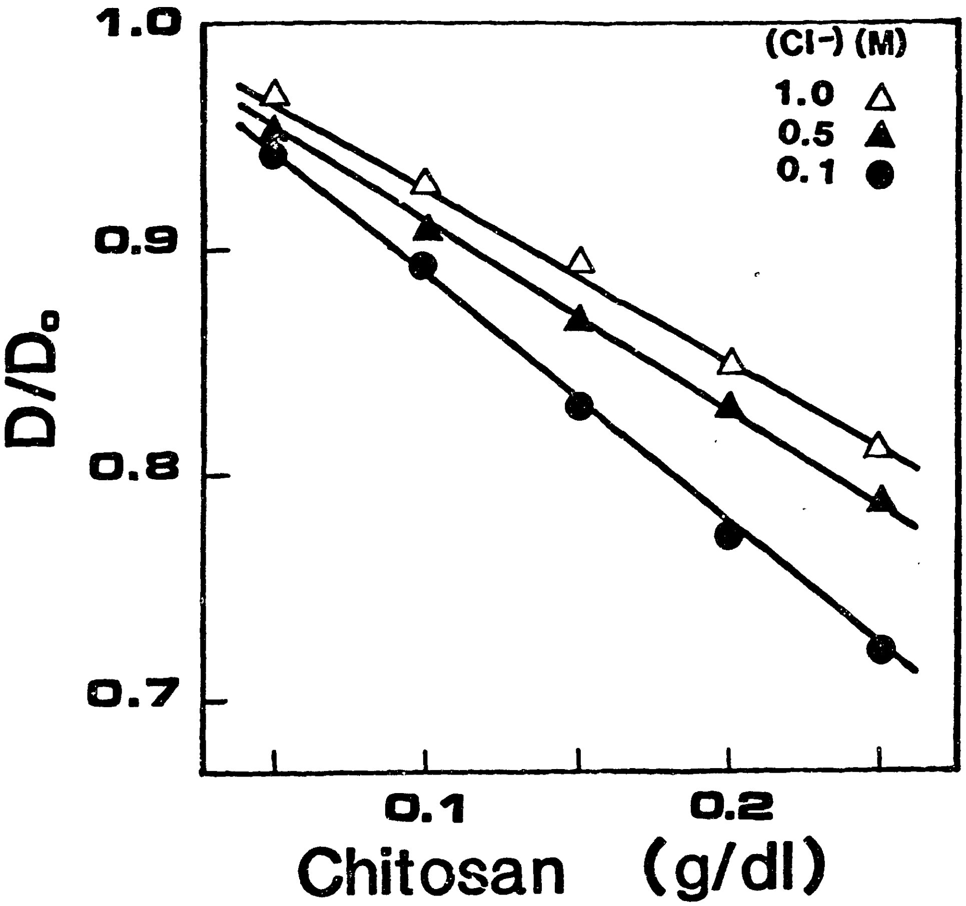


Figure 39. Relative diffusion coefficient of glucose in semi-concentrated chitosan solutions as affected by chitosan concentration and ionic strength of the media for pH value of 6.0 .

ted pH values and counterion concentrations.

2. The ratio D/D_o decreases with increasing polymer concentration.
3. For a given polyelectrolyte concentration, the ratio D/D_o increases as the counterion concentration increases.
4. For a given chitosan concentration and counterion concentration, D/D_c increases with increase in the pH of the solution.

These results are explained by the context of the obstruction theory developed by Wang (Wang, 1954), where the diffusion data are related to the concentration of the long chain polymer molecule by the equation:

$$\frac{D}{D_o} = 1 - f(\bar{v})c \tag{98}$$

where c = polyelectrolyte concentration

$f(\bar{v})$ = function of the polyelectrolyte's specific volume

$$f(\bar{v}) = f(T, v_h, H) \tag{99}$$

where T = a parameter related to the geometric shape of the polymer molecule.

$\bar{v}_h$ = partial specific volume of the polymer.

H = the "hydration term" defined as the mass of solvent bound per unit mass of polymer.

According to the obstruction theory, the polyelectrolyte solution is envisioned as a composite of two distinct regions: a polymer rich region consisting of the polymer-bound solvent, and a pure solvent region. Equation 98 predicts, first of all, that D/D_o is always lower than unity, and secondly, the D/D_o must decrease with increasing the polymer concentration for a selected medium condition. The validity of this relationship has been experimentally proved for several polyelectrolytes (Ogston and Sherman, 1961; Farag _et al_., 1976a,b; Namikawa _et al_., 1977; Nystrom and Roots, 1980; Ishida _et al_., 1981).

Moreover, my experimental results cast additional information with respect to the behavior of the system under examination:

1. From the linear relationship between D/D_o and chitosan concentration, at constant pH and ionic strength, it can be concluded that $f(\bar{v})$ is independent of polymer concentration. The insensitivity of the polymer specific volume toward polymer concentration can be related to a compensating effect between the increase in the overlapping of the macromolecular domains, as polyelectrolyte

concentration increases, and the reorganization of the surrounding solvation layers, as a result of increasing electrostatic repulsive forces when intermolecular interactons start to appear. This balance, in turn, made the diffusion pattern of the diffundant molecule remain unchanged with changes in the polyelectrolyte concentration. The independency of the partial specific volume with polymer concentration has been observed in other polyelectrolytes in solution, for instance carboxymethylcellulose with a degree of substitution between 0.98 and 2.65 (Tondre and Zana, 1972); dextran and dextran derivatives (Gekko and Noguchi, 1974); polyacrylic acid and polyacrylamide aqueous solutions (Phanibhusan and Kalidas, 1970) among others. In this sense, I can postulate that in semiconcentrated polyelectrolyte solutions a balance exists between the overlapping of the hydration shells on the polyions and the number of counterions bound per monomer as polymer concentrations increase. This relationship between the number of counterions bound per glucosamine monomer and chitosan concentration has been earlier discussed when evaluating the relative counterion activity coefficient.

2. The increase in D/D_o by both increasing the counterion concentration for a selected pH value and chitosan concentration, as well as, by increasing the pH values for

fixed counterion and chitosan concentration, indicates the contribution of the molecular volume in the transport properties of the polyelectrolyte solution. Thus, at constant polymer concentration, the hydrodynamic volume of chitosan in solution increases with either decreasing the counterion concentration and pH. This phenomenon is also observed in the previous studies in which the chitosan intrinsic viscosity ([η]) decreases with increasing the chloride concentration and pH (Kienzle-Sterzer *et al.*, 1983; Rodriguez-Sanchez *et al.*, 1982).

In order to quantify the effect of pH and counterion concentration on the transport properties of small molecules through the polyelectrolyte solution, the relative self-diffusion coefficient of glucose D/D_o is plotted against the overlapping parameter, represented by the term $C[\eta]$ where C is the chitosan concentration in g/dl and [η] is the intrinsic viscosity in dl/g. In figures 40-42, the ratio D/D_o is plotted as a function of $C[\eta]$ for all the experimental conditions. The most important features from the figures 40 to 42 are that D/D_o decreases linearly with increasing the overlapping parameter and that this linear relationship is maintained for all the counterion concentrations and pH values. Thus, these results agree with the two region model for polymer solutions used by Wang (Wang, 1954). Similar phenomenological relationship has

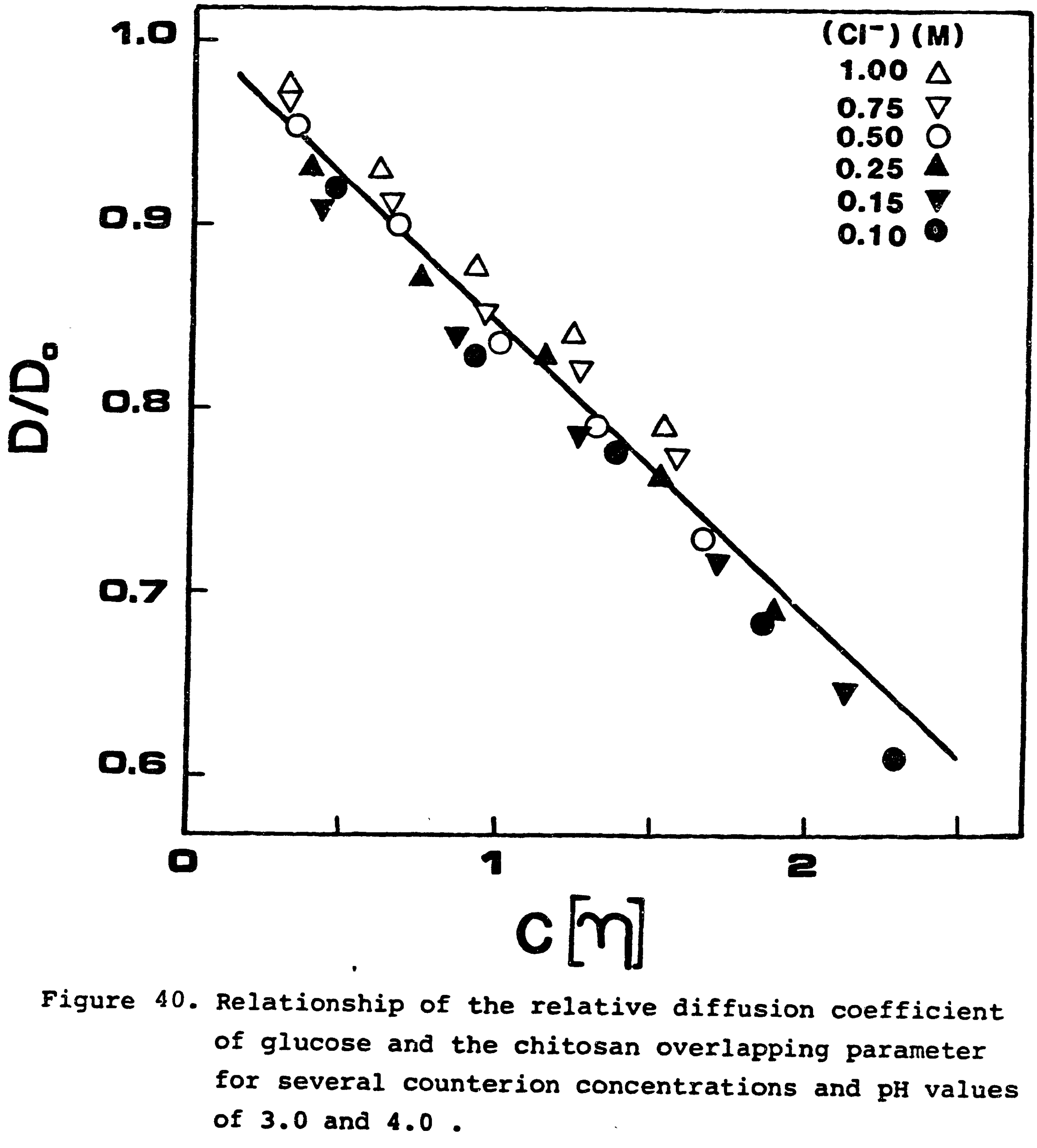


Figure 40. Relationship of the relative diffusion coefficient of glucose and the chitosan overlapping parameter for several counterion concentrations and pH values of 3.0 and 4.0 .

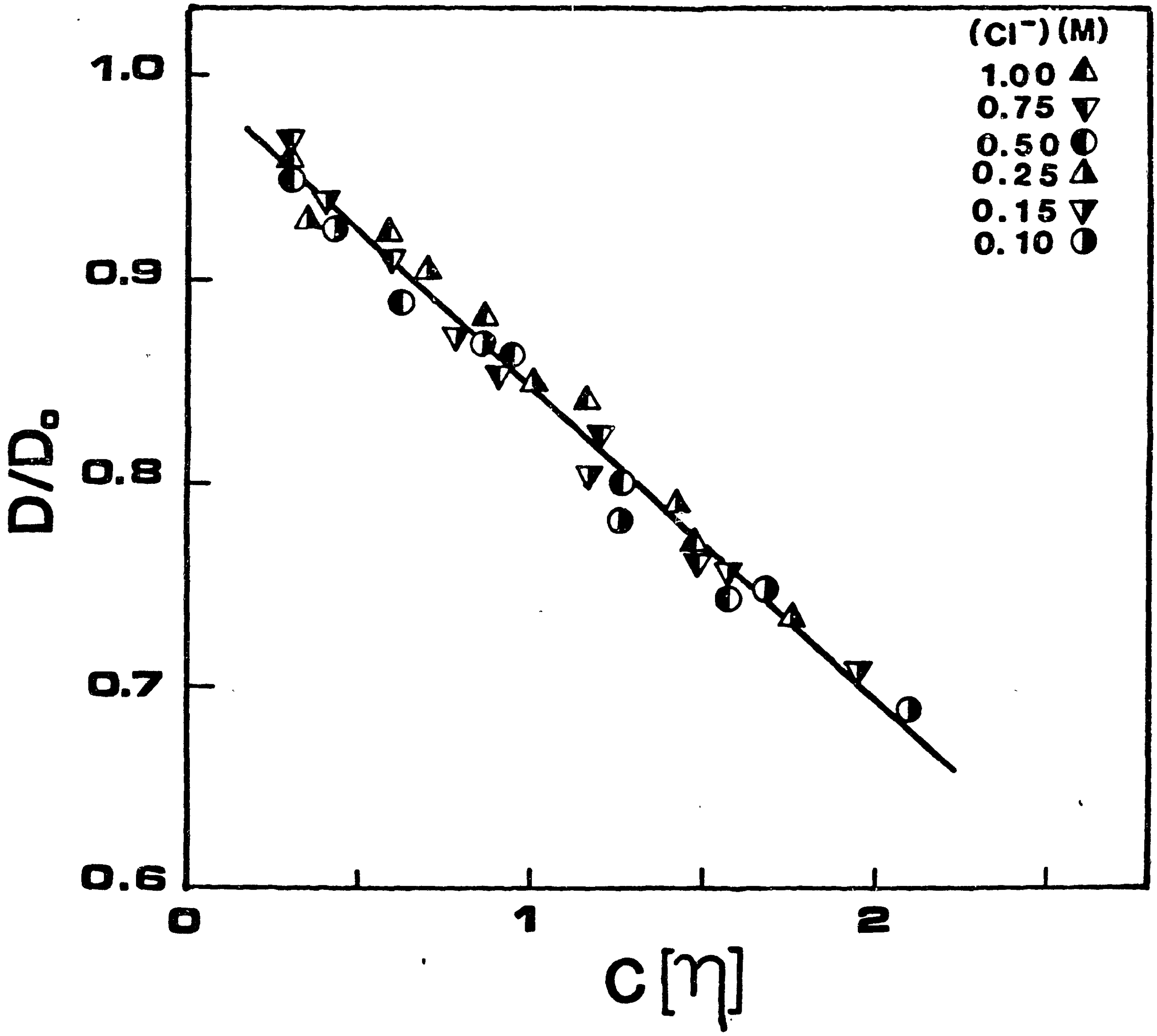


Figure 41. Relationship of the relative diffusion coefficient of glucose and the chitosan overlapping parameter for several counterion concentrations and pH value of 5.0 .

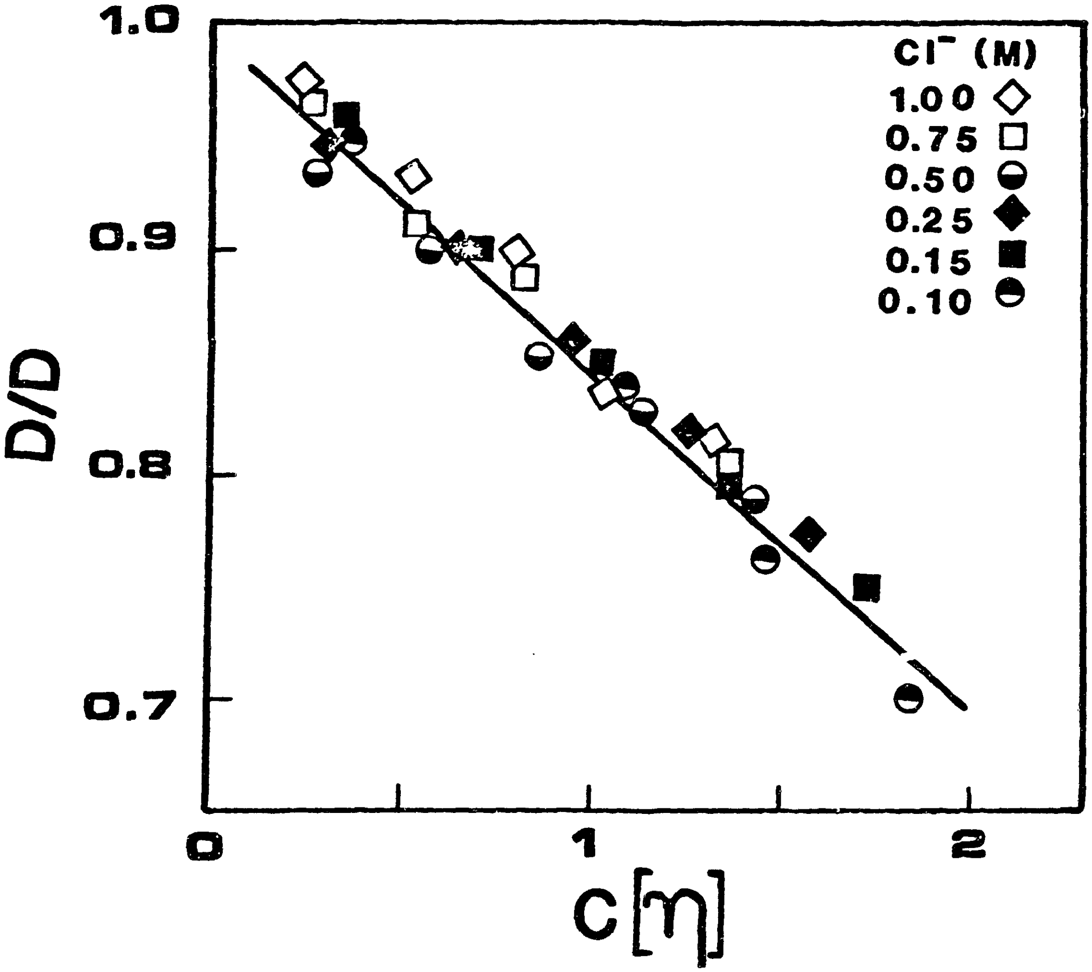


Figure 42. Relationship of the relative diffusion coefficient of glucose and the chitosan overlapping parameter for several counterion concentrations and pH value of 6.0 .

been elsewhere reported between solvent self-diffusion in polystyrene solutions and the polystyrene volume fraction (Nystrom _et_ _al_., 1982). Thus, it can be concluded that in the semiconcentrated region the polyelectrolyte molecules create a "long-range interacting system" which gains importance as the overlapping parameter increases.

It has been established that the diffusion coefficient of small molecules in a solution relates to the reciprocal of the viscosity of the media (Green, 1952; Rha, 1975). Figure 43 shows the linear relationship between the logarithm of the self-diffusion coefficient and the logarithm of the relative viscosity. A linear relationship is obtained for each value of the intrinsic viscosity. Therefore, the macroscopic viscosity, represented by the relative viscosity (η_r) cannot sufficiently describe the resistance to molecular motion, and thus, the molecular hydrodynamic volume must be considered. This effect can be observed in Figure 44 where the relationship between D/D_o and intrinsic viscosity is plotted for selected values of the macroscopic viscosity. Figure 44 shows that the role of the intrinsic viscosity on the relative self-diffusion coefficient of glucose increases with increasing the overall viscosity. This phenomenon suggests the importance of the polyelectrolyte hydrodynamic volume when the overlapping of the hydrodynamic do-

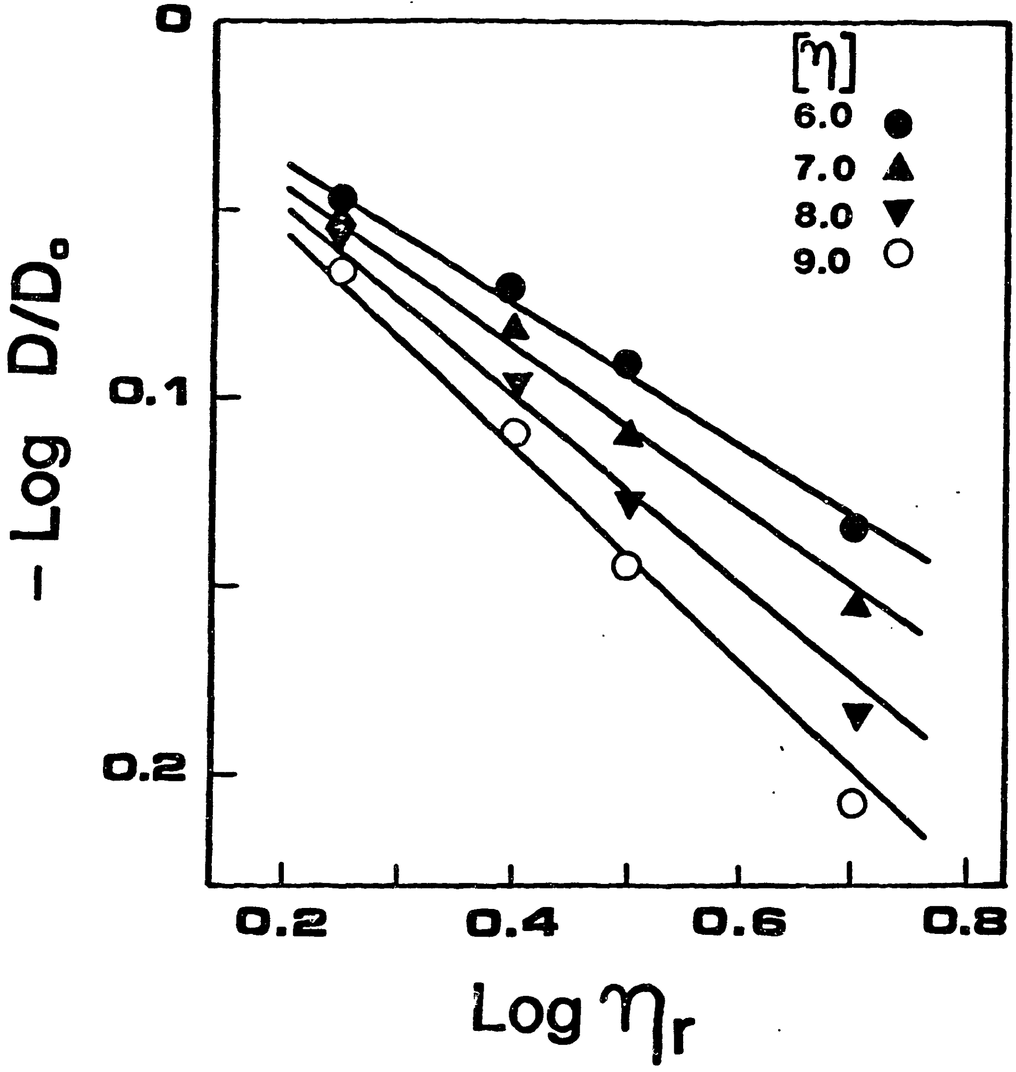


Figure 43. Logarithmic relationship of the relative diffusion coefficient of glucose and the macroscopic viscosity (represented by the relative viscosity "η_r") as a function of the chitosan intrinsic viscosity.

