# APPLICATION OF CHITOSAN AND PASS FOR THE REMOVAL OF TURBIDITY AND COLOUR FROM WATER

Thesis submitted to the
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By

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# DECLARATION

I hereby declare that the work presented in this thesis entitled "Application of chitosan and PASS for the removal of turbidity and colour from water" is based solely on the original work done by me under the guidance of Dr. V N. Sivasankara Pillai, Professor and Director, School of Environmental Studies, Cochin University of Science and Technology, and that no part of this thesis forms part of any other thesis or has been submitted previously for the award of any other degree.

Kochi 682 016,

15th January 2002.

Ravi Divakaran

# CERTIFICATE

This is to certify that the contents of this thesis entitled "Application of chitosan and PASS for the removal of turbidity and colour from water" are based entirely on the work done by Mr. Ravi Divakaran under my guidance in the School of Environmental Studies, Cochin University of Science and Technology, and that no part of this thesis forms part of any other thesis or has been submitted previously for the award of any other degree.

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**- Ravi Divakaran**.

# PREFACE

Water is the most critical input for any sustainable human activity. The amount of water consumed by the human population of a country can be taken as an indicator of the development achieved by it. For example, the average per capita use of water in the United States rose from 40 L per day in 1900 to 600 L per day in 1990 [Manahan, 1994]. In India, the estimated per capita use of water is 150 L per day in the metropolitan cities [Manual on Water Supply and Treatment, 1999]. For Kochi city with a population of 1.2 million (1991 census), the daily requirement is 180 million litres per day (MLD). The installed capacity is about 200 MLD, and after a transmission loss of about 30%, about 140 MLD is available to the public [Water Authority, personal communication].

Of all the water available on earth, only less than 2.5%, ($3.37 \times 10^7$ km$^3$) is fresh water required for human activities. Of this, readily accessible fresh water is limited to rivers (0.004%, $1.1 \times 10^3$ km$^3$), fresh water lakes (0.36%, $1.25 \times 10^5$ km$^3$) and accessible groundwater (12.4%, $4.2 \times 10^6$ km$^3$) [Skinner, 1969; Kitano, 1975]. The currently exploited fresh water is apportioned among irrigation (46%), industries (46%) and domestic use (8%) [Manahan, 1994]. The limited availability of fresh water in a locality even necessitates reuse and recycling of wastewater.

Every type of water use requires a defined quality for safety and best performance in a user environment. For example, feed water for boilers used in industries for generating steam must be free of minerals since their presence will lead to the formation of 'boiler scales' which adversely affect heat transfer. Potable water should essentially contain small quantities of minerals, should be aesthetically pleasing with no colour or odour and totally devoid of pathogenic microorganisms and harmful chemical contaminants. Water for use in laundries must be soft and free of iron, which may produce stains on cloth. Accordingly, raw water is amended by appropriate treatment.

Treatment methodology is dictated by target impurities present in raw water. One of the most significant impurities in raw water, which gives sensory judgement and needs removal, is suspended solids (SS), quantified by optical technique as turbidity. Suspended solids like microorganisms or organic and inorganic materials may cause turbidity. Turbidity assumes significance in water quality considerations since it can protect pathogenic microorganisms during disinfection, cause derating in sensory judgement and can cause scaling and consequent deterioration in the engineering properties of materials.

Suspended particulate matters are of colloidal size and therefore not removed by filtration. The suspension is stable because suspended particles are negatively charged, causing repulsion among them preventing agglomeration and settling. This makes special treatment necessary for bringing particles together to form larger aggregates that can settle easily and be removed. Charge neutralization, sweep-floc and bridging by polymeric substances are the main mechanisms by which colloids are destabilized and agglomerated into heavier particles, which then settle under gravity. The term “coagulation” is commonly used for this process when charge neutralization is the principal mechanism of colloid destabilization (L. *coagulare* – to be driven together), but the term “flocculation” is preferred in the other cases (L. *flocculus* – a small tuft of wool) [Faust and Aly (1983)]. Terms such as “aggregation”, “agglomeration”, “agglutination” and “conglomeration” are also sometimes used [Eisma, 1993].

The most common and conventional method for removing turbidity from water is by coagulating with alum or iron salts, and settling the precipitate in suitably designed clarifiers followed by filtration. But the sludge produced is bulky, difficult to dewater and accumulates in the dumping grounds causing environmental problems. Synthetic polymers such as polyacrylamide and polyethyleneoxide have been investigated for their ability to remove turbidity. They overcome many of the disadvantages of conventional methods, but are cost-effective only when rapid flocculation and reduction in sludge volume are demanded.

Considering the aforementioned situation, it was felt that more easily available and eco-friendly materials must be developed for removing turbidity from water. The results of our studies in this direction are presented in this thesis.

The thesis comprises of nine chapters, with a common bibliography at the end. Chapter 1 gives an introduction to the nature of turbidity and colour usually present in water. Chapter 2 discusses the nature and availability of the principal material used in these studies, namely chitosan. Chapters 3 to 8, which deal with the actual experimental work, are further subdivided into (a) introduction, (b) materials and methods, (c) results and discussion and (d) conclusions. Chapter 9 summarises the entire work so as to put the results and conclusions into proper perspective.