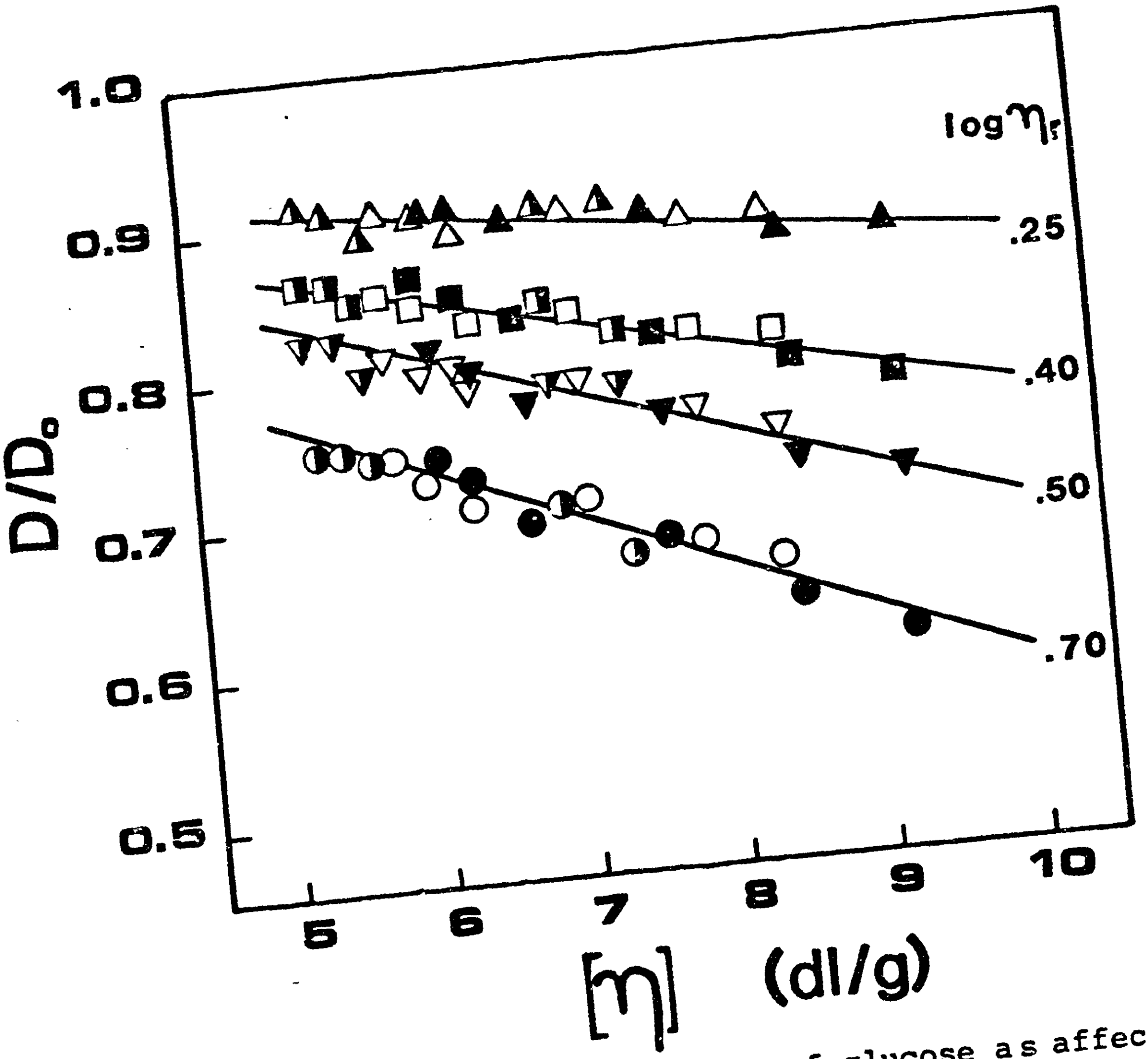


Figure 44. Relative diffusion coefficient of glucose as affected by the hydrodynamic volume of chitosan in solution (represented as intrinsic viscosity [η]) for several overall viscosities (represented by the relative viscosity "η_r"). pH 3.0 and 4.0 : black; pH 5.0 : open; pH 6.0 : half black.

mains start to appear. A more quantitative analysis of this behavior is represented in Figure 45, where the ratio η_r^*/η_r is plotted against the overlapping parameter. In this plot η^*_r is the relative microviscosity which represents the reciprocal of the ratio D/D_o. Using the Stokes-Einstein expression (Bird _et al_., 1960):

$$D = \frac{RT}{6\ r\eta_r^*} \tag{100}$$

where r is the radius of diffusant molecules, η_r^* is the microviscosity, D is the diffusion coefficient.

Figure 45 shows that the contribution of the microviscosity on the macroviscosity of the solution decreases as the overlapping parameter increases; and that this contribution is due exclusively to the domain overlapping as depicted by the independency on pH and counterion concentration. An additional interesting point is that η_r^*/η_r decreases stepwise for low values of $C[\eta]$ but a plateau region is observed for high $C[\eta]$ values. This feature indicates that at relatively high concentrations the macroviscosity is primarily controlled by the intermolecular interactions as the product of an increase in the overlapping, meanwhile as the polymer concentration is decreased, the Brownian motion, represented by the micro-

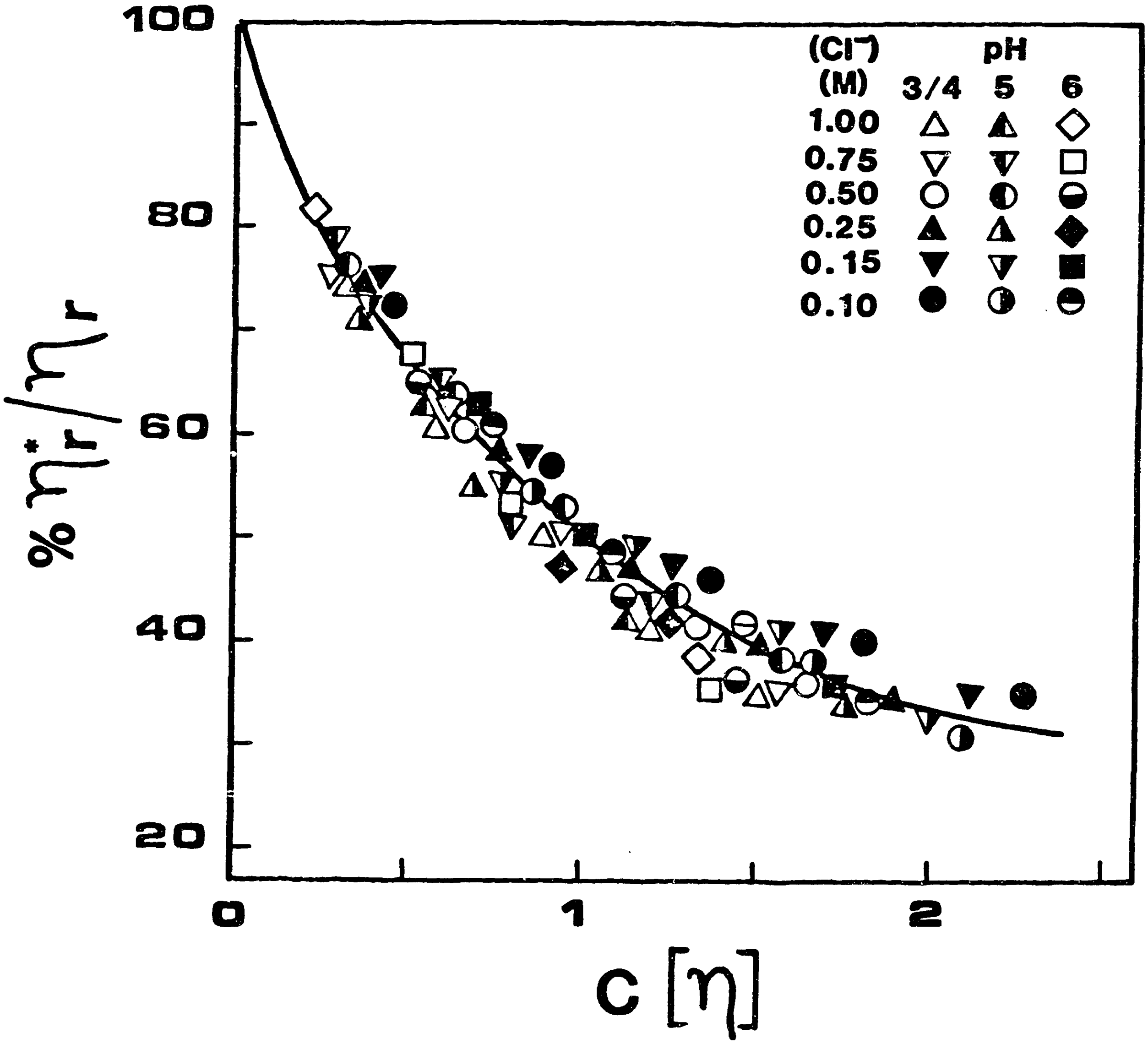


Figure 45. Relationship of the relative microviscosity to relative overall viscosity ratio and the chitosan overlapping parameter as affected by pH and counterion concentration.

viscosity, plays an increasing role. These findings corroborate the small contribution of the microviscosity on the overall solution viscosity reported on concentrated polystyrene solutions (Bagdonaite et al., 1981).

Self-diffusion coefficients of small molecules in polymer solutions can be used to calculate the polymer solvation, defined as the grams of solvent per gram of dry polymer. Applying the Wang expression for the self-diffusion values of glucose (Wang, 1954):

$$\frac{D}{D_o} = \left\{ 1 - \alpha \left[\frac{v_2 + H/\rho_1}{v_2 + (1 - W_2)/\rho_1 W_2} \right] \right\} \left[1 - \frac{W_2}{1 - W_2} \right] H \quad , \qquad (101)$$

where V_h = partial specific volume of the polymer

H = hydration term

ρ_1 = solvent viscosity

w_2 = mass fraction of the polymer

α = geometrical factor; 3/2 for spheres and 5/3 for rods

I estimate the amount of solvent bound per gram of dry chitosan. Table 21 shows that the hydration term increases with decreasing the ionic strength and with decreasing the pH of the media. However, it must be pointed out that the hydration term of chitosan gives a linear re-

Table 21

Hydration Term of Chitosan Molecule in Solution as Function of pH and Ionic Strength of the Media

	H (g solvent/g polymer)					
Cl^-(M)	1.00	0.75	0.50	0.25	0.15	0.10
pH						
3-4	36.08	39.08	44.20	47.64	50.84	56.47
5	34.60	36.80	40.08	43.80	46.68	49.40
6	30.65	32.00	34.69	36.50	40.10	45.50

lationship when plotted against the intrinsic viscosity of chitosan for a given conditions, independently of the pH and ionic strength values of the media, as shown in Figure 46. This result is expected given that the intrinsic viscosity term represents the hydrodynamic volume of the polymer molecule which, clearly, is composed by both polymer backbone and its associated solvent (Tanford, 1961).

Also, Table 21 shows that the hydration term of chitosan is between 30 grams of solvent per gram of chitosan and 60 grams of solvent per gram of chitosan. These moderately high values for the hydration term of chitosan closely match with the high hydration numbers (27.8 g H_2O/g polymer and 51.0 g H_2O/g polymer) for polygalactomana (Nordgren et al., 1973) and other reported values for polysaccharides such as carboxymethylcellulose of similar molecular weight (50-25 g H_2O/g polymer), carrageenan, (100-50 g H_2O/g polymer), and guar gum (50-40g H_2O/g polymer). These hydration values were obtained by differential scanning calorimetry (Biswas et al., 1975; Kumsah et al., 1976). All these values indicate that polysaccharides bind appreciably more solvent per gram of solute when compared with the hydration numbers of glucose (10.3 g H_2O/g sugar); fructose (12.8 g H_2O/g sugar) and other sugars (Hollenberg and Hall, 1983; Biswas et al., 1975).

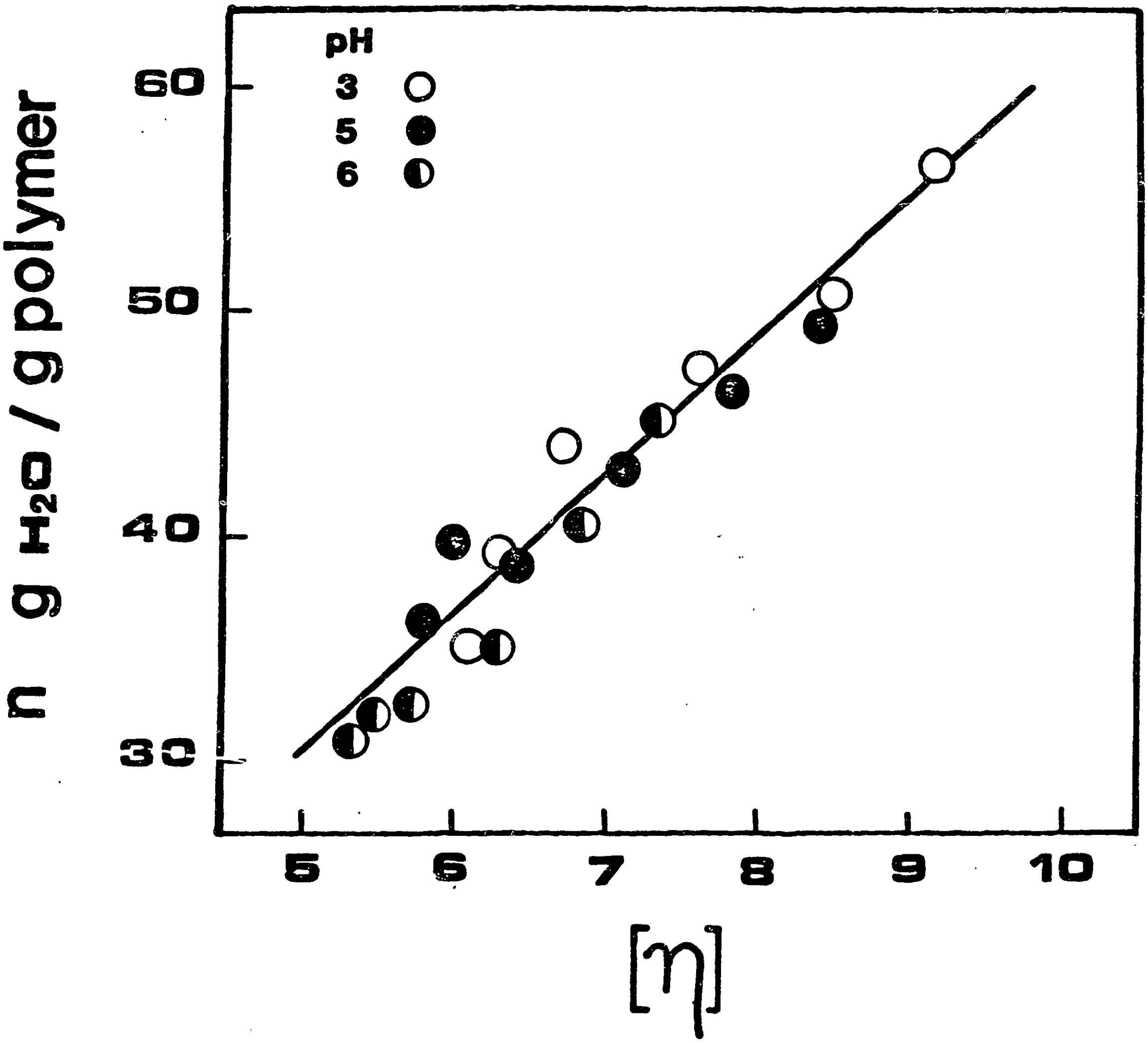


Figure 46. Relationship of the hydration term "n" and the intrinsic viscosity of chitosan as a fuction of pH and ionic strength.

4.5 Rheology of Concentrated Chitosan Solutions

In water or other strongly polar solvents, some polysaccharides can ionize forming polyions. In general, polyions can exhibit rheological properties which are quite different from that of non-charged polymers. Two classes of solution behavior can be distinguished:

1. The very dilute regime where the polymer chains are isolated. Under this condition the polymer chains are considered not to interact directly with one another, and therefore, only the polyelectrolytic nature of the polysaccharide must be considered.
2. The concentrated regime where individual polymer chains interact with each other. Entanglement and specific orientations of the macromolecular chains take place. These interactions can be affected directly by both the degree of ionization of the polyion and polyion-counterions interactions.

The behavior of chitosan in dilute solutions has been studied previously (Kienzle-Sterzer *et al.*, 1982; Rodriguez-Sanchez *et al.*, 1982). These studies demonstrated the characteristic polyelectrolytic behavior of chitosan at low ionic strength solutions, represented by the increase of the reduce viscosity with dilution; and that this behavior is supressed by increasing the salt concentration. It is established the controlling role of the solution conditions on the rheological response of chitosan at low concentration regime.

In order to evaluate the effect of the solution conditions on the rheological response of concentrated chitosan solutions, the influence of varying: pH's values between 3.0 and 5.0, counterion concentration between 0.1 M and 0.4 M and chitosan concentration between 0.50 g/dl and 1.50 g/dl, on the viscosity-shear rate response of chitosan solutions are analyzed.

Figures 47 to 58 show the relationship between the shear viscosity and the shear rate for concentrated chitosan solutions. In each case the following features are shown independently of the pH and ionic strength of the media:

1. The viscosity increases as chitosan concentration increases.
2. At low chitosan concentration, the viscosity of chitosan solutions is not shear rate dependent.
3. Increasing chitosan concentration, the viscosity-shear rate relationship shown two regions: one at low shear rates where the viscosity is independent of the shear rate, and a second

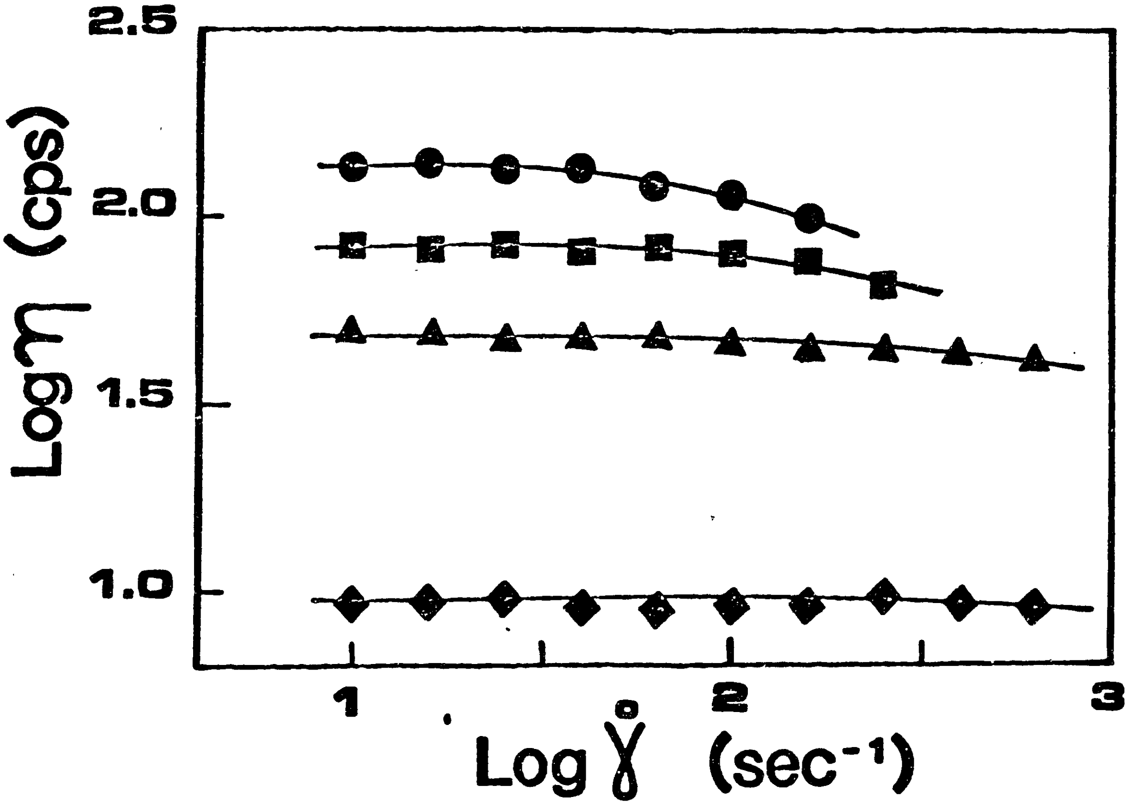


Figure 47. Relationship between the logarithm of the shear viscosity of semiconcentrated chitosan solutions and the logarithm of the shear rate: pH = 3.0; NaCl = 0.10 M. Symbols: ◆: 0.50 g/dl; ▲ : 1.00 g/dl; ■ : 1.25 g/dl; ● = 1.50 g/dl chitosan concentration.

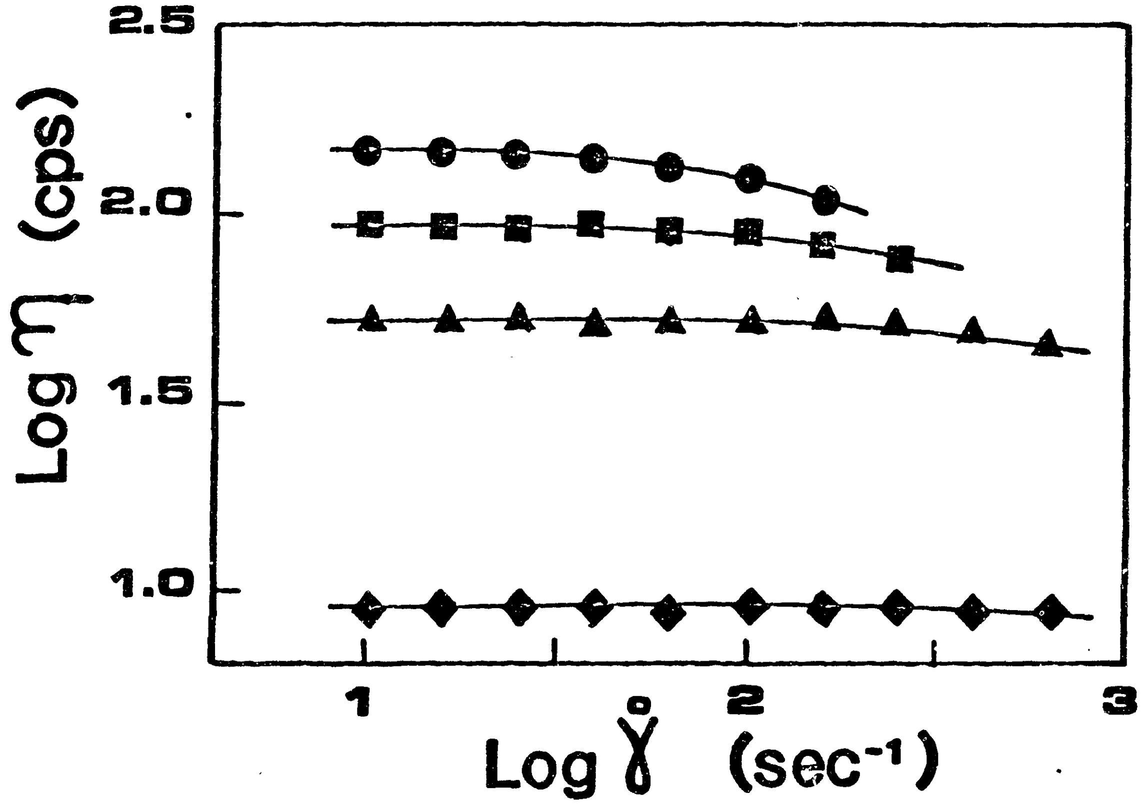


Figure 48. Relationship between the logarithm of the shear viscosity of semiconcentrated chitosan solutions and the logarithm of the shear rate: pH = 3.0; NaCl = 0.20 M. Symbols: ◆: 0.50 g/dl; ▲ : 1.00 g/dl; ■: 1.25 g/dl; ● = 1.50 g/dl chitosan concentration.

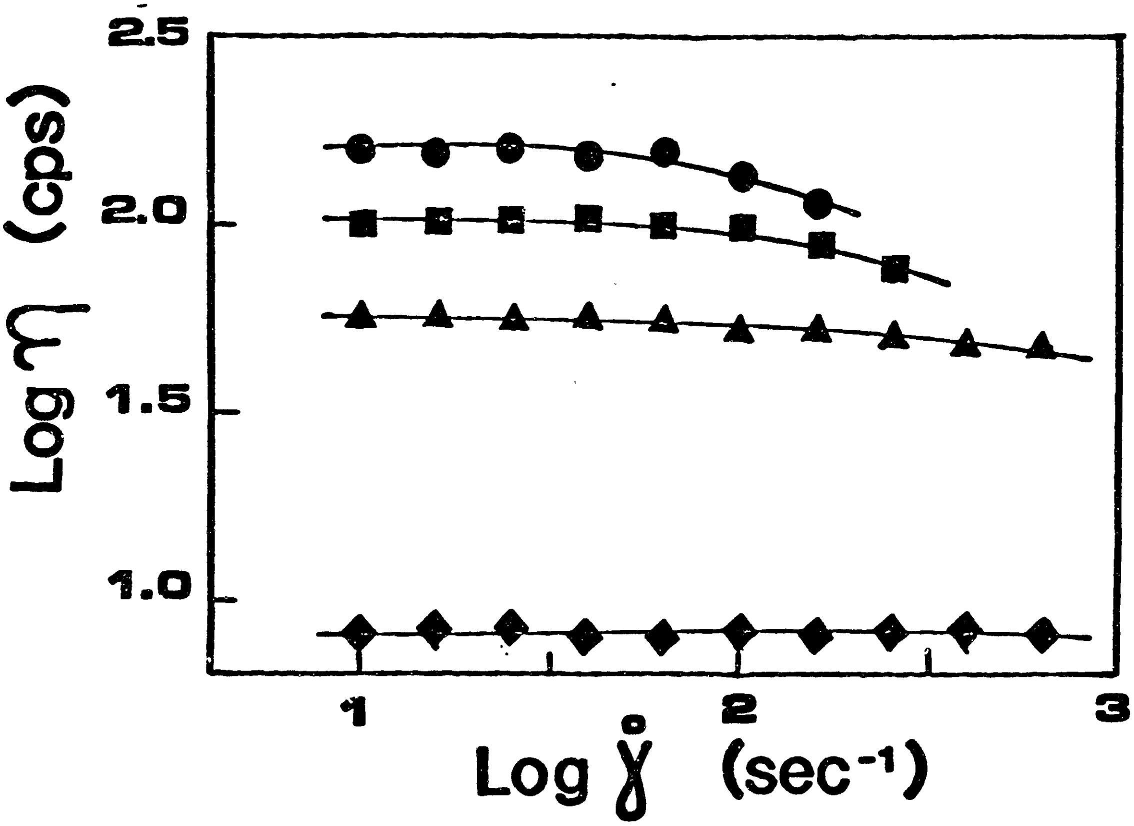


Figure 49. Relationship between the logarithm of the shear viscosity of semiconcentrated chitosan solutions and the logarithm of the shear rate: pH = 3.0; NaCl = 0.30 M. Symbols: ◆: 0.50 g/dl; ▲ : 1.00 g/dl; ■ : 1.25 g/dl; ● = 1.50 g/dl chitosan concentration.

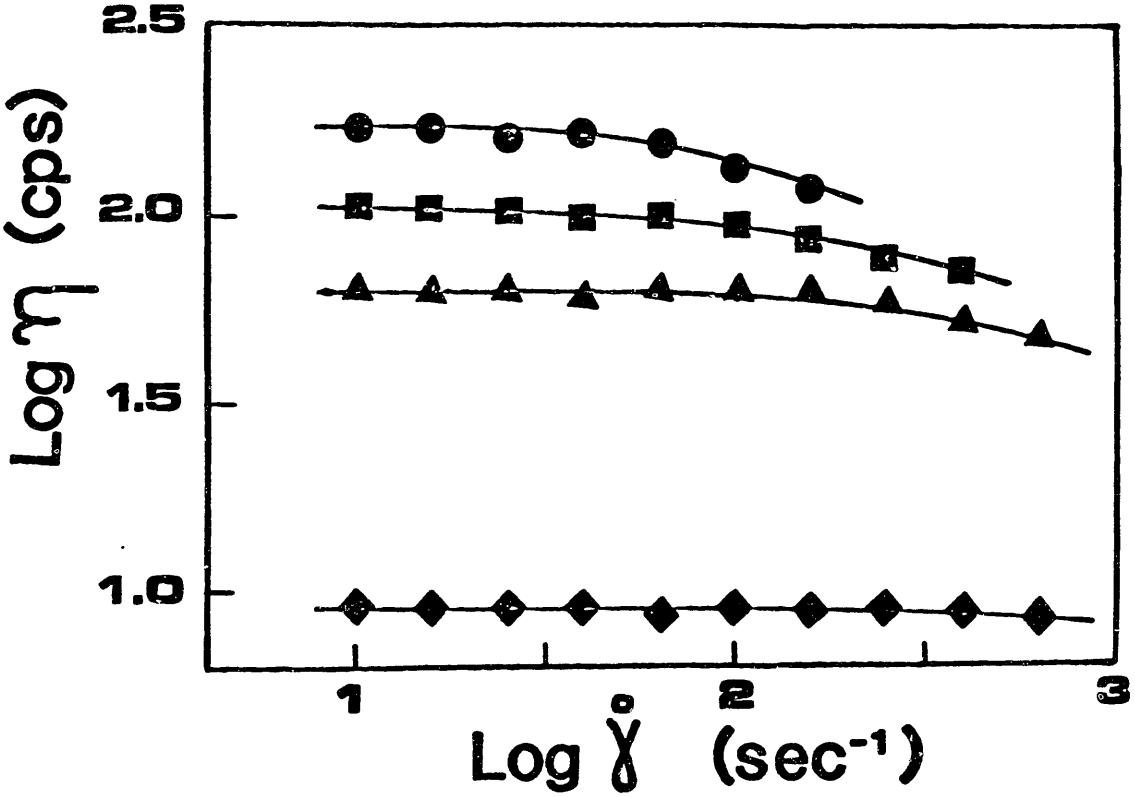


Figure 50. Relationship between the logarithm of the shear viscosity of semiconcentrated chitosan solutions and the logarithm of the shear rate: pH = 3.0; NaCl = 0.40 M. Symbols: ◆: 0.50 g/dl; ▲ : 1.00 g/dl; ■ : 1.25 g/dl; ● = 1.50 g/dl chitosan concentration.

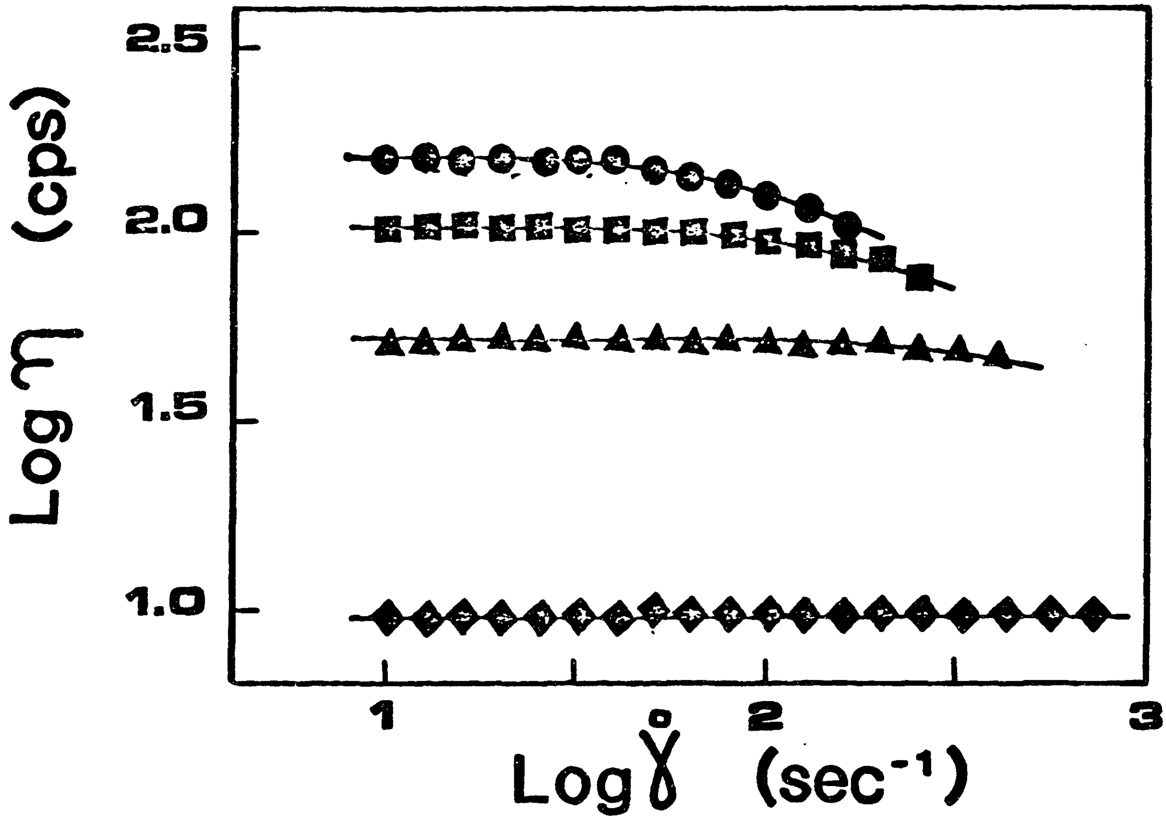


Figure 51. Relationship between the logarithm of the shear viscosity of semiconcentrated chitosan solutions and the logarithm of the shear rate: pH = 4.0; NaCl = 0.10 M. Symbols: ◆: 0.50 g/dl; ▲ : 1.00 g/dl; ■: 1.25 g/dl; ● = 1.50 g/dl chitosan concentration.

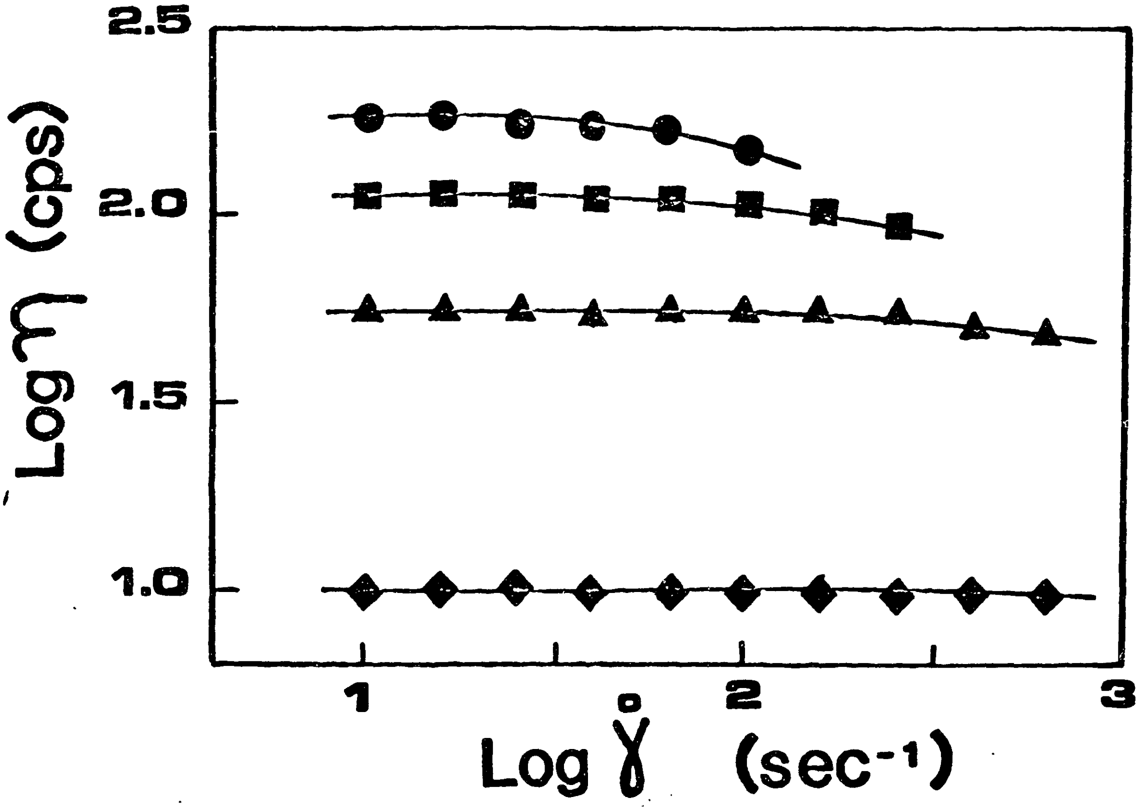


Figure 52. Relationship between the logarithm of the shear viscosity of semiconcentrated chitosan solutions and the logarithm of the shear rate: pH = 4.0; NaCl = 0.20 M. Symbols: ◆: 0.50 g/dl; ▲ : 1.00 g/dl; ■ : 1.25 g/dl; ● = 1.50 g/dl chitosan concentration.

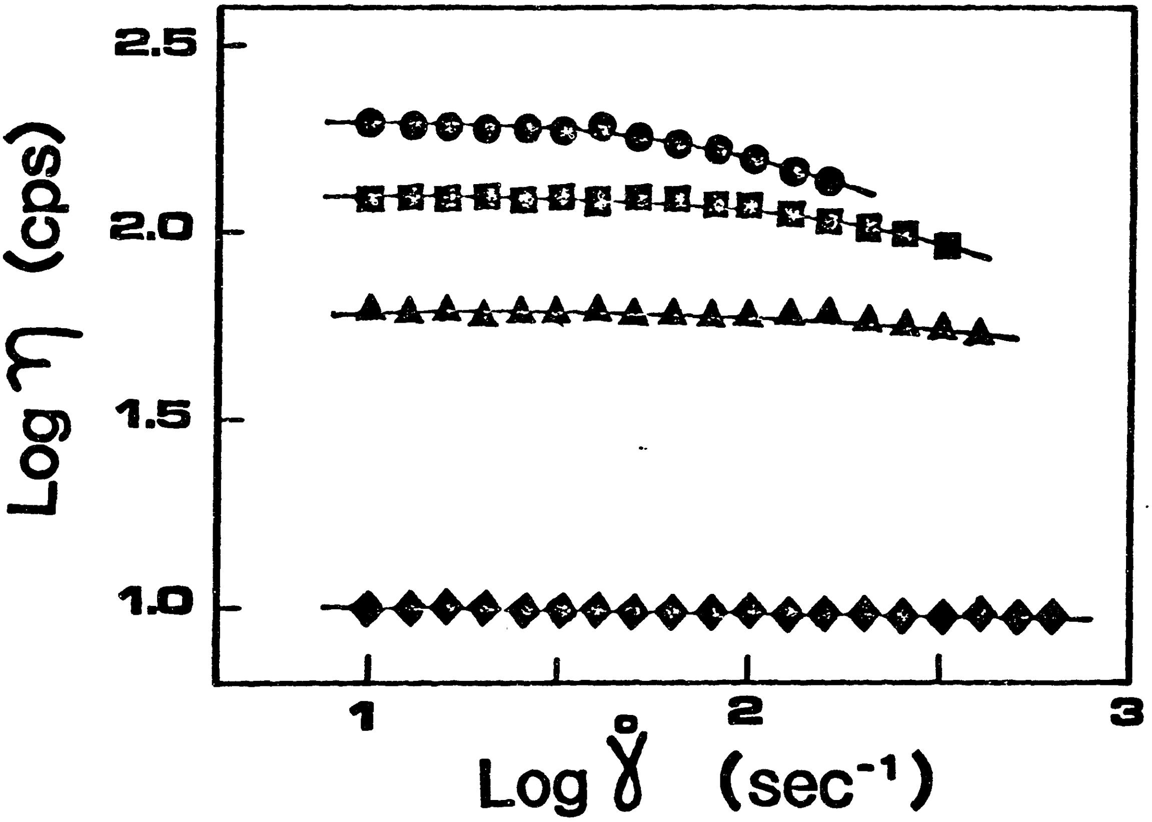


Figure 53. Relationship between the logarithm of the shear viscosity of semiconcentrated chitosan solutions and the logarithm of the shear rate: pH = 4.0; NaCl = 0.30 M. Symbols: ◆: 0.50 g/dl; ▲ : 1.00 g/dl; ■ : 1.25 g/dl; ● = 1.50 g/dl chitosan concentration.