**List of papers presented / published:**

(1) A portion of the work presented in chapter 3 of this thesis has been published in the following form:
Divakaran R and Pillai V N S (2001) Flocculation of kaolinite suspensions in water by chitosan. *Water Research*, 35:16, 3904-3908.

(2) Work presented in chapter 4 of this thesis has been accepted for publication in *Water Research* and is now in the press under the title "Flocculation of river silt using chitosan" by Divakaran R and Pillai V N S, Ref. No. WR3652.

(3) Work presented in chapter 5 of this thesis has been accepted for publication in the *Journal of Applied Phycology* and is now in the press under the title "Flocculation of algae using chitosan" by Divakaran R and Pillai V N S.

(4) Work presented in chapter 8 of this thesis has been presented as paper No. 1.8. titled "Investigations on the applicability of an industrial ferrous effluent for water treatment" by Divakaran R and Pillai V N S, at the *National Seminar on Eco-friendly Environment for Sustainability (SEFES)*, sponsored by the University Grants Commission and held at the Department of Zoology, Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu, India during 27 & 28 March 2000.

# CONTENTS

## Chapter 1

# Nature of turbidity and colour in water - an overview.

### 1.1 Introduction

The word “turbidity” means the opalescence or loss of transparency of water caused by the presence of minute suspended particles in it. Suspended matter is present in all natural waters. It may be a very small amount, as in the crystal-clear waters in caves and in some parts of the ocean, but microscopic inspection up to now has always indicated the presence of at least some suspended particulate material. Because of this ubiquitous presence, and because of the physical and chemical properties of the particulate material itself, the suspended matter forms an integral part of the worldwide geochemical, biological and geological cycles in the aquatic environment. By convention, particulate matter in suspension is defined as the material that is retained on a 0.4 to 0.5 μm pore size filter, although smaller particles are sometimes found. Smaller material is considered to be dissolved, and no upper limit has been fixed [Eisma, 1993]. Particles larger than 0.1 mm in size usually settle very rapidly unless they are present in highly turbulent waters or have very low densities. Even when present, they can be rapidly removed by filtration through sand beds. Dissolved molecules in water usually are smaller than 10 nm. Therefore particulate matter that require special treatment to be removed fall in the range from 10 nm to 0.1 mm and are of particular interest to our work. The approximate time required by particles of various sizes to settle when left

Table 1.1

Surface area and settling times for particles of different diameters

[Adapted from Powell, 1954]

| Diameter of particles (mm) | Examples | Total surface area* ($m^2$) | Time needed to settle by 1 m |
|---|---|---|---|
| 10 | Gravel | $3.14 \times 10^{-4}$ | 1 s |
| 1 | Coarse sand | $3.14 \times 10^{-3}$ | 10 s |
| 0.1 | Fine sand | $3.14 \times 10^{-2}$ | 2 min |
| 0.01 | Silt | $3.14 \times 10^{-1}$ | 1.8 hr |
| 0.001 | Bacteria | 3.14 | 7.5 days |
| 0.0001 | Colloidal particles | 31.4 | 2 years |
| 0.00001 | Colloidal particles | $3.14 \times 10^{2}$ | 20 years |
| 0.000001 | Colloidal particles | $3.14 \times 10^{3}$ | 200 years |

*Area for particles of indicated size produced from a particle 1 cm in diameter.

undisturbed in still waters, assuming them to be perfectly spherical so as to follow Stoke's law, and a specific gravity of 2.65, have been calculated and are presented in Table 1.1

Particles of a random shape, flakes etc. encounter more drag in the aqueous medium. These and other particles of lesser density will obviously remain suspended for a longer time. Figure 1.1 gives a comparison of the size spectrum of various kinds of waterborne particles with the pore-sizes of various kinds of specialised filters [Faust and Aly, 1983].

Turbidity is quantified by an optical method called nephelometry and is expressed in nephelometric turbidity units (NTU). The method makes use of the scattering of light by suspended particles in a direction perpendicular to the incident beam when a strong beam of white light is passed through the suspension (Tyndall effect). The nephelometer is calibrated against a formazine suspension prepared as per specifications [Standard Methods, 1995] to which an arbitrary but fixed value of turbidity is assigned.

## 1.2 Sources of suspended particles

The total amount of suspended matter in the rivers and lakes of the world is estimated to be about $4.25 \times 10^8$ kg [Eisma, 1993]. Virtually all suspended matter is supplied either by terrestrial erosion or through the production of organic matter, biogenic carbonate and biogenic opal (amorphous silica). Volcanism supplies an insignificant amount, but can be regionally and temporarily an important source.