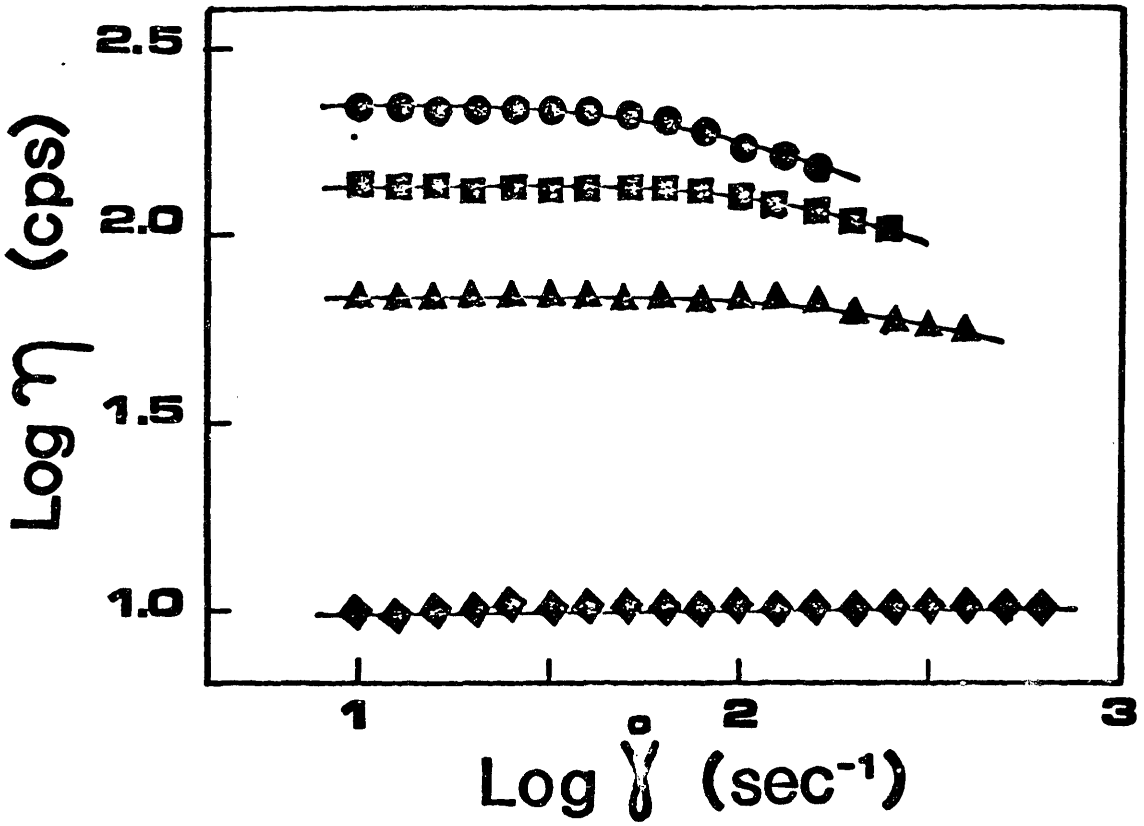


Figure 54. Relationship between the logarithm of the shear viscosity of semiconcentrated chitosan solutions and the logarithm of the shear rate:' pH = 4.0; NaCl = 0.40 M. Symbols: ◆: 0.50 g/dl; ▲: 1.00 g/dl; ■: 1.25 g/dl; ● = 1.50 g/dl chitosan concentration.

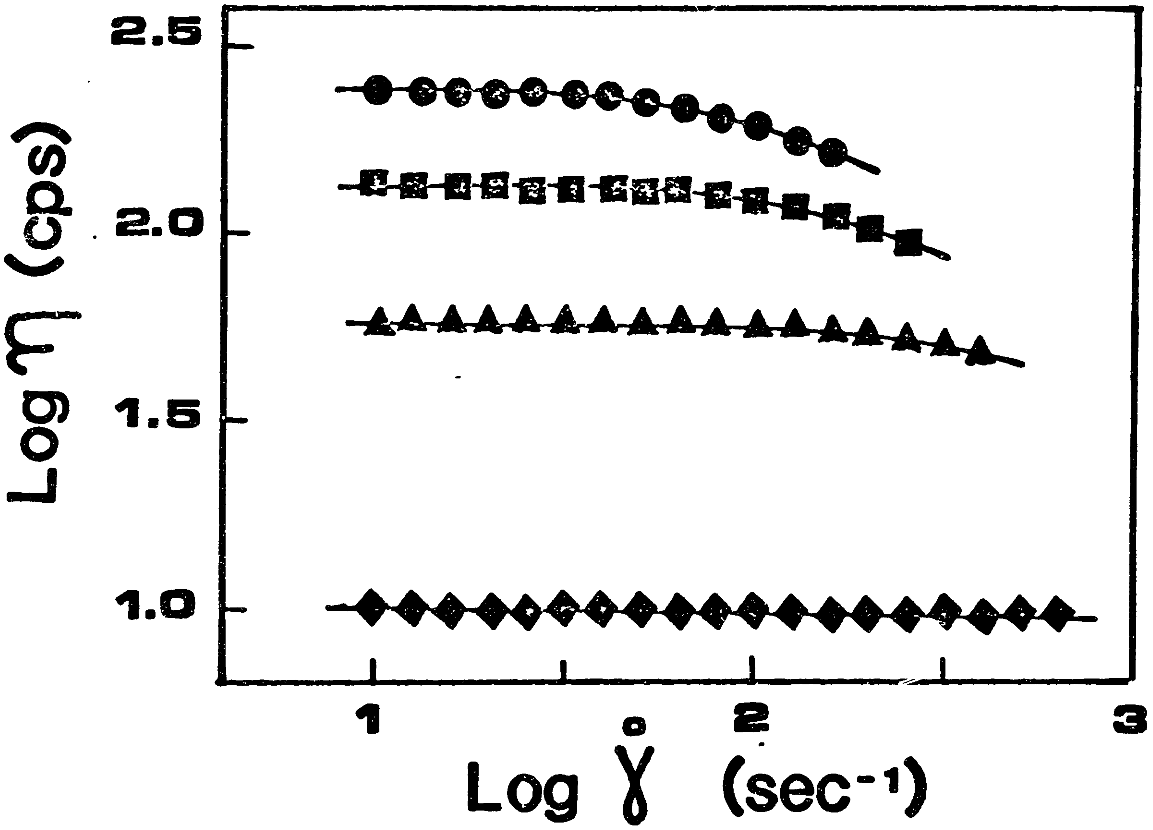


Figure 55. Relationship between the logarithm of the shear viscosity of semiconcentrated chitosan solutions and the logarithm of the shear rate: pH = 5.0; NaCl = 0.10 M. Symbols: ◆ : 0.50 g/dl; ▲ : 1.00 g/dl; ■ : 1.25 g/dl; ● = 1.50 g/dl chitosan concentration.

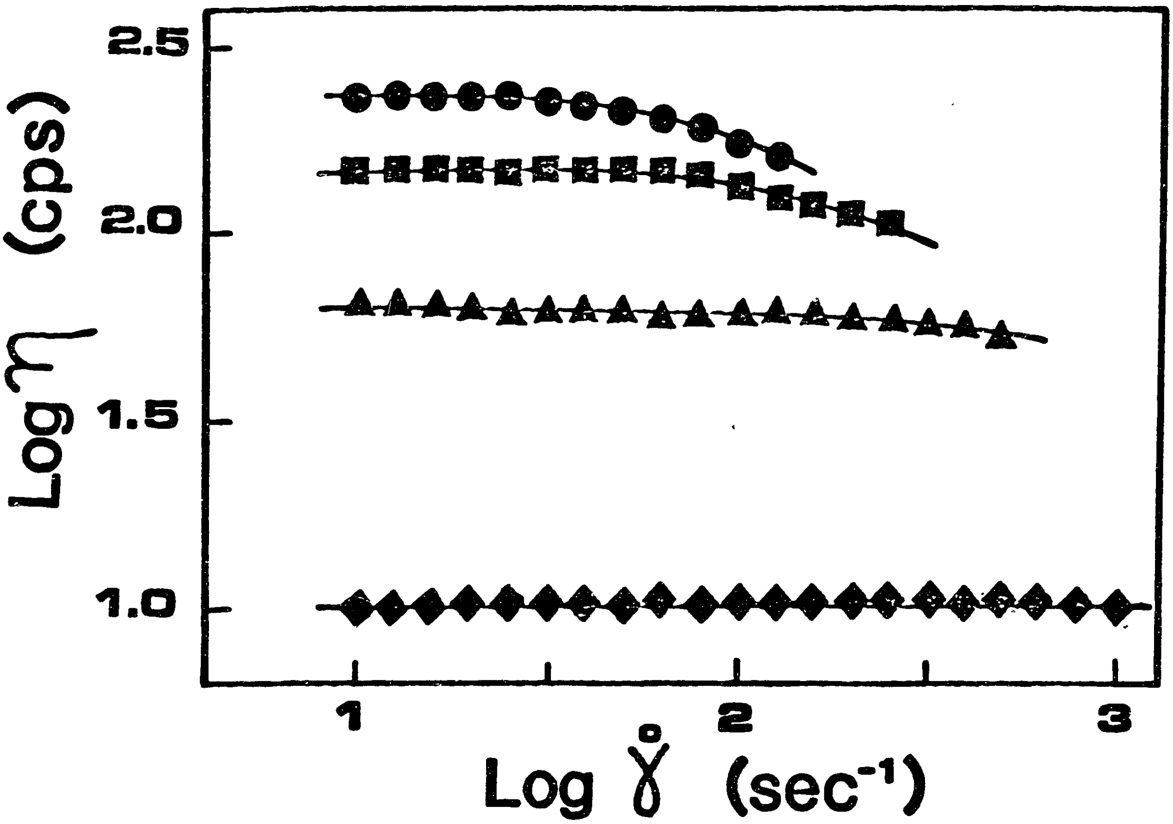


Figure 56. Relationship between the logarithm of the shear viscosity of semiconcentrated chitosan solutions and the logarithm of the shear rate: pH = 5.0; NaCl = 0.20 M. Symbols: ◆: 0.50 g/dl; ▲: 1.00 g/dl; ■ : 1.25 g/dl; ● = 1.50 g/dl chitosan concentration.

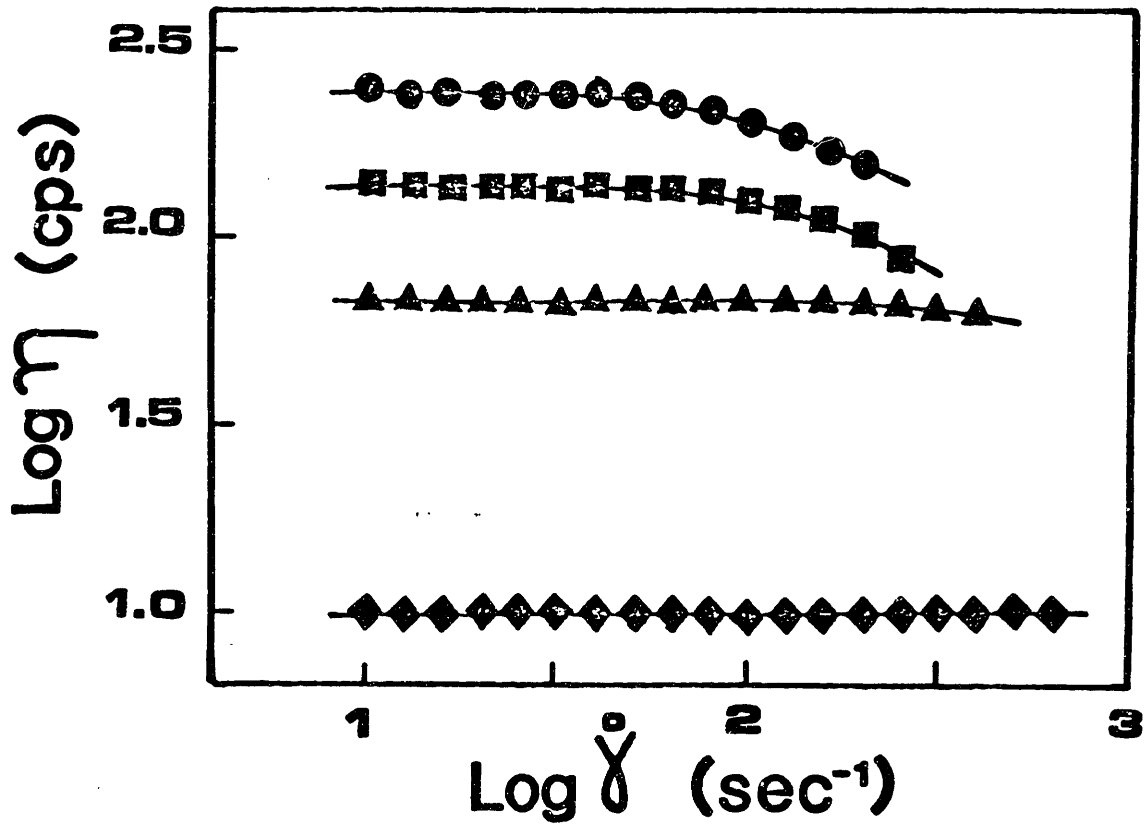


Figure 57. Relationship between the logarithm of the shear viscosity of semiconcentrated chitosan solutions and the logarithm of the shear rate: pH = 5.0; NaCl = 0.30 M. Symbols: ◆: 0.50 g/dl; ▲: 1.00 g/dl; ■: 1.25 g/dl; ● = 1.50 g/dl chitosan concentration.

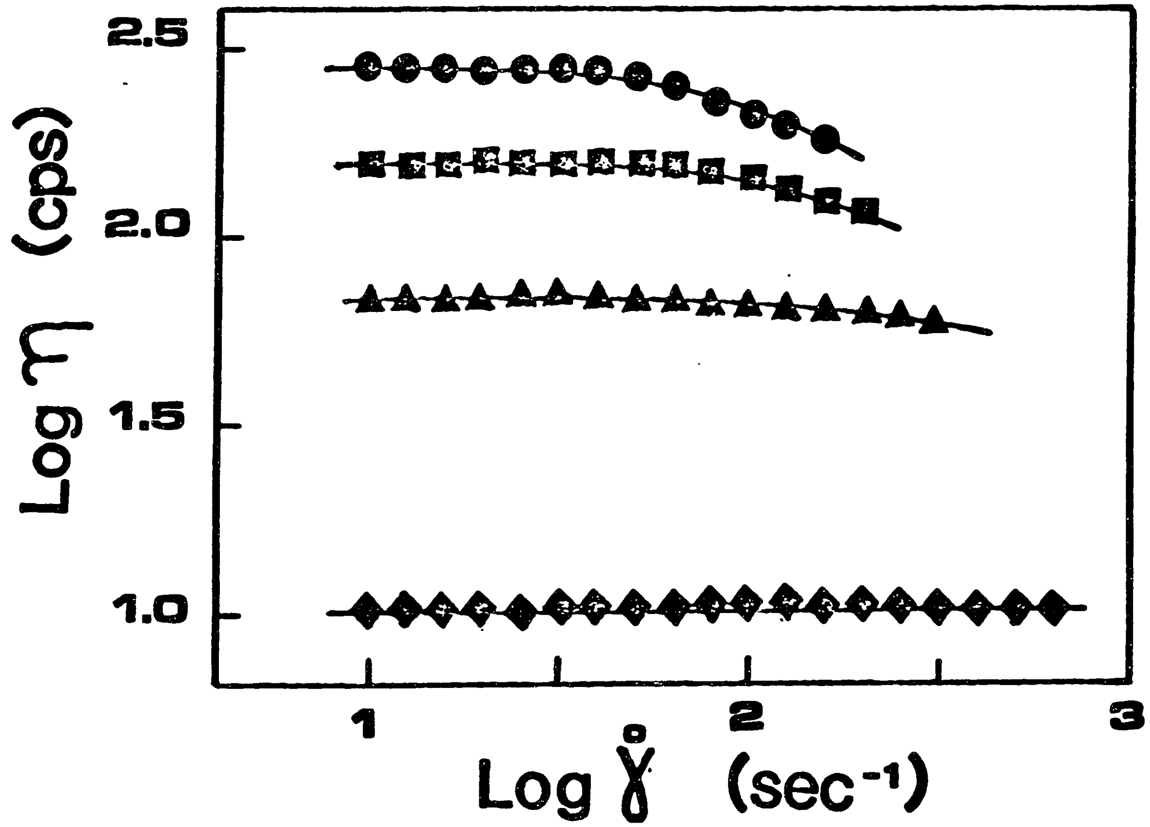


Figure 58. Relationship between the logarithm of the shear viscosity of semiconcentrated chitosan solutions and the logarithm of the shear rate: pH = 5.0; NaCl = 0.40 M. Symbols: ◆: 0.50 g/dl; ▲: 1.00 g/dl; ■: 1.25 g/dl; ● = 1.50 g/dl chitosan concentration.

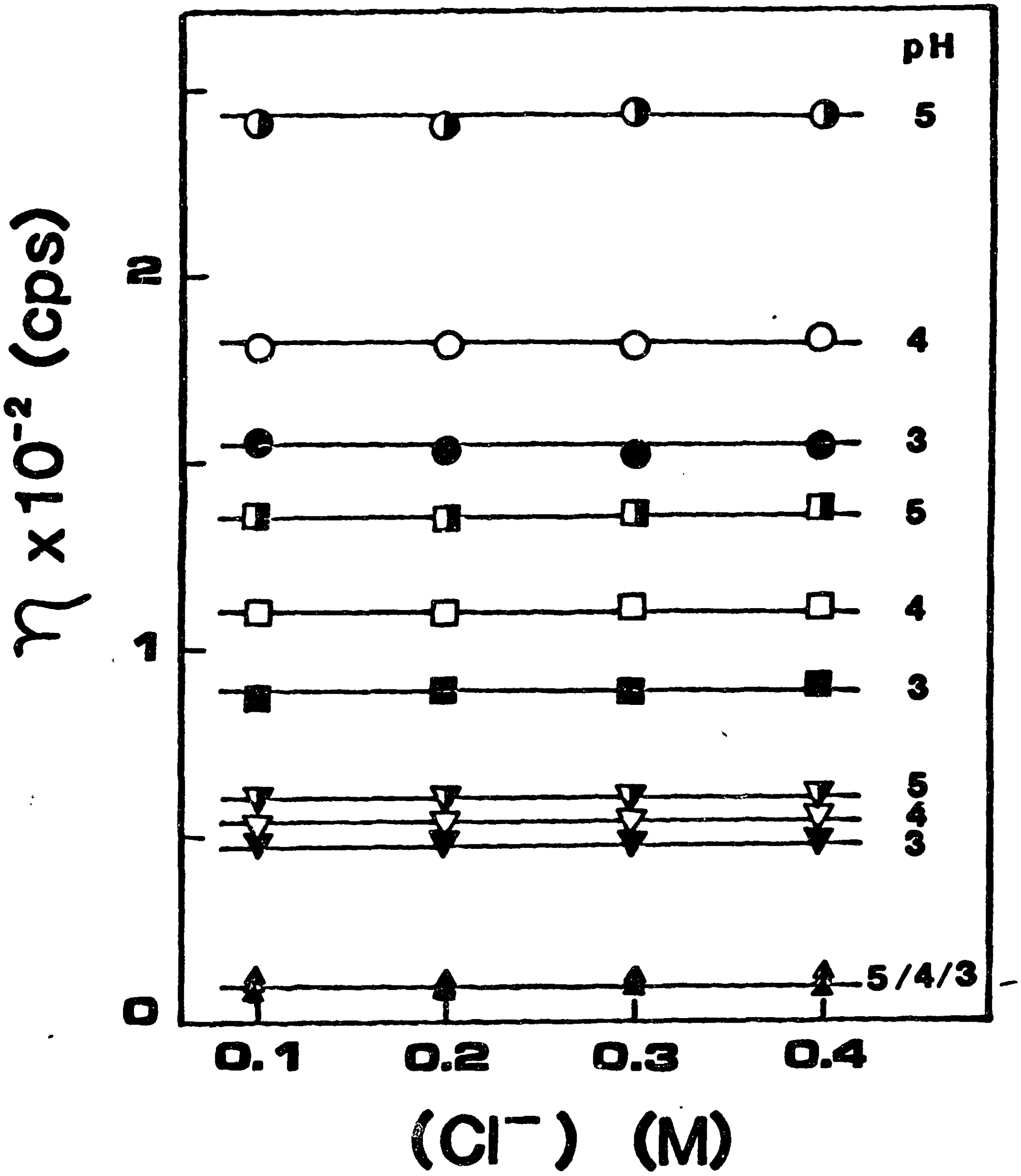


Figure 59. Zero shear viscosity of semiconcentrated chitosan solutions as affected by both pH and counterion concentration. Symbols: ▲ :0.50 g/dl; ▼ : 1.00 g/dl; ■ :1.25 g/dl; ● :1.50 g/dl chitosan concentration.

region where increasing the shear rate the viscosity of chitosan solutions decreases.

This behavior can be explained as an overall effect of the polymer concentration on the entanglement in polymer solutions. It is expected that as polymer concentration increases the intermolecular interactions start to appear, increasing the resistance to flow. Moreover, as the polymer concentration increases ($\geq$ 0.50 g/dl) a pseudoplastic behavior dominates the rheological response, as opposite to the Newtonian response for lower chitosan concentration. The pseudoplasticity is caused by the breaking of the entanglement by shear.

In order to study the effect of pH and counterion concentration upon the rheological response of concentrated chitosan solutions, the zero shear viscosity is plotted against chloride concentration for the entire range of pH and chitosan concentration, Figure 59. This figure shows that at low chitosan concentration (0.50 g/dl) the Newtonian viscosity is independent of pH and ionic strength of the media. For higher chitosan concentrations ($\geq$ 0.75 g/dl) the viscosity increases as pH increases, but remains unaltered to the counterion concentrations.

This insensitivity of the viscosity to the salt concentration is atypical for polyelectrolytes. Generally, the viscosity of charged polysaccharides in solution decreases as salt concentration increases as the results of smaller hydrodynamic radii. This is the case for chitosan in dilute solutions (Kienzle-Sterzer et al., 1983). However, the insensitivity of the viscosity of concentrated chitosan solution with respect to salt concentration is consistent with the phenomena of physical entanglement of chitosan molecules. Thus, it is expected from the potentiometric studies (Kienzle-Sterzer et al., 1984) that changes in the counterion concentration do not change significantly the surface charge density on the chitosan backbone. Therefore, it can be concluded that changes in the counterion concentration do not change the density of the intermolecular physical entanglements in concentrated chitosan solutions. The nature of this behavior is also supported by the effect of salt concentration on the relaxation mechanism of chitosan molecules in the concentrated regime (Rodriguez-Sanchez, 1983) and with the liquid-crystalline structure reported in chitosan solutions 30% to 90% by weight (Ogura et al., 1982). This anomalous behavior has been also found in some rod-like polysaccharides such as xanthan gum (Southwick et al., 1982, 1983; Whitcomb and Macosko, 1978) and in stiff alginate in KCl solutions (Seale et al., 1982).

In the evaluation of the effect of pH on the viscosity of concentrated chitosan solutions I observed that for the lower chitosan concentration (0.50 g/dl) the viscosity is independent of the pH of the solution. Moreover, by increasing the chitosan concentration a controlling role of pH on the viscosity is observed which increases as chitosan concentration increases. This influence of the pH at high chitosan concentration is due to the increase in the physical entanglement of chitosan molecules as pH increases. The degree of intermolecular entanglement is favored by the decrease in the charge surface density on the chitosan backbone as pH is increased. These results are prefectly in agreement with the effect of pH on the storage and loss modulus of concentrated chitosan solution (Rodriguez-Sanchez, 1983).

The insensitivity of the viscosity with pH (chitosan concentration equal to 0.50 g/dl) can be explained as due to the low degree of entanglement present at this concentration. The low degree of entanglement at this condition is shown by the independency of the shear viscosity to changes in the shear rate, as shown in Figures 47 to 58.

It must be pointed out at this point that the controlling effect of the physical entanglement in concentrated chitosan solutions on their rheological response confirms the previous finding of the small contribution of the microviscosity on the shear viscosity at higher values of the overlapping parameter.

The most common method of generalization of experimental results for different conditions consists in the formulation of scaled functional relationship of the form (Simha, 1952; Utracki and Simha, 1981):

$$\frac{\eta_{sp}}{c[\eta]} = \bar{\eta} = f(\, c[\eta] \,) = f(\, \bar{c} \,) \qquad (102)$$

where $\bar{\eta}$ is the reduced viscosity function, and $c[\eta]$ is the dimensionless polymer concentration. In order to represent our viscosity data in both the dilute regime (Kienzle-Sterzer _et_ _al_., 1983) and the concentrated regime, I used the Martin's equation (Utracki and Simha, 1963):

$$\bar{\eta} = \exp\,(\, K_M\, C \;\; [\eta]) \qquad (103)$$

where K_M is obtained from the slope of the linear relationship between the logarithm of the reduced viscosity function and the logarithm of the dimensionless concentration, for dilute chitosan solutions. The values of K_M plotted versus the electrostatic contribution to the excluded volume, $\alpha^3_{\eta e}$, reported previously (Kienzle-Sterzer _et_ _al_., 1983) are shown in Figure 60. Figure 60 indicates that K_M decreases as $\alpha^3_{\eta e}$ increases. The relationship of K_M to the electrostatic contribution to the expansion of

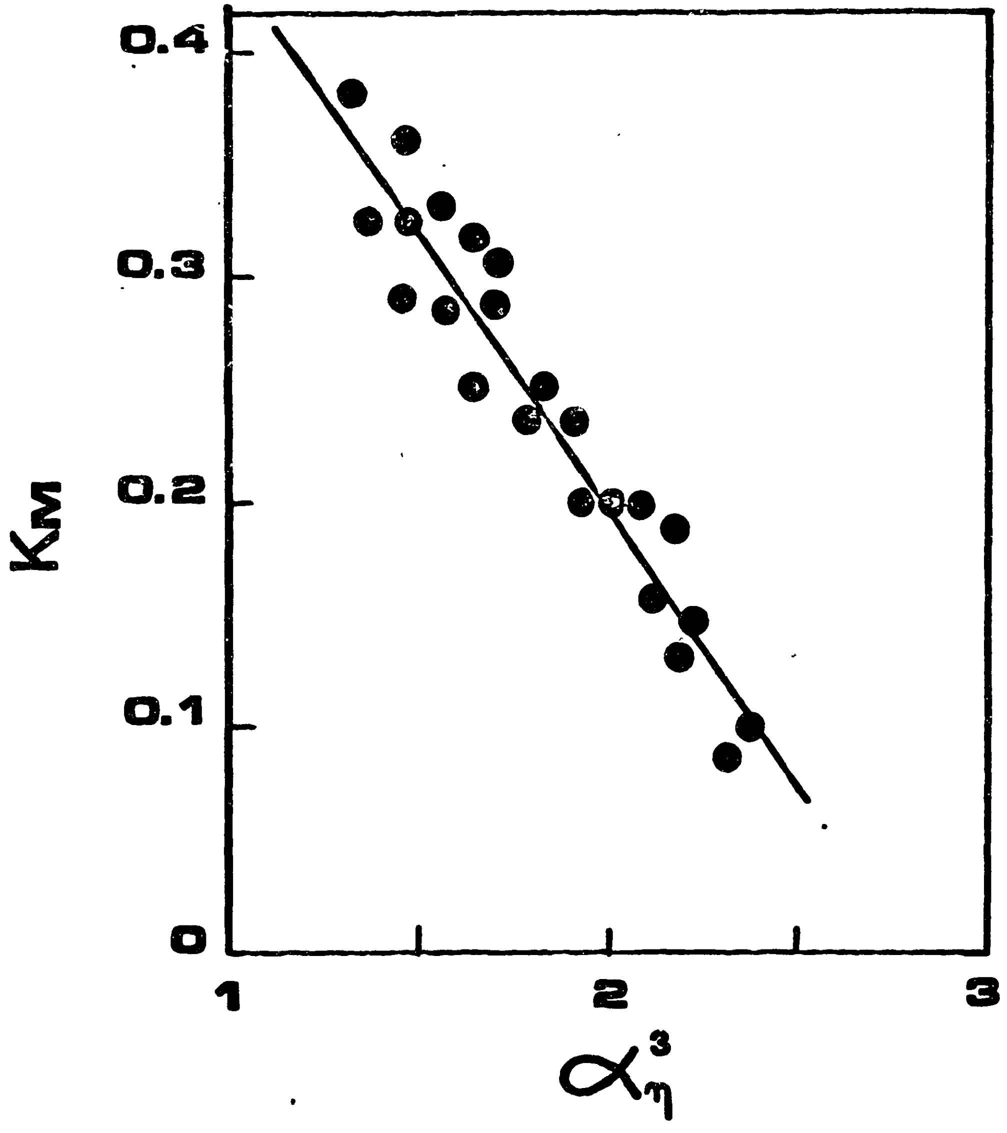


Figure 60. K_M from the Martin's equation versus the electrostatic contribution to the expansion coefficient of chitosan in solution.

chitosan molecules indicates the dependency of K_M on the interactions between the polyion and the solvent, as proposed by Dreval et al. (1973a) for a number of polymer solutions.

Figures 61 and 62 represent the relationship between the logarithm of the reduced viscosity function and $K_M C[\eta]$ for the dilute and concentrated regime respectively. A single line is obtained for all the range of pH, ionic strength and chitosan concentration. However, it must be pointed out that for $K_M C[\eta]$ higher than 2.0 a shift from the linearity between the logarithm of $\bar{\eta}$ and $K_M C[\eta]$ is observed. This deviation is explained as the result of a macromolecular association effect between chitosan molecules as chitosan concentration increases. Similar behavior has been observed for flexible-chain polymers such as polyisobutylene and polybutadine above their glass temperature (Dreval et al., 1973a,b).

This study indicates the effect of changing the pH and the counterion concentration on the rheological response of concentrated solutions. The relationship between viscosity and solution conditions shows the importance of the inter- and intramolecular interactions as well as polymer-solvent interactions on the physical entanglement of chitosan molecules as chitosan concentration increases and how these entanglements affect the response of chitosan solution to shear rate.

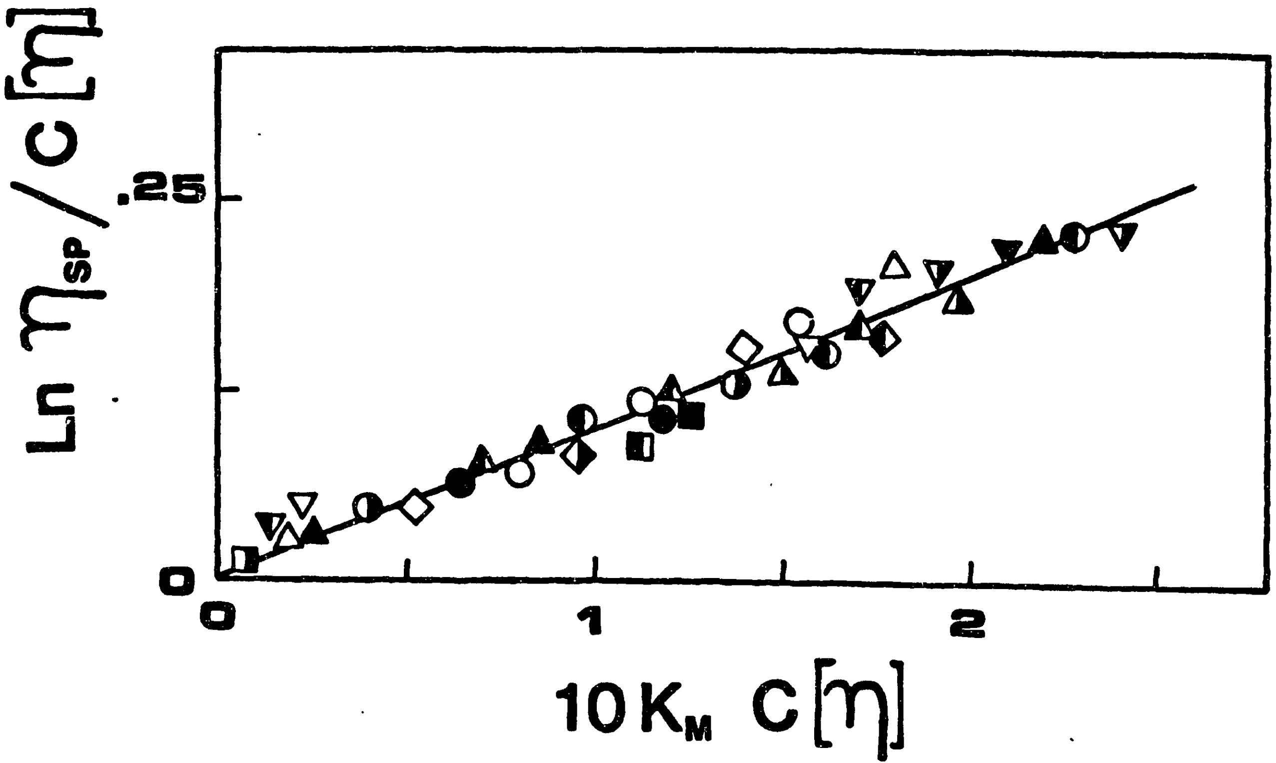


Figure 61. Logarithm of the reduced viscosity function ($\bar{\eta}$) versus $K_M C[\eta]$ for dilute chitosan solutions. Symbols:

△ pH = 3.0; NaCl = 0.50 M
▲ pH = 5.0; NaCl = 0.50 M
▽ pH = 3.0; NaCl = 0.30 M
▼ pH = 5.0; NaCl = 0.30 M
□ pH = 3.0; NaCl = 0.20 M
■ pH = 5.0; NaCl = 0.20 M
○ pH = 3.0; NaCl = 0.10 M
● pH = 5.0; NaCl = 0.10 M

◭ pH = 4.0; NaCl = 0.50 M
◮ pH = 6.0; NaCl = 0.50 M
⧨ pH = 4.0; NaCl = 0.30 M
⧩ pH = 6.0; NaCl = 0.30 M
◧ pH = 4.0; NaCl = 0.20 M
◨ pH = 6.0; NaCl = 0.20 M
◐ pH = 4.0; NaCl = 0.10 M
◑ pH = 6.0; NaCl = 0.10 M

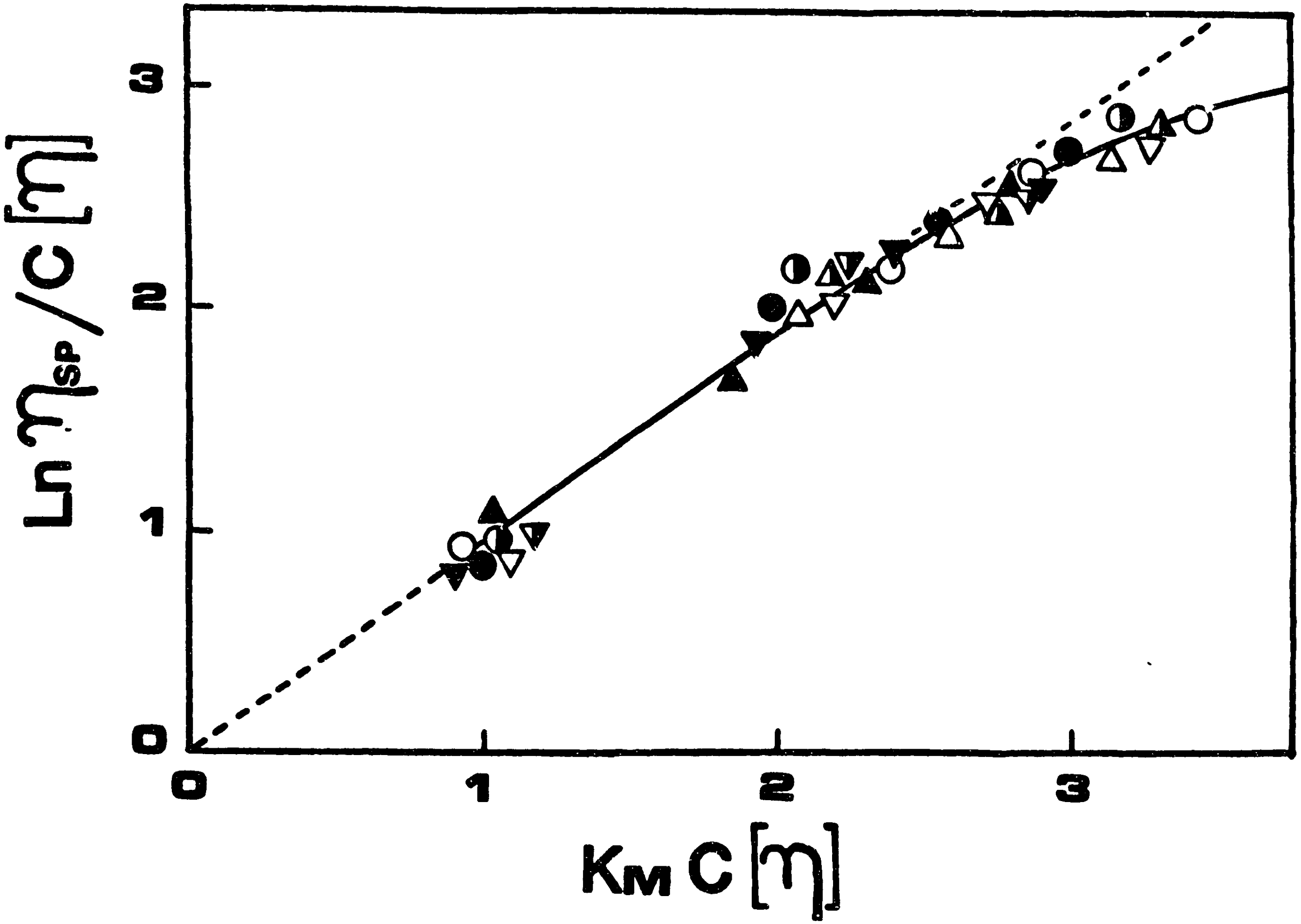


Figure 62. Logarithm of the reduced viscosity function ($\bar{\eta}$) versus $K_M C[\eta]$ for concentrated chitosan solution. Symbols represent the same conditions as described in Figure 61.

5. SUMMARY AND CONCLUSIONS

5.1 Summary

Intrinsic Viscosity of Chitosan at Low Ionic Strength of the Media

The dilute solution behavior of a cationic polyelectrolyte was studied with respect to acetic acid concentration. The reduced viscosity of chitosan solutions increases with dilution. Meanwhile, for a given chitosan concentration, the reduced viscosity is higher at lower acetic acid concentration indicating the expansion of the polyelectrolyte chains due to increases in the electrostatic repulsion between neighboring segments. The relationship between $[\eta]$ and the acetic acid concentration suggests no conformational transition of the chitosan molecules and the possible formation of intramolecular hydrogen bonds during the shrinking process of the hydrodynamic domain of chitosan.

Intrinsic Viscosity of Chitosan at High Ionic Strength of the Media

The hydrodynamic properties of chitosan as affected by the degree of ionization and the counterion concentration were studied. The intrinsic viscosity of chitosan decreases with decreasing the degree of ionization and increasing the counterion concentration. This behavior demonstrates the effect of both the repulsive electrostatic interactions between neighboring segments, and the counterion charge shielding process on the hydrodynamic volume of chitosan molecules in solution.

Chitosan in dilute solution has a flexibility representative of a β(1-4) glucan derivative as indicated by the values of the stiffness parameter ($B = 0.08$).

In order to describe the local conformational freedom of chitosan the ratio between the radius of gyration of the non-charged polyion and the theoretical radius of gyration is calculated. This coefficient is compared with the characteristic ratio under unperturbed (Θ) conditions, thus indicating that the nonelectrostatic contribution to the excluded volume does not play a significant role in the hydrodynamic behavior of dilute chitosan solutions.

Rheological Model of Chitosan in Solution

The hydrodynamic properties of dilute chitosan solutions as affected by degree of ionization, ionic strength of the media and molecular weight has been used to describe the relationship between chemical structure and solution properties of polysaccharides.

Chitosan molecule has been modeled as a non-draining equivalent sphere composed of a series of rigid rods with elastic bonding joints. This molecular model is in agreement with the chemical structure of chitosan, because of both, the stiff nature of the glucosamine ring and the limited rotational possibility of the β(1-4) glucosidic linkages. This model is also supported by the obtained values of the characteristic ratio (C_∞= 15) and stiffness parameter (B = 0.08) of chitosan when compared with other polysaccharides.

The persistance length of chitosan molecules in dilute solutions is calculated (Yamakawa and Fujii, 1974) to describe the effect of intramolecular interactions and counterion-polyion interactions on the local conformation of chitosan. The linear relationship between the molecular persistance length and the Debye-Huckel screening parameter is used to quantify the electrostatic contribution to the persistance length, which in turn is related to the bending capability of the linkage. Therefore, it is concluded that, the molecular stiffness increases as the degree of ionization increases and the ionic strength of the media decreases.

The experimental values of the electrostatic contribution to the excluded volume is determined from the relationship between the intrinsic viscosity and the ionic strength of the media. These experimental values are compared with those theoretically calculated (Fixman and Skolnick, 1978). The results indicated that the Debye-Huckel approximation is adequate to describe the effect of counterion concentration on the excluded volume, and that the interactions between neighboring segments, perhaps hydrogen bonding, play a major role as counterion shielding increases.

Examination of the relationship between intrinsic viscosity and molecular weight demonstrates the significance of intramolecular hydrogen bonding in the molecular conformation of chitosan in solution. Thus, the exponent of Mark-Houwink equation varies from "a" = 0.147 to "a" = 0.71 with the addition of urea. These values of "a" represent a conformational change from a compact hydrodynamic sphere to an extended random coil when urea is present.

The relationship between intrinsic viscosity and molecular weight of chitosan is evaluated (Ptitsy and Eizner, 1962; Leon _et al._, 1982) and the unperturbed Kuhn statistical segment length is calculated. The Kuhn segment length changes from 72.8 Å for the compact spherical conformation to 121.6 Å for the extended random coil conformation.