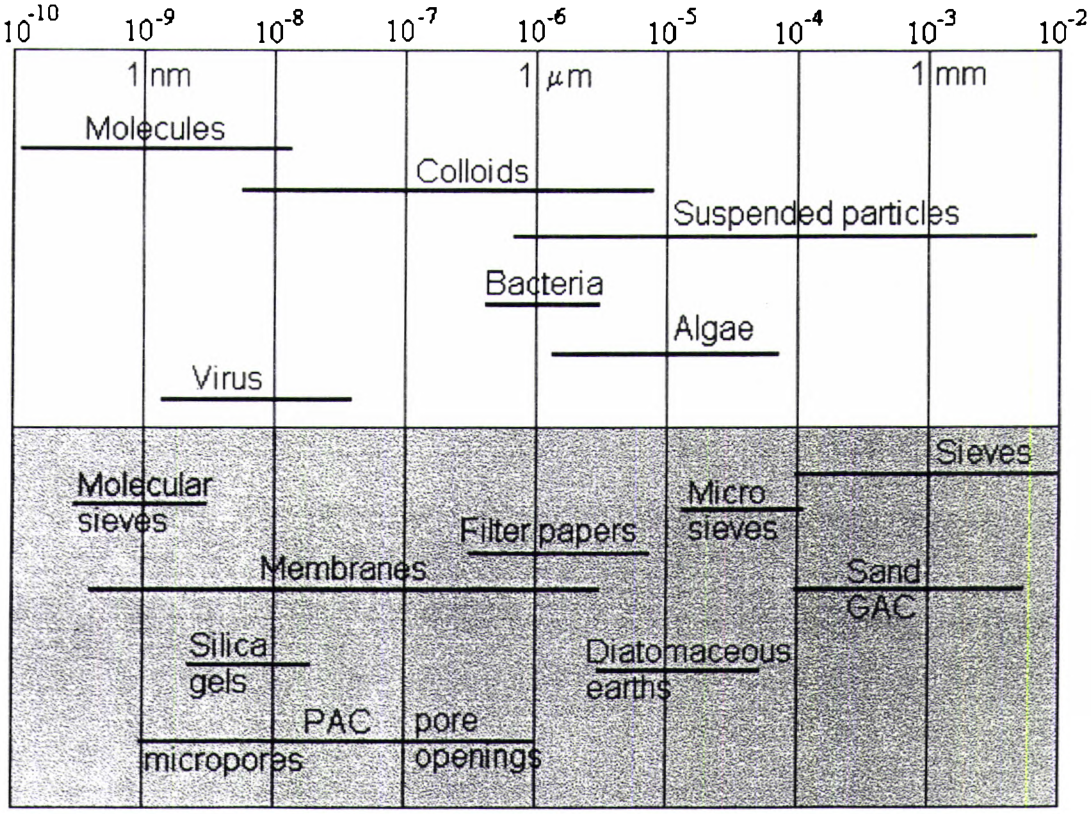


Figure 1.1

Size spectrum of water-borne particles (in metres) and pore sizes of filter media [Faust and Aly, 1983].

The mineral particles in water bodies are an assemblage of particles of different types, which reflects the rock type and weathering conditions in the source areas. Mineral particles found in suspended matter are those that are resistant to weathering. Table 1.2 indicates the composition of the principal minerals in suspended matter. In actual existence in natural waters, these may contain adsorbed constituents.

Besides mineral material, rivers transport particulate organic matter in suspension. The average organic content of suspended matter is about 4.5%. Organic matter in water bodies come from primary and secondary production in rivers and lakes, erosion from soil, human waste discharges, industrial effluents and agricultural run-offs [Eisma, 1993]. In common terminology, these include decaying animal and plant matter, humic and fulvic acids, colouring matter, pathogenic bacteria and viruses, algae, pesticides and other industrial chemicals.

## 1.3 Problems caused by suspended matter in water

The presence of suspended particles makes water opaque, coloured and sometimes malodourous. It thus becomes objectionable for human use by virtue of sensory quality. The opacity reduces penetration of light in water bodies and affects photosynthesis of submerged aquatic plants. The suspended matter is also a nuisance in industrial applications, affecting product quality and damaging equipment, leading to financial loss.

Table 1.2.

Composition of minerals in suspended matter [Eisma, 1993].

| | |
|---|---|
| Quartz | $SiO_2$ |
| *Feldspars* | |
| Orthoclase | $KAlSi_3O_8$ |
| Albite | $NaAlSi_3O_8$ |
| Anorthite | $CaAl_2Si_2O_8$ |
| *Clay minerals* | |
| Kaolinite | $Al_4(OH)_8[Si_4O_{10}]$ |
| Chlorite | $(Al, Mg, Fe)_3(OH)_2\,[(Al, Si)_4O_{10}]\,Mg_3(OH)$ |
| Illite | $(K, H_2O)\,Al_2(H_2O, OH)_2\,[AlSi_3O_{10}]$ |
| Montmorillonite | $\{(Al_{2-x}Mg_x)(OH)_2\,[Si_4O_{10}]\}^{-x}\,Na_x\,.\,n\,H_2O$ |
| Calcite / aragonite | $CaCO_3$ |
| Opal | $SiO_2$ (amorphous) |

The suspended particles, being very small in size, have a very large surface area per unit mass. This makes them good adsorbents and catalysts. Many poisonous chemical substances are thus adsorbed on the particle surfaces and transported over long distances. They also keep bacteria and viruses adsorbed on their surface and shield them from the effect of disinfectants applied during water treatment processes. There is almost a universal presence of coliform bacteria in surface water bodies. Thus they pose a threat to human health when drawn for domestic use.

Governing bodies of various communities have therefore made separate recommendations or specification of standards regarding maximum permissible turbidity in potable water supplied by public water supply units, and water for various industrial and recreational purposes.

## 1.4 Regulations regarding turbidity for drinking water supply

The WHO recommendations regarding turbidity for drinking water is as follows [Guidelines for drinking-water quality, 1993]:

"The appearance of water with a turbidity of less than 5 nephelometric turbidity units is usually acceptable to consumers, although this may vary with local circumstances. However, because of its microbiological effects, it is recommended that turbidity be kept as low as possible. No health-based guideline value for turbidity has been proposed."

The U.S. Environmental Protection Agency (EPA) regulation [Faust and Aly, 1983] for drinking water supply is that:

1) The monthly average turbidity may not exceed 1 NTU.
2) The two-day average turbidity may not exceed 5 NTU.

Manual on Water Supply and Treatment 3rd edn, CPHEEO, Government of India, (1999) recommends that:

1) Turbidity should be less than 1 NTU to be generally acceptable.
2) The limit may be extended up to 10 NTU in the absence of alternate sources.

## 1.5 Necessity for removal of suspended matter

Sand, gravel etc. settle rapidly on standing, but finer particles like silt, bacteria and plankton must be coagulated before they can be made to settle. Thus clarification of water becomes necessary for the following reasons:

1) To remove colour and odour to make water aesthetically pleasing.
2) To remove bacteria, virus etc. and make water more hygienic. Even apparently harmless suspended particles may act as vehicles for concentrating and transporting harmful pollutants.
3) To prevent formation of trihalomethanes (THM) in subsequent disinfection of the water using chlorine.
4) To make water more transparent so that photosynthesis is not affected. Thus aquatic plants and animals in ponds and aquaria may have better living conditions.

5) To meet specifications recommended for drinking water supplies and various industrial and recreational applications such as process and boiler feed waters, swimming pools etc.

## 1.6 Nature of colouring matter in water

The presence of various kinds of algae may impart blue, green, yellow or sometimes even reddish-brown colour to water bodies and impart a grassy, musty or even fishy odour to the water [Faust and Aly, 1983]. Apart from the presence of suspended matter as described earlier, the presence of a wide variety of water-soluble organic and inorganic compounds from various sources may also produce objectionable colour in the water. Dye-house effluents are one of the most obvious among these.

Pickling wastes and effluents from electrochemical industries and tanneries contain coloured inorganic salts of iron, nickel, chromium etc. Such inorganic material may also be leached out into water through the weathering of rocks or through volcanic activity.

But the most ubiquitous of all colouring matter present in natural water bodies is organic matter from the soil. Most surface water sources have a certain amount of organic matter of natural origin, the so-called humic substances. These compounds are degradation products of plant detritus and present in high concentrations in lake water and ground water giving the water an yellowish-brown colour [Gjessing, 1976]. These contain a large number of oxygenated functional groups like carboxyl, phenolic and alcoholic hydroxyls, aldehydic and keto groups as well as some nitrogenous groups. These are therefore very good chelating agents and may play a role in binding and transporting heavy metals. These are also adsorbed to the surface of clay minerals.

Other sources of organic colouring matter can be agricultural run-offs, wash-water from pig, cattle and poultry farms, and effluents from breweries, tanneries and pharmaceutical industries. The coir industry in Kerala extracts fibre by soaking coconut husks (outer covering of the fruits of the palm tree *Cocus nucifera*) by submerging them in shallow water bodies for several months. The process is known as coir retting. Retting is based on the action of bacteria and moisture to dissolve or rot away much of the cellular tissues and gummy substances surrounding bast-fibre bundles, thus facilitating separation of the fibre [Encyclopaedia Britannica]. Natural water retting employs stagnant or slow-moving waters, such as ponds, bogs, slow streams and rivers. Water in such bodies aquire a dark brown colour from the lignaceous materials leached out from the husks, becomes highly polluted and requires treatment. Engineered tank retting is practiced now a days for greater control and better product quality.

The hue and intensity of the colour in water contaminated by such organic substances are influenced greatly by the pH conditions in the water. Conversely, their presence also considerably affects the pH of the water body.

## 1.7 Regulations regarding colour in drinking water supply

The WHO recommendation regarding the maximum permissible limit of colour in drinking water is as follows [Guidelines for drinking-water quality, 1993]:"Colours above 15 TCU (true colour units) can be detected in a glass of water by most people. Colours below 15 TCU are usually acceptable to consumers, but acceptability may vary according to local circumstances. No health-based guideline value is proposed for colour in drinking water."

Manual on Water Supply and Treatment 3rd edn, CPHEEO, Government of India, (1999) recommends that:

1) Colour should be less than 5 units on the Pt-Co scale to be generally acceptable.

2) The limit may be extended up to 25 units in the absence of alternate sources.

## 1.8 Removal of colouring matter

The most obvious method for removing all types of colouring matter from water is adsorption by granular activated carbon (GAC) or powdered activated carbon (PAC). The method is usually very expensive and removal of the adsorbed material can become a problem especially when PAC is used. Since the organic matter gets easily adsorbed to the suspended particulate matter (including PAC), their removal by flocculation and settling can be cost effective.

Table 1.3.