Degree of Ionization of a Cationic Polyelectrolyte in Solution

In order to determine the degree of ionization of chitosan in solution the potentiometric titration was carried out for dilute and semiconcentrated solution regime. Polymer concentrations and ionic strength of the media were studied. The potentiometric titration curves provide the relationship between the degree of ionization and pKapp. In all cases, two distinct regions were present in the potentiometric titration curve. For pKapp greater than 3.5, pKapp value increased with decrease in α , but for pKapp lower than 3.5, α is independent of the pKapp. This behavior may be an indication of a conformational transition of chitosan with the change in surface charge. The change in surface charge certainly can relate to the changes in the hydrodynamic volume as a function of pH. For a given pKapp, α shifts to higher values at higher salt concentrations due to the shielding of charges in the polyions. With varying polyelectrolyte concentration, two distinct zones are observed for a constant pKapp value: one for low polymer concentration where α is independent of chitosan concentration, and the other at high polymer concentration where α decreases as the polymer concentration increases. This demonstrates the influence of the repulsive electrostatic forces on the intermolecular interactions between charged points as the overlap increases between the hydrodynamic domain of the polyion.

The potentiometric titration study established that 1. inter - intramolecular interactions in chitosan solutions are electrostatic in nature; 2. the surface charge potential plays a critical role in the potentiometric behavior of the polyelectrolyte solutions, and 3. it is possible to determine the polyelectrolyte concentration at which overlapping starts to occur from potentiometric titration studies.

Counterion Activity in a Cationic Polyelectrolyte Solution

The counterion transport properties, represented by the chloride ion activity coefficient in polyelectrolyte solutions were studied. The effect of polymer concentration and ionic strength of the media in the non-dilute regime were examined. The relative counterion activity coefficient (γ/γ_o) decreased with a decrease in ionic strength, but remained unchanged with polymer concentrations. Chloride ions bound per monomer of polymer (n) were estimated from the activity coefficient. The number of bound ion (n) decreased with decreases in ionic strength and increases in polymer concentration. This phenomenon demonstrates the effect of intermolecular interactions in polyelectrolytes on the charge shielding process of polyions.

Diffusion of Small Molecules in Polyelectrolyte Solutions

The diffusion of small non-interacting molecules in chitosan solutions was studied. The effect of polyelectrolyte concentration, pH and ionic strength on the diffusion properties of glucose in chitosan solutions was determined. The relative self-diffusion coefficient of glucose decreased linearly with increasing chitosan concentration indicating a compensating effect between the overlapping of the macromolecular domains and the size of surrounding solvation layers. Also, the relative self-diffusion coefficient of glucose increased with increasing ionic strength and pH for fixed chitosan concentration. This indicated the role of the polyelectrolyte hydrodynamic volume on the transport properties of polymer solutions.

From the relationship between the relative self-diffusion coefficient of glucose and the overlapping parameter, semiconcentrated chitosan solutions have been envisioned as consisting of two distinct regions: a polymer rich region consisting of the polymer-solvation layer, and a free solvent region. This concept suggested the existence of a "loose network" which increases as polymer concentration increases, and that in turn controls the behavior of polyelectrolyte solutions. This conclusion is clearly represented by the diminishing influence of the microviscosity on the overall macroviscosity as the overlapping term increases.

Finally, the values of the relative self-diffusion coefficient of glucose were used to estimate the solvation of chitosan molecules in solution. The solvation value of chitosan varies from 30 g of solvent/g of polymer to 60 g of solvent/g of polymer, and it is compared with respect to other polysaccharides in solution.

Rheology of Concentrated Chitosan Solutions

The rheological response of concentrated chitosan solutions (chitosan concentrations between 0.50 g/dl and 1.50 g/dl) as affected by the pH (pH's values between 3.0 and 5.0), ionic strength of the media (Cl^- concentration between 0.1 M and 0.4 M) and shear rate up to 10^3 reciprocal seconds has been studied. The results indicate that the viscosity of chitosan solution increased as chitosan concentration increased and that for high chitosan concentrations the viscosity decreases as shear rate increases. Also, it is shown that the viscosity remains unchanged with changes in the counterion concentration and increases as pH increases for the high concentrations. These tendencies are explained by the influence of different surface density on the chitosan backbone with changes of pH and ionic strength, upon the physical entanglement of chitosan molecules in concentrated regime.

The evaluation of the concentration dependence of the zero shear viscosity shows that a generalize viscosity curve results by plotting the logarithm of the reduce viscosity function versus $K_M C[\eta]$. However, as chitosan concentration increases a deviation from the linearity is observed as product of increasing the association between molecules.

5.2 Conclusions

The rheological evaluation of chitosan in dilute solution indicates:

1. Chitosan molecules can be modeled as a series of rigid rods joined together for flexible joins (wormlike molecule) that behave as a non-draining hydrodynamic sphere. This is supported by both the linear relationship between $[\eta]^{2/3}$ and the logarithm of the reciprocal of the counterion concentration and the low value of the exponent

(a = 0.147) in the Mark-Houwink equation.

2. The intrinsic viscosity of chitosan molecules in solution increases with increasing the degree of ionization and/or decreasing the counterion concentration. This behavior demonstrates the effect of both the repulsive electrostatic interactions between neighboring segments, and the counterion charge shielding process upon the hydrodynamic volume of chitosan.
3. Chitosan has a flexibility typical of a $\beta(1-4)$ glucan derivative as shown by the value of the characteristic ratio ($C_\infty = 14.2$) and the "stiffness parameter" (B = 0.08).
4. The chitosan backbone flexibility increases with increasing counterion concentration and decreasing the degree of ionization. The degree of flexibility is evaluated by the relationship between the persistence length, obtained by the Yamakawa-Fujii equation, and both the salt concentration and the degree of ionization.
5. The non-electrostatic contribution to the excluded volume does not influence the rheology of dilute chitosan solutions. This is evidence by comparing the value of the chitosan characteristic ratio at conditions ($C_\infty = 14.2$) and at in-

finite salt concentration ($C_\infty = 15$).

6. The repulsion between the molecule counterion clouds increases as the result of the chitosan collapsing with increasing salt concentration at a constant degree of ionization. This is evidence by comparing the higher values of the experimental electrostatic contribution to the excluded volume to those theoretically obtained from the Fixman-Skolnick approach.

The potentiometric titration studies of chitosan in both diluted and concentrated solutions show:

1. Counterion condensation occurs for all pKapp values lower than 3.5 independently of both the polyelectrolyte concentration and the counterion concentration. This phenomenon is shown by the independency of the degree of ionization for pKapp values below 3.5. This critical degree of ionization corresponds to the critical length between charges (b = 7.2 Å) for monovalent salt solutions.
2. A "loose network" results at high chitosan concentration as product of increasing intermolecular interactions. The existence of this "loose network" is suggested by the decrease in the

value of the critical degree of ionization with increasing chitosan concentration.

The rheological and transport experiments of concentrated chitosan solutions show:

1. Concentrated chitosan solutions, where the hydrodynamic domains overlap extensively, can be envisioned as a "sponge-like" through which the solvent and small molecules can freely flow. This model is suggested by the obtained master curve between the relative self-diffusion coefficient of glucose and the overlapping parameter (C[η]) independently of the pH and ionic strength of the media.
2. Chitosan molecules expand as a product of increasing intermolecular interactions behaving as rod-like molecules for high chitosan concentration. This behavior is supported by:
    i. the independency of the zero shear viscosity with changes in salt concentration;
    ii. the decrease in the number of counterion associated per monomer with increasing the overlapping parameter (C[η]) and;
    iii. the linearity between the self-diffusion

coefficient of glucose and chitosan concentration.

3. Intermolecular interactions control the rheological response of concentrated chitosan solution as indicated by the increase in the zero shear viscosity, at given chitosan concentration and ionic strength, with increasing pH values.
4. The contribution of the Brownian motion to the overall viscosity, as represented by the microviscosity of chitosan solution, decreases as the overlapping parameter increases up to $C[\eta] \geq 1.5$ where a plateau is obtained, $(\eta_r^*/\eta_r = 30\%)$, independently of the pH and ionic strength of the media.

6. SUGGESTIONS FOR FUTURE RESEARCH

This research studies the effect of inter- and intramolecular interactions on the rheological response of diluted and concentrated chitosan solutions. It, also, establishes the relationship between degree of ionization and counterion concentration upon the hydrodynamic volume and flexibility of chitosan in solution. A common factor in this work is that chitosan is dissolved in high polar solvents (mixtures of water-Cl^- solutions). Therefore, the following future research must be conducted in order to understand chitosan and chitosan complexes:

1. Studies incorporating dynamic and static light scattering in dilute chitosan solutions to understand the effect of intramolecular interactions on both the self-diffusion coefficient and the second viral coefficient of chitosan molecules in solution.
2. Evaluate the effect of pH and ionic strength on the dynamic surface tension of dilute and concentrated chitosan solution. The results of this study must be analyzed together with the results from light scattering in order to interpret the mechanisms of interface formation.

3. Rheological studies as well as phase separation experiments of complex chitosan-water-nonpolar solvents. This study will bring the opportunity of a more complete understanding of potential industrial application for chitosan.
4. Evaluate the reactivity of chitosan (a cationic polyelectrolyte) with anionic polysaccharides such as alginate, xanthan gum, etc. This study, together with the rheological and tensile properties of the complexes will help us to find possible applications for chitosan in the complex recovery processes of polysaccharides in the biotechnology industry.
5. Use of chitosan and/or chitosan derivatives as drag reducing agents. Study the effect of extensibility and flexibility of polymers upon their drag reducing capabilities using chitosan as a model polyelectrolyte.

REFERENCES

Adamson, A.W. 1976. Physical Chemistry of Surfaces. John Wiley & Sons, New York.

Adler, R.S. and K.F. Freed. 1980. On the dynamic scalling theories of polymer solutions at nonzero concentration. J. Chem. Phys. 72:4186-4191.

Alfrey, T.; Berg, P.W., and H. Morawetz. 1951. Counterion distribution in solutions of rod-shaped polyelectrolytes. J. Polym Sci. 7:43-51.

Allan, G.C.; Fox, J.R., and N. Kong. 1978. A critical evaluation of the potential sources of chitin and chitosan. Proc. 1st. Int. Conf. on Chitin and Chitosan. R.A.A. Muzzarelli - E.R. Parisier Ed. pp. 64-69.

Allan, G.C.; Johnson, P.G.; Lay, Y.Z., and K.V. Sarkasen. 1971.Solubility and reactivity of marine polymers in dimethylformamide + dinitrogen tetroxide. Chem. Ind., 1971; 127-135.

Ander, P.; Gangi, G., and A. Koblansky. 1978. Interactions of small ions with heparin and dextran sulfate by self-diffusion measurements. Macromolecules, 11, 904-909.

Ander, P.; Leung-Louie, L., and F. Silvestry. 1979. Polycations. 1. Sodium, calcium and sulfate ion diffusion coefficients in aquous salt solutions containing ioene bromides. Macromolecules, 12, 1204-1210.

Anderson, C.F. and M.T. Record. 1980. The relationship between the Poisson-Boltzmann model and the condensation hypothesis: An analysis based on the low salt form of the Donnan coefficient. Biophys. Chem., 11:353-360.

Ando, T. and S. Kataoka. 1979. Japan Kokai Tokkyo Koho 79,110,267.

Archer, B.G.; Craney, C.L., and H. Krakauer. 1972. The interactions of Na ions with synthetic polynucleotides. Biopolymers, 11:172-181.

Arena, B.J. 1983. U.S. Patent 4,367,355.

Armstrong, R.W., and U.P. Strauss. 1969. Encycl. Polym. Sci. Technol. p. 781-802.

Ascoli, F.; Botre, C., and A.M. Liquori. 1961. On the polyelectrolyte behavior of heparin. I. Binding of sodium ions. J. Phys. Chem., 65:1991-1992.

Ashford, N.A.; Hattis, D., and A.E. Murray. 1977. Industrial properties for chitin and protein from shellfish wastes. M.I.T. Sea Grant Report MITSG 77-3 M.I.T., Cambridge, Mass.

Astrup, T.; Galsmar, I., and M. Volkert. 1944. Polysaccharide sulfuric acid and related compounds. Acta Physiol. Scand., 9:215-226.

Austin, P.R. 1975. U.S. Patent 3,892,731.

Austin, P.R.; Brine, C.J.; Castkle, J.E., and J.P. Zikakis. 1981. Chitin: New facets of research. Science, 212:749-752.

Averbach, B.L.A. 1977. Film-forming capability of chitosan. M.I.T. Sea Grant Report MITSG 78-7. M.I.T., Cambridge, Mass.

Bagdonaite, V.A.; Juskeviciute, S.S., and Yu.A. Shlyaprikov. 1981. On macro- and microviscosity of high polymer solutions. Polymer, 22:145-146.

Benoit, H. and P.M. Doty. 1953. Light scattering from non-gaussian chains. J. Phys. Chem., 57:958-963.

Berger, M.; Byers, C.H., and J.J. Magenheimer. 1983. U.S. Patent 4,386,151.

Bergstrom, s. 1936. Polysaccharide sulfuric acids with heparin action. Z. Physiol. Chem., 238:163-168.

Berkovich, L.A.; Timofeyeva, G.I.; Tsyurupa, M.P., and V.A. Davankov. 1980. Hydrodynamic and conformational parameters of chitosane. Vysokomol. Soyed., A22:1834-1844.

Bird, R.B.; Steward, W.E., and E.N. Lightfoot. 1960. Transport Phenomena. John Wiley and Sons, New York.

Birnbaum, S.; Pendleton, R.; Larsson, P.O., and K. Mosbach. Covalent stabilization of alginate gels for the entrapment of living whole cells. Biotechnol. Letters, 3:393-397.

Biswas, A.B.; Kumsah, C.A.; Pass, G., and G.O. Phillips. 1975. The effect of carbohydrates on the heat of fusion of water. J. Solution Chem. 4:581-590.

Blackwell, J.; Parker, K.D., and K.M. Randall. 1967. Chitin fibers of the diatoms Thalassiosira fluviatilis and Cyclotella cryptica. J. Mol. Biol., 28:383-385.

Bloomfield, V.A.; Crothers, D.M., and I.Jr. Tinoco. 1974. Physical Chemistry of Nucleic Acids. Harper and Row Ed., New York.

Bloys van Treslong, C.J. and A.J. Staverman. 1974. Poly-(ethyleimine). II Potentiometric titration behavior in comparison with other weak polyelectrolytes. Recl. Trav. Chim. Pays-Bas., 93:171-178.

Bloys van Treslong, C.J. 1978. Evaluation of potentiometric data of weak polyelectrolytes taking account of nearest neighbor interaction. Recl. Trav. Chim. Pays-Bas., 97:13-22.

Bloys van Treslong, C.J. and P. Moonen. 1978. Distribution of counterions in solutions of weak polyelectrolytes. A study to the effects of neighbor interactions between charged sites and the structure of the macromolecule. Recl. Trav. Chim. Pays-Bas., 97:22-27.

Bloys van Treslong, C.J. and B.J. Jensen. 1983. (Vynyl amine) - (Vynyl alcohol) copolymers. Synthesis, characterization and potentiometric behavior. Eur. Polym. J. 19:131-134.

Bokanga,P. 1984. S.M. Thesis, Food Science and Technology, Cambridge, Ma.

Bomstein, R.A. 1974. U.S. Patent 3,833,744.

Boyd, G.E. and D.P. Wilson. 1982. Counterion binding in polyelectrolytes in dilute solutiopns. Macromolecules, 15:78-82.

Brant, D.A. and K.D. Boebel. 1975. A general treatment of the configurational statistics of polysaccharides. Macromolecules, 8:522-538.

Broussignac, P. 1968. Un haut polymere naturel peu connu dans l'industrie: le chitosane. Chim. Ind. Genie. Chim., 99:1242-1250.

Brown, W.; Henley, D., and J. Ohman, 1963. Studies on cellulose derivatives. Part II. The influence of solvent and temperature on the configuration and hydrodynamic behavior of hydroxyethyl cellulose in dilute solution. Makromol. Chem., 64:49-67.

Cappoza, R.C. 1975. Ger. Patent 2,505,305.

Chibata, I.; Tosda, T.; Sato, T.; Yamamoto, K.; Tokota, I., and Y. Nishida. 1978. New method for immobilization of microbial cells and its industrial application. Enzyme Eng., 3:335-341.

Chien, H.W.; Isihara, C.H., and A. Isihara. 1976. Intrinsic viscosity of polyelectrolytes in salt solutions. Polym. J., 3:288-293.

Clark, G.L. and A.F. Smith. 1935. X-ray diffraction studies of chitin, chitosan and derivatives. J. Phys. Chem., 40:863-879.

Cleland, R.L. and J.L. Wang. 1970. Ionic polysaccharides. III. Dilute solution properties of hyaluronic acid fractions. Biopolymers, 9:799-810.

Cleland, R.L.; Wang, J.L., and D.W. Dtweiler. 1982. Polyelectrolyte properties of sodium hyaluronate. 2. Potentiometric titration of hyaluronic acid. Macromolecules, 15:386-392.

Cox, R.A. 1960. The influence of ionic strength on the viscosity of ribonucleic acid and other polyelectrolytes. J. Polym. Sci., 47:441-456.

Cushing, I.B. and E.J. Kratovil. 1956. U.S. Patent 2,755,275.

Cushing, I.B.; Davis, R.V.; Kratovil, E.J., and D.W. McCorquodale. 1954. The sulfonation of chitin in chlorosulfonic and dichloroethane. J. Am. Chem. Soc., 76:4590-4591.

Dayan, S.; Maissa, P.; Vellutini, M.J., and P. Sixou. 1982. Intrinsic viscosity of cellulose derivatives and the persistent cylinder model of Yamakawa. Polymer, 23:800-804.

Danilov, S.N. and E.A. Plisko. 1954. Chitin. Action of acids and alkalis on chitin. Zhur. Obs. Khim., 24: 1761-1769.

Danilov, S.N. and E.A. Plisko. 1961. The study of chitin. 4. Preparations and properties of carboxymethylchitin. Zhur. Obs. Khim., 31: 426-430.

Delville, A. and P. Laszlo. 1983. Ion binding or condensation to polyelectrolytes. 2. A detailed comparison on the example of sodium heparinate. Biophys. Chem., 17:119-124.

Domard, A. and M. Rinaudo. 1983. Preparation and characterization of fully deacetylated chitosan. Int. J. Biol. Macromol., 5:49-52.

Dondos, A. and G. Staikos. 1980. Treatment of high molecular weight wormlike polymers as random coiled polymers., Eur. Polym. J., 16:1215-1221.

Dreval, V.E.; Malkin, A.Ya., and G.O. Botvinnik. 1973a. Approach to generalization of concentration dependence of the zero-shear viscosity in polymer solutions. J. Polym. Sci., Polym. Phys. Ed., 11:1055-1076.

Dreval, V.E.; Malkin, A.Ya.; Vinogradev, G.V., and A.A. Tager. 1973b. Effect of the solvent nature on the rheological properties of concentrated solutions of various polymers. Eur. Polym. J., 9:85-99.

Dubin, P.L. and V.P. Strauss. 1970. Hydrophobic bonding in alternating copolymers of maleic acid and alkyl vinyl ethers. J. Phys. Chem., 74:2842-2847.

Durand, G. and J.M. Navarro. 1978. Immobilized microbial cells. Process Biochem., 13: 14-20.

Edsall, J. and J. Wyman. 1958. Biophysical Chemistry. Academic Press Inc., New York.

Eisenberg, H. and D. Woodside. 1962. Multicomponent polyelectyrolyte solutions. Part II. Excluded volume study of polyvinylsulfonate alkalihalide system. J. Chem. Phys., 36:1844-1848.

Farag, A.A.; Farag, H.A.; Sedahmed, G.H., and A.F. Nagawi. 1976a. Diffusion in aqueous polymer solutions. J. Appl. Polym. Sci., 20;3247-3253.

Farag, A.A.; Sedahmed, G.H.; Farag, H.A., and A.F. Nagawi. 1976b. Diffusion of some dyes in aqueous polymer solutions. British Polym. J,. 8:54-57.

Fillar, L.J. and M.G. Wirick. 1978. Bulk and solution properties of chitosan. Proc. 1st. Int. Conf. on Chitin and Chitosan. R.A.A. Muzzarelli - E.R. Parisier Ed., pp. 169-176.

Fixman, M. 1964. Polyelectrolytes: A fuzzy sphere model. J. Chem. Phys., 41:3772-3778.

Fixman, M. 1979. The Poisson-Boltzman equation and its application to polyelectrolytes. J. Chem. Phys., 70:4995-5005.

Fixman, M. and J. Skolnick. 1978. Polyelectrolyte excluded volume paradox. Macromolecules, 11:863-867.

Fleming, I.D.; Turner, M., and E.J. Napier. 1974. German Patent 2,422,374.

Florence, A.T. and J.R. Johnson. 1975. Transport of anions in aqueous polymer solutions. J. Pharmacol. 27 Supplement, 70P.

Flori, P.J. 1953. Principles of Polymer Chemistry. Cornell University Press, Ithaca, New York.

Fouss, R.M. 1948. Electrostatic interactions of polyelectrolytes and simple electrolytes. J. Polym. Sci., 3: 602-611.