Commonly used coagulants and flocculants [Kemmer, 1979].

| Common name | Formula | pH of 1% soln. | Physical form | Availability of active constituent. |
|---|---|---|---|---|
| Alum | $Al_2(SO_4)_3.\ 14\ H_2O$ | 3.4 | Lump | 17% $Al_2O_3$ |
| | | | Liquid | 8.5% $Al_2O_3$ |
| Lime | $Ca(OH)_2$ | 12 | Lump | > 90% CaO |
| | | | Powder | 93 to 95% CaO |
| | | | Slurry | 15 to 20% CaO |
| Ferric chloride | $FeCl_3.\ 6\ H_2O$ | 3 to 4 | Lump | 20% Fe |
| | | | Liquid | 20% Fe |
| Ferric sulfate | $Fe_2(SO_4)_3.\ 3\ H_2O$ | 3 to 4 | Granular | 18.5% Fe |
| Copperas | $FeSO_4.\ 7\ H_2O$ | 3 to 4 | Granular | 20% Fe |
| Sodium aluminate | $Na_2Al_2O_4$ | 11 to 12 | Flake | 46% $Al_2O_3$ |
| | | | Liquid | 2.6% $Al_2O_3$ |

## 1.9 Conventionally used coagulants and flocculants

A list of conventionally used coagulants are given in Table 1.3 [adapted from Kemmer, 1979] along with their chemical composition and important properties.

## 1.10 Mechanism of actiou of coagulants

Particles in most natural waters acquire a negative charge because of the negatively charged phenolic, carboxylic acid and sulfonic acid groups present on the adsorbed lignins, tannins, humic acids, detergents etc. and functionality present on bacterial cell walls. All particles have the same type of charge, hence they repel each other and do not aggregate.

There are two layers of ions around the particle. One is at the surface of the particle itself. This is a dense layer of positive charges collected from the surrounding medium (since the negatively charged particles attract positive charges towards them) and is called the Stern layer. It is a rigid layer and supposed to move constantly along with the particles through the medium (see Figure 1.2) This layer then attracts and maintains a diffuse layer of negative charges from the medium, called the Guoy-Chapman layer. Since the particle surface is charged and the bulk of the medium is neutral, there is a potential difference between the surface and the bulk medium some distance away from it. The potential difference between the Stern layer and the bulk medium is called the Nernst potential and that between the Guoy-Chapman layer and the bulk medium is called the Zeta potential.

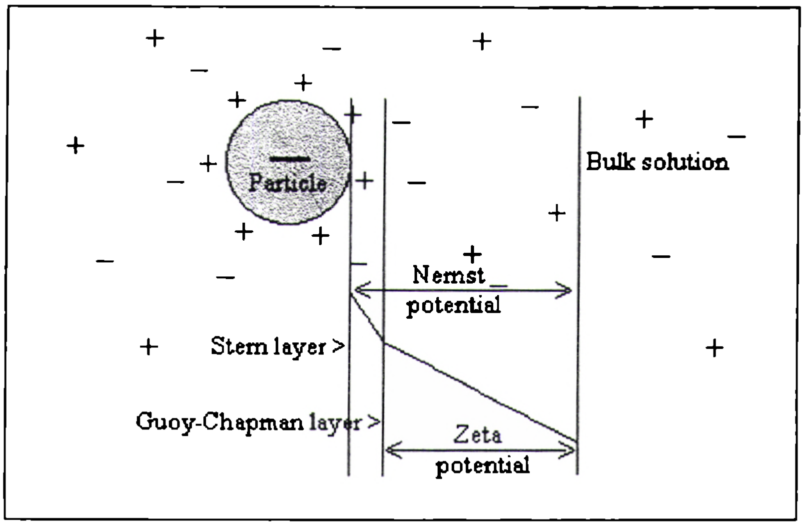


Figure 1.2.

Charge distribution around a suspended particle

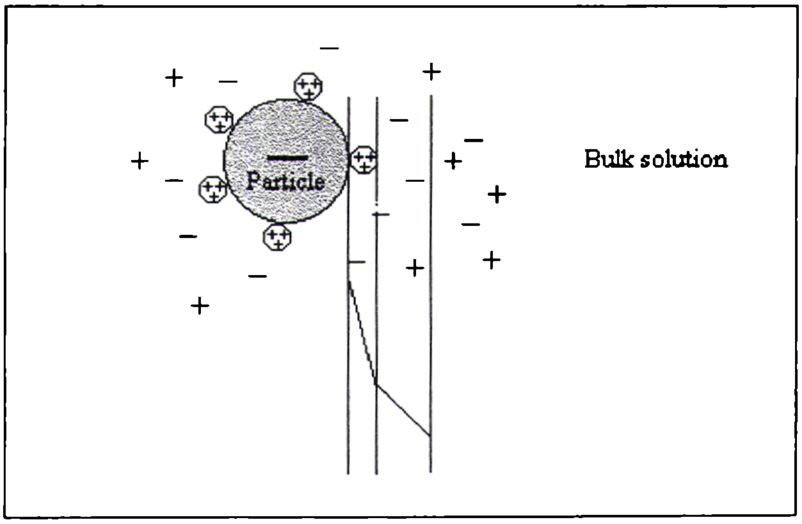


Figure 1.3.

Compression of charged layers by highly charged cations

- Illustration of Hardy-Schulze rule.

Measurement of the Zeta potential is important in the study of kinetics of coagulation.