Fouss, R.M. and V.P. Strauss. 1949. Electrostatic interactions of polyelectrolytes and simple electrolytes. Ann. N.Y. Acad. Sci., 51:836-839.

Fouss, R.M.; Katchalsky, A., and S. Lifson. 1951. Potential of an infinite rodlike molecule and the distribution of the counterions. Proc. Nat. Acad. Sci. U.S.A., 37:579-583.

Fox, T.G. and P.J. Flory. 1949. Intrinsic viscosity/molecular weight relationship for polyisobutylane. J. Phys. Colloid. Chem., 53:197-207.

Fowler, R. and E.A. Guggenheim. 1960. Statistical Thermodynamics. Cambridge University Press, Cambridge.

Fujita, T. 1970. Japanese Patent 7,013,599.

Gamzazade, A.I.; Skylyar, A.M.; Pavalora, S.S.A., and S.V. Rogozhin. 1981. Viscosity properties of chitosan solutions. Vysokomol. Soedin., A23:594-597.

Gardner, K.H. and J. Blackwell. 1974. The styructure of native cellulose. Biopolymers, 13:1975-2001.

Gardner, K.H. and J. Blackwell. 1975. Refinement of the structure of -chitin. Biopolymers, 14:1481-1495.

Gekko, K. and H. Naguchi. 1974. Hydration behavior of ionic dextran derivatives. Macromolecules, 7:224-229.

Gekko, K. and H. Noguchi. 1978. Counterion activity and adiabatic compressibility of dextran phosphate. Rpts. Progr. Polym. Sci. Jpn., 21:683-684.

Goebel, K.D. and D.A. Brant. 1980. The configuration of amylose and its derivatives in aqueous solutions. Experimental results. Macromolecules, 3:634-643.

Goebel, K.D.; Dimpfl, W.L., and D.A. Brant. 1970. The conformational energy of maltose and amylose. Macromolecules, 3:644-651.

Green, H.S. 1952. The Molecular Theory of Fluids. Interscience, New York.

Gross, P.; Konrad E., and H. Mager. 1982. Investigations on chitosan as a natural film forming ingridients in hair cosmetic products under the consideration of ecological aspects. Chitin and Chitosan, Proc. 2nd. Int. Conf. on Chitin and Chitosan. S. Hirano and S. Tokura, Ed., p. 205-209.

Grourke, M. and J.H. Gibbs. 1971. Comparison of helix stabilities of poly-L-lysine, poly-L-ornithine, and poly-(L-diaminobutyric acid). Biopolymers, 10:795-808.

Gueron, M. and G. Weisbach. 1979. Polyelectrolyte theory. 2. Activity coefficients in Poisson-Boltzman and in condensation theory. The polarizability of the counterion sheat. J. Phys. Chem., 83:1991-2000.

Hackman, R.H. 1954. Chitin. I. Enzymatic degradation of chitin and chitin esters. Austr. J. Biol. Sci., 7: 168-178.

Hall, L.D. and M. Yalpani. 1980. Enhancement of the metal-chelating properties of chitin and chitosan. Carbohyd. Res., 83:C-5.

Harawith, A.: Roseman, S., and H.J. Blumenthal. 1957. The preparation of glucosamine oligosaccharides. I. Separation. *J. Am. Chem. Soc.*, 79:5046-5059.

Hauge, E. and P.C. Hemmer. 1971. Yang-Lee distribution of zeros for a van der Waals gas. *Phys. Norvegica*, 5:209-218.

Hayakawa, K. and J.C.T. Kwak. 1982. Surfactant/polyelectrolyte interactions. 1. Binding of dodecyltrimethylammonium ions by sodium dextrant sulfate and sodium poly(styrenesulfonate) in aqueous solution in the presence of sodium chloride. *J. Phys. Chem.*, 86:3866-3870.

Hiemenz, P.C. 1977. *Principles of Colloid and Surface Chemistry*. Marcel Dekker Inc., New York.

Hill, T.L. 1955. Virial expansion of the osmotic pressure in the Donnan membrane equilibrium. *Arch. Biochem. Biophys.*, 57:229-232.

Hirano, I.; Einaga, Y., and H. Fujita. 1979. Curdlan (Bacterial -1,3-Glucan) in a cadoxen-water mixture. *Polymer J.*, 11:901-904.

Hirano, S.; Tobetto, K., and Y. Noishiki. SEM ultrastructure studies of N-acyl- and N-benzylidine chitosan and chitosan membranes. *J. Biomed. Mater. Res.*, 15:903-911.

Hoffmann-LaRoche, F. & Co. 1957. N-formulated chitosan sulfonic acid polyesters. Brit. 777,204.

Hollegberg, J.L. and D.O. Hall. 1983. Hydration numbers by near-infrared spectrophotometry. 2. Sugars. *J. Phys. Chem.*, 87:695-696.

Horowitz, S.T.; Roseman, S., and H.J. Blumenthal. 1957. Preparation of glucosamine oligosaccharide. 1. Separation. *J. Am. Chem. Soc.*, 79:5046-5049.

Huggins, M.L. 1942. Viscosity of dilute solutions of dilute solutions of long-chain molecules. IV. Dependence on concentration. *J. Am. Chem. Soc.*, 64:2716-2719.

Ikegami, A. 1964. Hydration and ion binding of polyelectrolytes. *J. Polym. Sci. Part A.*, 2:907-910.

Imai, N. and S. Sasaki. 1980. Dielectric increment in polyion solutions due to the distorsion of counterion distribution. Biophys. Chem., 11:361-367.

Ishida, N.; Ogino, K., and T. Nakagawa. 1965. Effect of salt on viscosity of poly(styrenesulfonic acid) solutions. J. Chem. Soc. Jpn., 86:1029-1037.

Ishida, S.; Nonomura, H.; Morimoto, Y., and K. Kaneko. 1981. Diffusion phenomena of small molecules in polyelectrolyte solutions. Polym. Bull., 6:35-39.

Ishikawa, M. 1979. The pH of weak polyacid solutions in the presence of mono- and divalent counterions. Macromolecules, 12:498-502.

Ivani, E.M. 1982. U.S. Patent 4,365,050.

Iwasa, K. and J.C.T. Kwak. 1977. The contribution of higher order cluster term to the activity coefficients of the small ions in polyelectrolyte solutions. J. Phys. Chem., 81:408-412.

Iwasa, D.; McQuarrie, D.A., and J.C.T. 1978. Higher-order limiting laws of polyelectrolyte solutions. J. Phys. Chem., 82:1979-1982.

Jeanloz, R., and E. Forchielli. 1950. Hyaluronic acid and related compounds. 3. Determination of the structure of chitin by periodate oxidation. Helv. Chim. Acta., 33:1690-1697.

Jones, A. and I.A. Veliky. 1981. Effect of medium constituents on the viebility of immobilized plant cells. Can. J. Bot., 59:2095-2104.

Josi, Y.M. and J.C.T. Kwat. 1979. Mean and single ion activity coefficients in aqueous mixtures of sodium chloride and sodium pectate, sodium pectinate and sodium carboxymethylcellulose. J. Phys. Chem.,83: 1978-1987.

Kamide, K. and Y. Miyazaki. 1978. The partially free draining effect of unperturbed chain dimensions of cellulose, amylose and their derivatives. Polym. J., 10: 409-412.

Kamide, K. and M. Saito. 1983. Excluded volume effect for solutions of cellulose derivatives evaluated by worm-like chain model. Eur. Polym. J., 19:507-510.

Kamide, K.; Saito, M., and H. Suzuki. 1983. Persistence length of cellulose derivatives in solution. Makromol. Chem. Rapid Commun., 4:33-39.

Kao Soap Co. 1982. Japanese Patent 8,282,576.

Katchalsky, A. and P. Spitnik. 1947. Potentiometric titrations of polymethacrylic acid. J. Polym. Sci., 2:432-446.

Katchalsky, A.; Shavit, N., and H. Eisenberg. 1954. Dissociation of weak polymeric acids and bases. J. Polym. Sci., 13:69-76.

Katchalsky, A.; Mazur, J., and P. Spitnik. 1957. Polybase properties of polyvinylamine. J. Polym. Sci., 23: 513-532.

Kato, N.; Nakagawa, T., and H. Akamata. 1960. Study of polystyrene-sulfonic acid in solution. Bull. Chem. Soc. Jpn., 33:322-325.

Kato, T.; Okamoto, T.; Tokuya, T., and A. Takahashi. 1982. Solution properties and chain flexibility of pullulan in aqueous solutions. Byopolymers, 21:1623-1633.

Kay, P.J. and F.E. Treloar, 1974. The intrinsic viscosity of poly(acrylic acid) at different ionic strengths: Random coil and rigid rod behavior. Makromol. Chem., 175:3207-3223.

Kerenzi, P.C.; Meurer, B.; Spegt, P., and G. Weill. 1979. Divalent paramagnetic counterions site binding in polyelectrolyte solutions. Anal;ysis of the frecuency dependece of the water protons magnetic relaxation and the characteristic param,eters of "site binding". Biophys. Chem., 9:181-194.

Khokhlof, A.R. 1980. On the collapse of weakly charged polyelectrolytes. J. Phys. A: Math. Gen., 13:979-987.

Kienzle-Sterzer, C.A. 1980a. Tensile properties of swollen chitosan fils. Master's of Sience Thesis, M.I.T. Cambridge, Ma. U.S.A.

Kienzle-Sterzer,C.A.; Rodriguez-Sanchez, D., and C.K. Rha. 1980b. Characterization of chitosan films. Rheology Applications. Vol. 3, p. 169. Plenum Publising, New York.

Kienzle-Sterzer, C.A.; Rodriguez-Sanchez, D., and C.K. Rha. 1982a. Mechanical properties of chitosan films: Effect of solvent acid. Makromol. Chem., 183:1353-1357.

Kienzle-Sterzer, C.A.; Rodriguez-Sanchez, D.; Karalekas, D., and C.K. Rha. 1982b. Stress-relaxation of a polyelectrolyte network as affected by ionic strength. Macromolecules, 15:631-634.

Kienzle-Sterzer, C.A.; Rodrigeuz-Sanchez, D., and C.K. Rha. 1982c. Dilute solution behavior of a cationic polyelectrolyte. J. Appl. Polym. Sci., 27:4467-4471.

Kienzle-Sterzer, C.A.; Rodriguez-Sanchez, D., and C.K. Rha. 1983. Hydrodynamic properties of a cationic polyelectrolyte. Submitted to Makromol. Chem.

Kierstan, M. and C. Bucke. 1977. The immobilization of microbial cells, subcellular organelles, and enzymes in calcium alginate gels. Biotechnol. Bioeng., 19: 387-397.

Knorr, D. 1984. Uses of chitinous polymers in food - a challenge for food research and development. Food Technology, 1:85-97.

Koene, R.S.; Nicolai, T., and M. Mandel. 1983. Scalling relations for aqueous polyelectrolyte-salt solution. 3. Osmotic pressure as a function of molar mass and ionic strength in semidilute regime. Macromolecules, 16:231-236.

Kojima, T. 1981 Polyelectrolyte behavior of a polybenzimidazole in formic acid. Polym. J., 13:85-87.

Kokufuta, E.;Matsumoto, W., and I. Nakamura. 1982. Use of polyelectrolyte complex for immobilization of microorganisms. J. Appl. Polym. Sci., 27:2503-2512.

Kong, N. 1975. A feasibility study of new routes to marine polymers chitin and chitosan. M.Sc. Thesis, University of Washington.

Kosterlitz, J.M. and D.J. Thouless. 1973. Long-rage order and metastability in two-dimensional solid and superfluids. J. Phys., C6: 1181-1196.

Kosugi, J.; Kato, T., and M. Funahashi. 1981. E.P. 3,862,8A2.

Kotin, L. and M. Nagasawa. 1962. Chain model for polyelectrolytes. VII. Potentiometric titration and ion binding in solutions of linear polyelectrolytes. J. Chem. Phys., 35:873-978.

Kowblansky, M. and P. Zema. 1981a. Interaction of sodium ions with the sodium salts of poly(acrylic acid/acrylamide) copolymers of varying charge density. Macromolecules, 14: 166-170.

Kowblansky, M. and P. Zema. 1981b. Effect of polyelectrolyte charge density on calcium ion activity coefficients and additivity in aqueous solutions of calcium acrylamide-acrylic acid copolymers. Macromolecules, 14:1448-1451.

Kraemer, E.O. 1938. Molecular weigths of cellulose and cellulose derivatives. Ind. Eng. Chem., 30:1200-1212.

Kratky, O. and G. Porod. 1949. Rontgenuntersuchung geloster fadenmolekule. Rec. Trav. Chim., 68:1106-1122.

Kumsah, C.A.; Pass, G., and G.O. Phillips. 1976. The interaction between sodium carboxymethylcellulose and water. J. Solution Chem., 5:799-806.

Kurata, M. and H. Yamakawa. 1958. Theory of dilute polymer solutions. II. Osmotic pressure and frictional properties. J. Chem. Phys,. 29:311-325.

Lampert, M.A. and R.S. Crandall. 1980. Nonlinear Poisson-Boltzman theory for polyelectrolyte solutions: The counterion condensate around a line charge as a - function. Chem. Phys. Letters. 72:481-486.

Landell, R.F.; Rembaum, A., and P.S. Yen-Shiao. 1981. U.S. Patent 4,285,819.

Lapanje, S. and F. Gubunsek. 1967. Activity coefficients of counterions in solutions of diethylaminoethyl dextran hydrochloride. Biopolymers, 5:351-356.

Laurent, T.C.; Preston, B.N., and L.O. Sandelof. 1979. Transport of molecules in concentrated systems. Nature, Lond., 278:60-62.

Lee, V. 1974. Solution and shear properties of chitin and chitosan. University Microfilms, Ann Arbor, 74/29,446.

Leon, L.M.; Quintana, J.R.; Martinez, A., and J. Landabidea. 1982. A study of Ptitsyn-Eizner -parameter for flexible polymers. Eur. Polym. J., 18:89-93.

Liberti, P.A. and S.S. Stivala. 1966. Viscosity of aqueous polyelectrolytes. J. Polym. Sci. Part B, 4:137-151.

Lifson, S. 1957. Potentiometric titration, association phenomena, and interaction of neighboring groups in polyelectrolytes. J. Chem. Phys., 26:727-732.

Lusena, C.V. and R.C, Rose. 1953. Preparation and viscosity of chitosan. J. Fish. Res. Board. Can., 10:521-522.

Malette, W.G.; Quigley, H.J.; Gaines, R.D.; Johnson, N.D., and W.G. Rainer. 1983. Achieving hemostasis using chitosan. Ann. Thorac. Surg., 36:55-59.

Mackie, J.S. and P. Meares. 1955. The diffusion of electrolytes in a cation- exchange resine membrane. I. Theoretical. Proc. Roy. Soc. Lond., A232:498-510.

Magdelenant, H.; Turr, P., and M. Chemla. 1974. Study of the self-diffusion coefficients of cations in the presence of an acidic polysaccharide. Biopolymers, 13: 1535-1549.

Magdelenant, H.; Turr, P.; Trivanti, P.; Chemla, M.; Menez, R., and M. Drifford. 1979. The effect of counterion substitution on the transport properties of polyelectrolyte solutions. Biopolymers, 18:187-201.

Manley, R.S. 1956. Properties of ethylhydroxyethycellulose molecules in solution. Arkiv. Kemi., 9:519-531.

Manning, G.S. 1969a. Limiting laws and counterion condensation on polyelectrolyte solutions. I. Colligative properties. J. Chem. Phys., 51:924-933.

Manning, G.S. 1969b. Limiting laws and counterion condensation on polyelectrolyte solutions. II. Self/diffusion of small ions. J. Chem. Phys., 51:3249-3257.

Maning, G.S. 1975. A limiting law for the conductance of the rod model of a salt-free polyelectrolyte solution. J. Phys. Chem., 79:262-270.

Manning, G.S. 1977. Limiting laws and counterion condensation in polyelectrolyte solutions. IV. The approach to the limit and the extraordinary stability of the charged fraction. Biophys. Chem., 7:95-102.

Manning, G.S. 1978. The molecular theory of polyelectrolyte solutions with applications to the electrostatic properties of polynucleotides. *Q. Rev. Biophys.*, 2: 179-249.

Manning, G.S. 1979. Counterion binding in polyelectrolyte theory. *Accts. of Chem. Res.*, 12:443-449.

Manning, G.S. 1981. Limiting laws and counterion condensation in polyelectrolyte solutions. 6. Theory of the titration curve. *J. Phys. Chem.*, 85:870-877.

Manning, G.S. and A. Holtzer. 1973. Application of polyelectrolyte limiting laws to potentiometric titration. *J. Phys. Chem.*, 77:2206-221.

Matsushima,Y. and N. Fujii. 1957. Studies on aminohexoses. 4. N-deacylation with hydrazine and deamination with nitrous acids, a clue to the structure of aminopolysaccharides. *Bull. Chem. Soc. Jpn.*, 30:48-50.

Messing, R.A. 1980. Immobilized microbes. *Annu. Reports Ferment. Processes*, 4:105-107.

Miyamoto, S. 1981. Application of electrostatic persistance length to polyelectrolyte solutions. *Makromol. Chem.*, 182:559-568.

Moorjani, M.N.; Khasim, D.I.; Rajalakshami, P.; Puharajappa, P., and B.L. Amla. 1978. Chitosan of high viscosity and protein as a valuable by-product from squilla. *Proc. 1st. Int. Conf. Chitin/Chitosan*, R.A.A. Muzzarelli - E.R. Parisier Ed.,pp.210-215.

Mosbach, K.H. and K.G.C. Nilsson. 1982. PCT International W08200660.

Muroga, Y.; Suzuki, K.; Kawagushi, Y., and M. Nagasawa. 1972. Potentiometric titration of polyelectrolytes having stiff backbones. *Biopolymers*, 11:137-140.

Muzzarelli, R.A.A. 1973. *Natural Chelating Polymers*. Pergamon Press, New York.

Muzzarelli, R.A.A. 1977. *Chitin*. Pergamon Press, Oxford, U.K.

Muzzarelli, R.A.A. 1980. Immobilization of enzymes on chitin and chitosan. *Enzyme Microb. Technol.*, 2:177-182.

Muzzarelli, R.A.A. 1983. Chitin and its derivatives: New trends of apply research. Carbohydr. Polym., 3:53-75.

Naczk, M.; Synowiecki, J., and Z.E. Sikorski. 1981. The gross chemical composition of Antartic krill shell waste. Food Chemistry, 7:175-183.

Nagasawa, M. 1971. Potentiometric titration and conformation of synthetic and natural polyelectrolytes. Pure Appl. Chem., 26:519-536.

Nagasawa, M. 1975. Thermodynamic and hydrodynamic properties of polyelectrolytes. J. Polym. Sci. Part C,49:1-32.

Nagasawa, M. and A. Holtzer. 1964. The use of the Debye-Huckel approximation in the analysis of protein potentiometric titration. J. Am. Chem. Soc.,86:531-535.

Nagasawa, K. and N. Tanoura. 1972. Reaction between carbohydrates and sulfuric acid. 3. Depolymerization and sulfonation of chitosan by sulfuric acid. Chem. Pharm. Bull., 20:157-162.

Nagasawa, M.; Murase, T., and K. Kondo. 1965. Potentiometric titration of stereoregular polyelectrolytes. J. Phys. Chem., 69:4005-4012.

Namikawa, R.; Ozazaki, H.; Nakanishi, K.; Matsuno, R., and T. Kamikubo. 1977. Diffusion of amino acids and saccharides in solutions of dextrans and its derivatives. Agric. Biol. Chem., 41:1003-1009.

Nilsson, K. and K. Mosbach. 1980. Sephadex-bound histimine in the catalysis of ester hydrolysis. FEBS. Letters, 118:145-152.

Noda, I.; Isuge, T., and M. Nagasawa. 1970. The intrinsic viscosity of polyelectrolytes. J. Phys. Chem., 74:710-719.

Nordgren, R.; Jones, D.A., and H.A. Wittcoff. 1973. U.S. Patent 3,723,408.

Numazaki, S.O. and K. Kito. 1975. Chitosans. Japan 126,787.

Nystrom, B. and J. Roots. 1980. Diffusion transport of sucrose, -alanine, and bovine serum albumin in aqueous solutions of hydroxypropyl cellulose. Eur. Polym. J., 16:201-204.

Nystrom, B.; Moseley, M.E.; Stilbs, P., and J. Roots. 1982. Solvent self-diffusion in semidilute polystyrene solutions. Pulsed-gradient spin-echo measurements on a standard Fourier transform n.m.r. spectrometer. Polymer 22:218-220.

Odijk, T. 1979. Possible scalling relations for semidilute polyelectrolyte solutions. Macromolecules, 12:688-693.

Odijk, T. 1983. On the limiting law solution of the cylindrical Poisson-Boltzman equation for polyelectrolites. Chem. Phys. Letters, 100:145-150.

Ogston, A.G. and T.F. Sherman. 1961. Effects of hyaluronic acid upon diffusion of solutes and flow of solvent. J. Physiol., 156:67-74.

Ogura, K.; Kanamoto, T.; Itoh, M.; Miyashiro, H., and K. Tanaka. 1980. Dynamic mechanical behavior of chitin and chitosan. Polym. Bull., 2:301-307.

Ogura, K.; Kanamoto, T.; Sannan, T.; Tanaka, K., and Y. Iwakura. 1982. Liquid crystalline phases based on chitosan and its derivatives. Proc. 2nd. Int. Conf. on Chitin-Chitosan. S. Hirano - S. Tokura Eds., pp. 39-44.

Ohmiya, K.; Ohashi, H.; Kobayashi, T., and S. Shimizu. 1977. Hydrolysis of lactose by immobilized microorganisms. Appl. Environ. Microbiol., 33:137-149.

Okimasu, S. 1958. Physicochemical behavior of carboxymethylchitosan as polyampholyte. 1. Molecular weight by ligth-scattering. Nippon Nogei Kagaku Kaishi, 32:298-302.

Oosawa, F. 1957. Theory of thermodynamic properties of polyelectrolyte solutions. J. Polym. Sci., 23:421-446.

Oosawa, F. 1971. Polyelectrolytes. Marcel Dekker, New York.

Overbeek, J. Th. 1948. Dissociation and titration constants for polybasic acids. Bull. Soc. Chim. Belges, 57:252-263.

Park, I.W.; Choi, K.H., and K.K. Park. 1983. Acid-base equilibrium properties of chitosan. Bull. Korean. Chem. Soc., 4:68-72.

Patel, J.R.; Patel, C.K., and R.D. Patel. 1967. Solution properties of sodium carboxymethyl amylose in salt solution. *Staerke*, 19:330-335.

Phanibhusan, R-C. and K. Kalidas. 1970. The adiabatic compressibility of poly(acrylicacid) and polyacrylamide in aqueous solution. *J. Appl. Polym. Sci.*, 14:2937-2946.

Pittalis, F.; Bartoli, F., and G. Giovannoni. 1983. E.P. 77092 A2.

Podlas, T.J. and P. Ander. 1969. Interaction of sodium and potassium ions with - and - carrageenan in aqueous solutions with and without added salt. *Macromolecules*, 2:432-436.

Podlas, T.J. and P. Ander. 1970. Interaction of sodium and potassium ions with sodium and potassium alginate in aqueous solutions with and without salt. *Macromolecules*, 3:154-158.

Prager, S. 1960. Diffusion in inhomogeneous media. *J. Chem. Phys.*, 33:122-131.

Pramanik, A.G. and P.K. Choudhary. 1968. Physicochemical and macromolecular properties af sodium amylose xanthate in dilute solutions. *J. Polym. Sci. Part. A-1*, 6:1121-1127.

Ptitsyn, O.B. and Yu.E. Eizner. 1959. Hydrodynamic properties of semirigid macromolecules in solution. *Zh. Tekh. Fiz.*, 29:1117-1120.

Ptitsyn, O.B. and Yu.E. Eizner. 1962. Hydrodynamic properties of semirigid macromolecules in solution. *Dokl. Akad. Nauk. SSSR*, 142:134-136.

Randall, J.M.; Randall, V.G.; McDonald, G.M.; Young, R.N., and M.S. Masri. 1979. Removal of trace quantities of nickel from solution. *J. Appl. Polym. Sci.*, 23:727-732.

Ramakrishnam, C. and N. Prasad. 1972. Rigid body refiment and conformation of -chitin. *Biotech. Biophys. Acta*, 261:123-135.

Revah-Moiseev, S. and A. Carroad. 1981. Conversion of enzymatic hydrolysate of shellfish wasre chitin to single-cell protein. *Biotechnol. Bioeng.*, 23:1067-1072.

Rha, C.K. 1975. Theories and Principles of Viscosity, In: Theory, Determination and Control of Physical Properties of Food Materials. C.K. Rha, Ed., D. Reidel, Publs. Holland.

Rice, S.A. and M. Nagasawa. 1961. Polyelectrolyte solutions. Academic Press, New York.

Ricoh Co. 1983. Japan Kokai Tokkyo Koho 8340545A2.

Rigby, G.W. 1936. U.S. Patent 2,030,880.

Rinaudo, M. and M. Milas. 1976. Activity coefficients of small ions in aqueous mixtures of polyelectrolytes and simple electrolytes. Chem. Phys. Letters, 41:456-459.

Ripoll, C.; Muller, G., and E. Selegny. 1971. Determination du pKa de la poly(vinyl-2 pyridine) et des cofficients d'activite des patits ions de la solution. Discussion et conclusion. Eur. Polym. J., 7:1393-1407.

Robinson-Lang, E. 1982. M.S. Thesis, Chemical Engineering, M.I.T., Cambridge, Mass.

Robinson, G.; Ross-Murphy, S.B., and E.R. Morris. 1982. Viscosity-molecular weight relationships, intrinsic chain flexibility and dynamic solution properties of guar galactomannan. Carbohydr. Res,. 107:17-32.

Rodriguez Sanchez, D. and C.K. Rha. 1981. Chitosan globules. J. Fd. Technol. (U.K.), 16:469-474.

Rodriguez-Sanchez, D.; Kienzle-Sterzer, C.A., and C.K. Rha. 1982. Intrinsic viscosity of chitosan solutions as affected by ionic strength. Proc. 2nd. Int. Conf. on Chitin-Chitosan. S. Hirano - S. Tokura, Eds., p. 30-34.

Rodriguez-Sanchez, D. and C.K. Rha. 1984. Patent Pending.

Ross, P.D. and L. Scruggs. 1968. Viscosity study of DNA. II. The effect of simple salt concentration on the viscosity of high molecular weight DNA and application of viscometry to the study of DNA isolated from T4 and T5 bacteriphage mutants. Biopolymers, 6:1005-1013.

Roth, L.W.; Shepperd, I.M., and R.K. Richards. 1954. Anticoagulants and other pharmacologic effects of sulfates chitin in animals. Proc. Soc. Exptl. Biol. Med., 86: 315-318.

Rudall, K.M. 1963. The chitin-protein complex of insect cuticles. *Adv. Insect Physiol.*, 1:257-263.

Rudall, K.M. 1968. Chitin and its association with other molecules. *J. Polym. Sci.*, 28:83-89.

Rudall, K.M. and W. Kenchington. 1973. The chitin system. *Biol. Rew.*, 48:597-636.

Ruiz-Herrera, J. 1978. The distribution and quantitative importance of chitin fungi. *Proc. 1st. Int. Conf. on Chitin-Chitosan*, R.A.A. Muzzarelli - E.R. Parisier Ed., pp. 11-15.

Russel, W.B. 1982. Polyelectrolyte solutyions: Counterion condensation and intermolecular interactions. *J. Polym. Sci., Polym. Phys. Ed.*, 20:1233-1247.

Rymdem, R. and J. Carlfors. 1982. Diffusion of glucose derivatives in aqueous solutions of hydroxypropyl cellulose. *Polymer*, 23:325-331.

Saif, S.R.; Tani, Y., and J. Ogata. 1975. Preparation of glucose phosphate through the transphosphorilation with immobilized cells. *J. Ferment. Technol.*, 53:380-397.

Saini, R. and W.R. Vieth. 1975. Preparation and characterization of several forms of immobilized invertase. *J. Appl. Chem. Biotechnol.*, 25:115-121.

Samuels, R.J. 1981. Solid State characterization of the structure of chitosan films. *J. Polym. Sci., Polym. Phys. Ed.*, 19:1081-1090.

Sanborn, R.H. and E.F. Arleman. 1955. Rates of solvolysis of triphenylmethyl, t-butyl and n-butyl halydes. *J. Am. Chem. Soc.*, 77:3726-3731.

Sasaki, S. and A. Minakata. 1977. Potentiometric titration and interaction of neighboring groups in polyelectrolytes. *Rpts. Progrss. Polym. Phys. Jpn.*, 20:73-76.

Sawayanagi, Y.; Nambu, N., and T. Nagai. 1982. Disolution behavior of poorly soluble drugs from ground mixtures with chitin and chitosan. *Chem. Pharm. Bull.*, 30:4216-4223.

Schorigin, P.P. and E. Hait. 1935. Acetylation of chitin. *Ber.*, 68-B:971-973.

Schrorigin, P.P. and N.N. Makarova-Semlyanskaya. 1935. Methyl athers of chitin. *Ber.*, 68-B:969-971.

Scott, K.G. 1977. U.S. Patent 4,056,392.

Seale, R.; Morris, E.R., and D.A. Rees. 1982. Interactions of alginates with univalent cations. *Carbohydr. Res.*, 110:101-112.

Senior, M.B.; Gorrell, S.L., and E. Hamori. 1971. Light-scattering and potentiometric titration studies on poly-L-Tyrosine in aqueous solutions. *Biopolymers*, 10: 2387-2396.

Shaefgen, J.R. and C.F. Trivisonno. 1952. Polyelectrolyrte behavior of polyamides. I. Viscosity of solutions of linear polyamides in HCOOH and in H SO . *J. Am. Chem. Soc.*, 74:2715-2727.

Sharp, P. and V.A. Bloomfield. 1968. Intrinsic viscosity of wormlike chains with excluded volume effect. *J. Chem. Phys.*, 48:2149-2153.

Shinoda, K.; Kanamori, K., and M. Hisashita. 1979. Japan Kokai Tokkyo Koho, 79:73463.

Shimizu, S.; Morioka, H.; Tani, Y., and J. Ogata. 1975. Metabolism of pantothenic acid in microorganisms. XI. Synthesis of coenzyme A by immobilized microbial cells. *J. Ferment. Technol.*, 53:77-91.

Simha, R. 1952. A treatment of the viscosity of concentrated suspensions. *J. Appl. Phys.*, 23:1020-1024.

Sinkovitz, D.M. and R. Slagel. 1973. U.S. Patent 3,770,673.

Sirica, A.E. and R.J. Woodman. 1974. Selective agregation of L-1210 leukemia cells by polycation chitosan. *J. Nat. Cancer Inst.*, 47:377-388.

Skerjanc, J. 1973. Concentration dependence of the apparent molal volumes of polyelectrolytes. *J. Phys. Chem.*, 77:2225-2228.

Skylyar, A.M.; Gamzazado, A.I.; Rogovina, L.Z.; Titkova, L.V.; Pavlova, S.S.A.; Rogozhin, S.V., and G.L. Slonimskii. 1981. Study of rheological properties of dilute and moderately concentrated solution of chitozane. *Visokomol. Soed.*, A23:1394-1403.

Slonitskii, S.V.; Frisman, E.V.; Valeev, A.K., and A.M. El'yashevich. 1980. Calculation of the intrinsic viscosity of synthetic and biological polyelectrolytes of different rigidity. *Molekuly. Biolog.*, 14:484-489.

Smidsrod, O. 1970. Solution properties of alginate. *Carbohydr. Res.*, 13:359-372.

Smidsrod, O. and A. Haug. 1971. Estimation of the relative stiffness of the molecular chain in polyelectrolytes from measurements of viscosity at different ionic strengths. *Biopolymers*, 10:1213-1221.

Smirnoff, W.A. 1975. U.S. Patent 3,862,007.

Southwick, J.G.; Jamieson, A.M., and J. Blackwell. 1982. Conformation of xanthan dissolved in aqueous urea and sodium chloride solutions. *Carbohydr. Res.*, 99:117-127.

Southwick, J.G.; Jamieson, A.M., and J. Blackwell. 1983. Relationships between molecular structure and rheology for xanthan, a naturally occurring bacteria-produced cellulose derivative. *J. Appl. Polym. Sci. Appl. Polym. Symp.*, 37:385-392.

Spegt, P.; Tondre, C.; Weill, G., and R. Zana. 1973. Selectivity of fixation of monovalent ions on a polyphosphate. *Biophys. Chem.*, 1:55-61.

Staiko, G. and A. Dondos. 1983. Determination of the statistical segment of wormlike polymers using viscometric data. *Eur. Polym. J.*, 19:555-560.

Stivala, S.S.; Zweig, J.E., and J. Ehrlich. 1981. Dilute solution properties of *Streptococcus salivaricus* levan and its hydrolysates. *A.C.S. Symposium Series. Solution Properties of Polysaccharides*, p. 101-110.

Stockmayer, W.H. and M. Fixman. 1963. On the estimation of unperturbed dimensions from intrinsic viscosities. *J. Polym. Sci. Part C*, 1:137-148.

Strauss, U.P. and Y.P. Leung. 1965. Volume changes as a criterion for site binding of counterions by polyelectrolytes. *J. Am. Chem. Soc.*, 87:1476-1480.

Suzuki, Y. and H. Uedaira. 1970. Hydration of potassium hyaluronate. *Bull. Chem. Soc. Japan.*, 43:1892-1894.

Swanson, G,R.; Dudley, E.G., and K.J. Williamson. 1980. The use of fish and shellfish wastes as fertilizers and feedstuffs. Handbook of Organic Waste Conversion, Ed. M.W.M. Bewick. Von Nostrand Reinhold Co., New York.

Takahashi, A. and M. Nagasawa. 1964. Excluded volume of polyelectrolytes in salt solutions. J. Am. Chem. Soc., 86:543-551.

Takahashi, A.; Kato, T., and M. Nagasawa. 1967. The second virial coefficients of polyelectrolytes. J. Phys. Chem., 712:2001-2009.

Takeda, M. and E. Abe. 1962. Isolation of crustacean chitin: Decalcification by disodium ethylenediaminotetraacetate and enzymatic hydrolysis of incidebtal proteins. Norisho Suisan Koshusho Kenkyu Hokoku, 11:339-406.

Tamura, M. and M. Kurata. 1952. Viscosity of binary mixture of liquids. Bull. Chem. Soc. Japan, 25:32-40.

Tan, J.S. and S.P. Gasper. 1974. Dilute solution behavior of polyelectrolytes. Intrinsic viscosity and light scattering studies. J. Polym. Sci., Polym. Phys. Ed., 12:1785-1804.

Tan, J.S. and P.R. Marcus. 1976. Ion binding of sulfonate containing polyelectrolytes. J. Polym. Sci. Polym. Phys. Ed., 14:239-250.

Tan, J.S. and P.R. Marcus. 1976. Ion binding in sulfonate containing polyelectrolytes. J. Polym. Sci., Polym. Phys. Ed., 14:239-250.

Tanford, C. 1961. Physical Chemistry of Macromolecules. John Wiley and Sons. New York.

Toda, K. 1975. Interparticle mass transfer study with a packed column of immobilized microbes. Biotechnol. Bioeng., 17:1729-1747.

Tondre, C. and R. Zana. 1971. Ultrasonic absorption as a probe for the study of site binding of counterions in polyelectrolytes solutions. J. Phys. Chem., 75:3367-3372.

Tondre, C. and R. Zana. 1972. Apparent molal volumes of polyelectrolyte solutions. J. Phys. Chem., 76:3451-3459.

Tondre, C.; kate, K.M., and R. Zana. 1978. Density and ultrasonic absorption studies of the interaction between polyions and hydrophobic counterions. *Eur. Polym. J.*, 14:139-143.

Tokura, S.; Nishi, N., and J. Noguchi. 1979. Studies on chitin. III. Preparation of chitin fibers. *Polymer J.*, 11:781-786.

Triebel, H.; Reinert, K.E., and J. Strassburger. 1971. Persistance length of DNA from hydrodynamic measurements. *Biopolymers*, 10:2619-2621.

Trifiletti, R. and P. Ander. 1979. Interaction of small ions with sodium deoxyribonucleic acid by self/diffusion studies. *Macromolecules*, 12:1197-1201.

Trivedi, H.C.; Patel, C.K., and R.D. Patel. 1981a. Studies on carboxymethylated cellulose: Potentiometric titration, 1. *Makromol. Chem.*, 182:3561-3567.

Trivedi, H.C.; Patel, C.K., and R.D. Patel. 1981b. Studies on carboxymethylated cellulose: Potentiometric titration. 2. *Makromol. Chem.*, 182:3569-3577.

Trivedi, H.C.; Patel, C.K., and R.D. Patel. 1981c. Studies on carboxymethylcellulose. *Angew Makromol. Chem.*, 95: 155-172.

Trivedi, H.C. and R.D. Patel. 1982. Studies on carboxymethyl cellulose: Estimation of the relative stiffness of polyions. *Makromol. Chem., Rapid Commun.*, 3:317-320.

Trujillo, R. 1968. Preparation of carboxymethyl chitin. *Carbohydr. Res.*, 7:483-485.

Tsukada, S. and Y. Inoue. 1981. Conformational properties of chito-oligosaccharides: Titration, optical rotation and carbon-13 n.m.r. studies of chito-oligosaccharides. *Carbohydr. Res.*, 88:19-38.

Ueda, T. and Y. Kobatake. 1973. Effective fixed charge density goberning membrane phenomena. VI. Activity coefficients and mobilities of small ions in aqueous solutions of poly(styrene sulfonic acid). *J. Phys. Chem.*, 77:2995-3007.

Unitika Ltd. 1982. Japan Kokai Tokkyo Koho 8206362A2.

Unitika Ltd. 1983. Japanesse Patent 58,134,101.

Utracki, L.A. and R. Simha. 1963. Corresponding state relations for the viscosity of moderately concentrated polymer solutions. *J. Polym. Sci. Part A*, 1:1089-1098.

Utracki, L.A. and R. Simha. 1981. Viscosity of polymer solutions: Scalling relationships. *J. Rheol.*, 25:329-350.

Vallin, D.; Huguet, J., and M. Vert. 1980. Partial methylation of poly[thio-1-(N,N-dimethylaminomethyl)-ethylene] and conformational behavior of resulting dibasic polyelectrolytes. *Polymer. J.*, 12:113-124.

van Dijk, W.; van der Touw, F., and M. Mandel. 1981. Model estimated for the relaxation time of the counterion distribution in the radical direction around a rodlike, charge macromolecule. *Macromolecules*, 14:1554-1558.

Vieth, W.R.; Wang, S.S., and R. Saini. 1973. Immobilization of whole cells in membraneous form. *Biotechnol. Bioeng.*, 15:565-573.

Von Weimarn, P.P. 1927. Coversion of fibroin, chitin, casein and simillar substances into the ropyplastic state and colloid solution. *Ind. Eng. Chem.*, 19:109-110.

Vorlop, K.D. and J. Klein. 1981. Formation of spherical chitosan biocatalysts by ionotropic gelation. *Biotechnol. Letters*, 3:9-16.

Wang, J.H. 1954. Theory of the self-diffusion of water in protein solutions. A new method for studying the hydration and shape of protein molecules. *J. Am. Chem. Soc.*, 76:4755-4765.

Weetall, H.H. and M.A. Bennett. 1976. Industrial applications of immobilized enzymes: Present state of the art. *5th Int. Ferment. Symp., Berlin*, pp. 229-238.

Wells, J.D. 1973. Thermodynamics of polyelectrolyte solutions. An empirical extension of the Manning Theory to finite salt concentration. *Biopolymers*, 12:223-227.

Wen, W.Y. and S. Saito. 1964. Apparent and partial molal volumes of five symmetrical tetraalkylammonium bromides in aqueous solutions. *J. Phys. Chem.*, 68:2639-2644.

Whitcomb, P.J. and C.W. Maccoko. 1978. Rheology of xanthan gum. *J. Rheol.*, 22:493-505.

Wolff, C. 1978. Viscosite des solutions des polyelectrolytes. *J. Physique*, *Colloque C2*, 39:169-173.

Wolfrom, M.L. 1958. U.S. Patent 2,832,766.

Wolfrom, M.L.; Vercellotti, J.R., and D. Horton. 1964. Methylation studies on carboxyl-reduced heparin: 2-amino-2-deoxy-3, 6-di-O-methyl- -glucopyranose from the methylation of chitosan. *J. Org. Chem.*, 29:547-550.

Wu, A.C.M.; Bough, W.A.; Holmes, M.R., and B.E. Perkins. 1978. Influence of manufactoring variables on the characteristics and effectiveness of chitosan products. III. Coagulation of cheese whey solids. *Biotechnol. Bioeng.*, 20:1957-1970.

Wu, A.C.M. and W.A. Bough. 1978. A study of the variables in chitosan manufacturing process in relation to molecular weight distribution, chemival characteristics and waste treatment effectiveness. *Proc. 1st. Int. Conf. Chitin/Chitosan*, R.A.A. Muzzarelli - E.R. Parisier Ed., pp. 88-94.

Yamakawa, H. 1971. *Modern Theory of Polymer Solution*. Harper and Row, New York.

Yamakawa, H. and M. Fujii. 1974. Intrinsic viscosity of wormlike chains. Determination of the shift factor. *Macromolecules*, 7:128-135.

Yeh, R. and A. Isihara. 1971. Intrinsic viscosity of polyelectrolytes in salt solutions. *J. Polym. Sci Part A-2*, 9:373-390.

Yoshida, N. 1978. Self-diffusion of small ions in polyelectrolyte solutions. *J. Chem. Phys.*, 69:4867-4874.

Yuan, L.; Dougherty, T.J., S.S. Stivala. 1972. Semiempirical approach to the viscvosity of polyelectrolyte solutions. *J. Polym. Sci. Part A-2*, 10:171-183.

Yuryev, V.P.; Braudo, E.E., and V.B. Tolstoguzov. 1983. Structural study of the solutions of acidic polysaccharide. III. Evaluation of the electrostatic and nonelectrostatic contributions to the counterion activity coefficient in the presence of low-molecular salt with the same ion. *Colloid and Polym. Sci.*, 261:210-214.