The charge and concentration of the counterions in the Stern layer help to reduce the effect of the surface charge and therefore reduce the repulsion between the particles. Generally, the only positive counterion obtainable from water molecule is the hydronium ion, which is present only in very low concentrations at neutral pH. If the water contains dissolved salts, the cations derived from them enter the Stern layer and provide a more effective neutralisation of the surface charge. This tends to bring the particles closer to each other and form aggregates. Thus addition of salts like NaCl can induce coagulation of suspended matter in water.

If the counterions are di- or trivalent cations, the effect on the neutralisation of surface charge is doubled or tripled, leading to more effective coagulation using lower concentrations of added metal salts. This is known as the Schulze-Hardy rule (see Figure 1.3). This is the reason why salts of trivalent metal ions such as iron and aluminum are used as coagulants in water treatment plants.

At least four distinct mechanisms of colloid destabilisation exist [Faust and Aly, 1983]. These are (1) compression of the double-layer, (2) neutralisation of charges by adsorption of counter-charged ion, (3) entrapment in a precipitate and (4) adsorption and inter-particle bridging by the adsorbate.

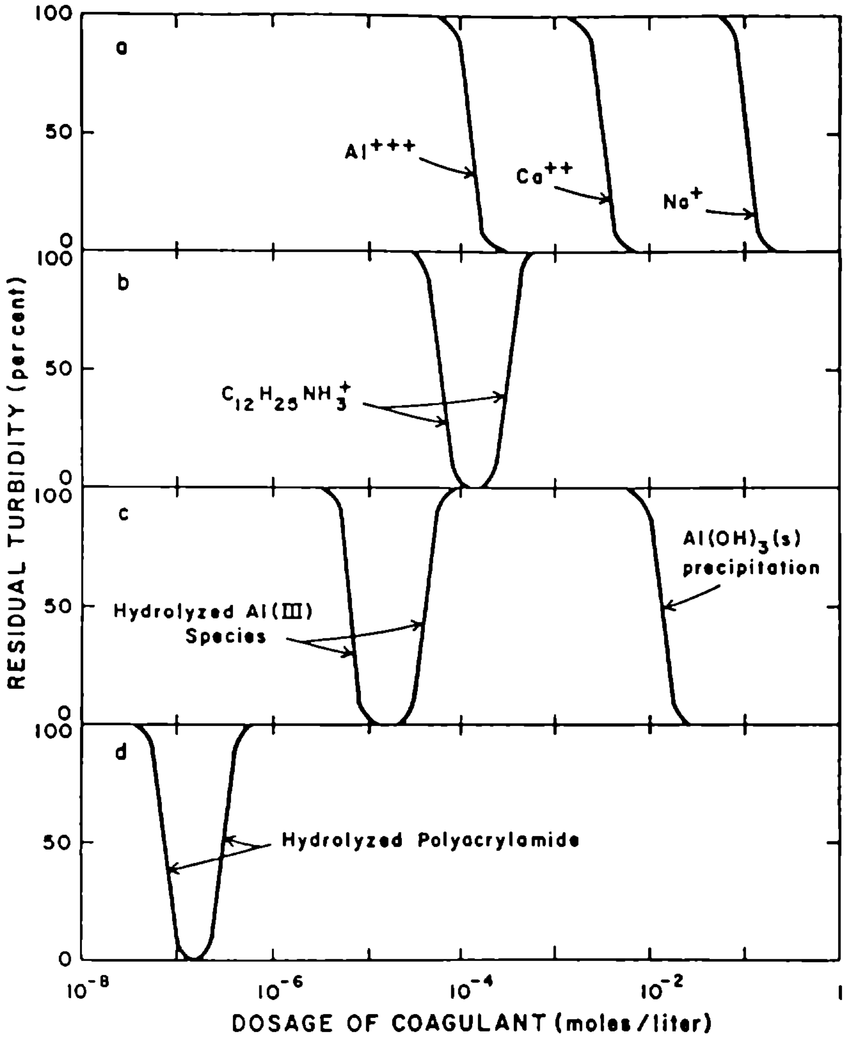


Figure 1.4

Coagulation curves for various types of coagulants.

[Faust and Aly, 1983]

### *Compression of the double-layer:*

When addition of an electrolyte (metal salt) causes no specific interaction between it and the particle, these counterions enter into the double layer and determine its nature. The double layer thickness will decrease, and the Nernst and Zeta potentials will decrease. Particles can come closer to each other, and if a sufficient amount of an "indifferent" electrolyte is added, coagulation occurs. This effect increases greatly as the valence of the counterion increases (Schulze-Hardy rule). That is, a lesser concentration of trivalent ion will bring about coagulation than a divalent ion, which in turn requires a lesser concentration compared to a monovalent ion. (See Figure 1.4 'a').

### *Adsorption and charge neutralisation:*

Colloidal suspensions of negatively charged silver iodide particles can be coagulated using dodecylammonium ions ($C_{12}H_{25}NH_3^+$). On the basis of electrostatic models, this monovalent cation should induce coagulation in a manner similar to $Na^+$ ions. But when about a decimole of $Na^+$ is required for coagulation, about $6 \times 10^{-5}$ moles of the organic amine is effective. This suggests a coagulation mechanism in addition to electrostatic interaction. Further, addition of more of the organic amine was found to *cause a restabilisation of the colloid accompanied by charge reversal.* This can be explained by an adsorption of excess of amine on the colloid particle. (See Figure 1.4 'b').

### *Entrapment in a precipitate*

Colloidal silver iodide can be coagulated using a low dosage of $6 \times 10^{-6}$ moles of aluminum nitrate. The colloidal system was restabilised and the charge reversed at higher dosages of about $4 \times 10^{-5}$ moles. On increasing the concentration further, there is another zone of coagulation at still higher doses of about $10^{-2}$ moles where $Al(OH)_3$ is precipitated (see Figure 1.4 'c'). In this second region of coagulation, the coagulant is precipitated rapidly, floc is formed, and an interaction occurs with the colloid. This is frequently called "orthokinetic coagulation" or the "sweep floc" formation. It is the entrapment of a low zeta potential colloid within a precipitate. The coagulants $Al(OH)_3$, $Fe(OH)_3$, etc. physically destabilise the colloid.

### *Adsorption and inter-particle bridging*

There has been a large increase recently in the use of synthetic organic polymers as coagulants or as coagulant aids. These are effective at very low concentrations (see Figure 1.4 'd'). Neutral or even anionic polymers are sometimes found to be very effective. Obviously, an electrostatic mechanism is not able to explain these observations. A "bridging" mechanism was proposed by La Mer and Healy to account for destabilisation of colloidal systems by high molecular weight organic polymers.

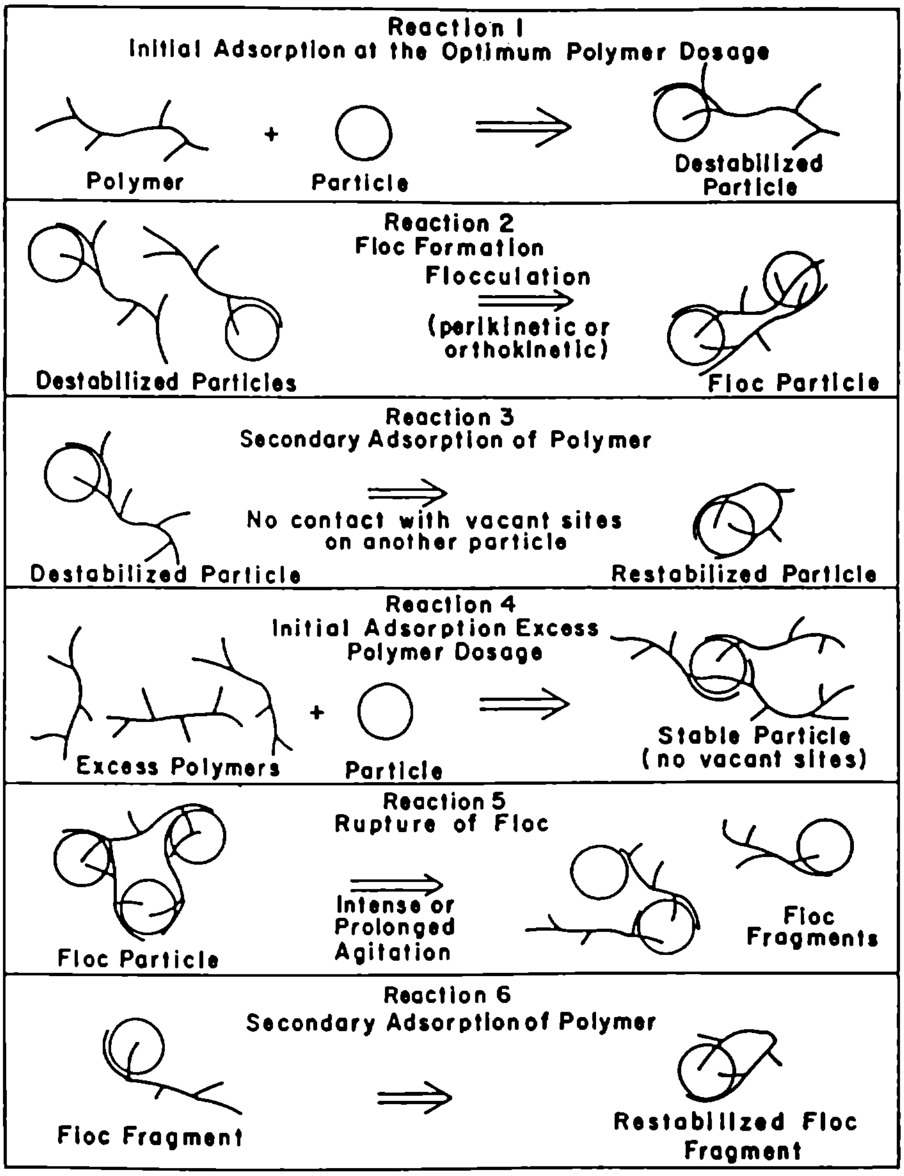


Figure 1.5

The bridging mechanism of coagulation.

[Faust and Aly, 1983]

In order for a polymer molecule to act as an effective destabiliser, it must contain constituents that can interact with sites on the colloidal particle. The attachment of the polymer to the particle may lead to coagulation. The unattached portion of the polymer can interact with a second particle, forming a bridge between the particles. Longer the polymer chain, larger will be the number of particles incorporated (see Figure 1.5). This may also lead to coagulation and an increase in the mass and density of the floc. A third possibility is that bridging may not occur, the entire polymer chain may surround the particle, leading to stabilisation of the particle. This type of restabilisation is not due to charge reversal in the case of an anionic polymer, but may be due to saturation of the particle's surface with excess polymer. Further, extended rapid agitation of the floc may lead to rupture of the bridge, fragmentation of the floc and possible restabilisation.

### 1.11 Kinetics of particle aggregation

Flocculation and coagulation of colloids, by whatever mechanism, depend on the frequency of collisions and on the efficiency of particle contacts. Whenever suspended particles collide, there are at least three mechanisms of transport bringing the particles together [Stumm and Morgan, 1981].

1. Particles are in motion because of their normal energy (Brownian motion). Any coagulation occuring by this means is called "perikinetic coagulation."

2. When the particles are large enough, or when the fluid shear rate (stirring rate) is high enough, the relative motion from velocity gradients exceeds that by thermal effects. This is called "orthokinetic coagulation."
3. In the sedimentation process, particles with different gravitational settling velocities may collide and aggregate.

### 1.12 Desirable characteristics in the flocs produced

1. Floc density – dense flocs settle fast.
2. Shear resistance – this is required to prevent breakage of flocs during stirring.
3. Filterability – good filterability is desirable for fast and complete removal of flocs.
4. Compressibility – good compressibility is desirable to reduce sludge volume.

### 1.13 Coagulant aids

Flocs formed by hydrolysed metal coagulants may not have all the aforementioned desirable characteristics. Coagulation may also become difficult in the presence of interfering substances. Therefore coagulant aids are sometimes used to aid primary coagulants. These are usually polyelectrolytes or activated silica.

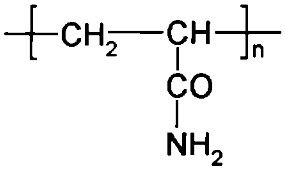
### 1.14 Polyelectrlytes used in coagulation

These are synthetic organic linear or branched polymers of high molecular weight. If the monomer contains an ionisable group such as carboxyl, amino or sulfonic, then the polymer is called a polyelectrolyte. There are cationic, anionic or

(1) Nonionic polymers:

$-\!\left[ CH_2-CH_2-O \right]_n\!-$

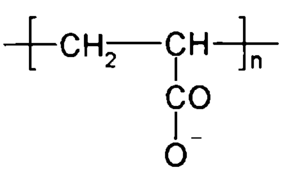
Poly ethyleneoxide (PEO)



$-\!\left[ CH_2-\underset{\underset{NH_2}{|}}{\underset{CO}{\underset{|}{CH}}} \right]_n\!-$

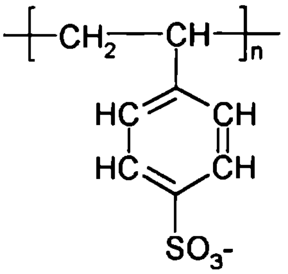
Poly acrylamide (PAM)

(2) Anionic polyelectrolytes:



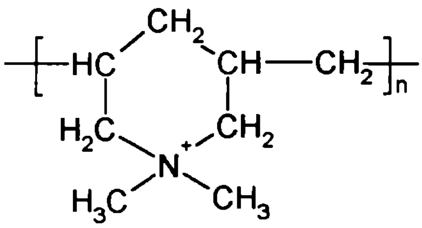
$-\!\left[ CH_2-\underset{\underset{O^-}{|}}{\underset{CO}{\underset{|}{CH}}} \right]_n\!-$

Poly acrylic acid (PAA)



Poly styrene sulphonate (PSS)

(3) Cationic polyelectrolyte:



Poly diallyl dimethyl ammonium

(PDADMA, Cat-Floc)

Figure 1.6.

Commercially available synthetic polymeric flocculants ampholytic (having both positive and negative groups) polyelectrolytes. Nonionic polymers are those without any ionisable groups. Some examples are presented in Figure 1.6 (Faust and Aly, 1983).

The use of high-molecular mass organic compounds as flocculants is not a new development. Many natural organic materials such as starch, cellulose, polysaccharide gums and proteins have been used as flocculant aids. Synthetic polyelecrolytes are relatively more expensive and their use is warranted only for specialized applications.

## 1.15 Jar Tests

The famous jar testing method developed by Cohen is extensively used for quality control of in-plant coagulation process. It is an extremely useful technique for determining parameters such as coagulant dosage, pH, alkalinity and flocculation time, but is not particularly useful for scaling up information.

Jar test results correlate reasonably well with plant tests. They are significant for a number of quality control techniques for the coagulation – filtration process. These are: time required for the appearance of first floc, visual evaluation of floc size, rate of settling of floc, visual or photometric measurement of supernatant or filtrate clarity and colour, and analytical determination of residual coagulant in the supernatant or filtrate. In-plant and continuous monitoring of turbidity is an extremely useful quality control technique for determining the optimum coagulant dosage.

## 1.16 Disposal of coagulation sludges

Direct discharge of sludges from coagulation process into water bodies is undesirable. In many countries this type of disposal is forbidden. Alum sludge is a non-Newtonian, bulky and gelatinous material. It is composed of hydrous aluminum oxide and other inorganic particles such as clay, sand or carbon, and organics such as colour colloids, waste particulates and various types of microorganisms. The total solid content is variable, but is in the range of 1 to 20 g $L^{-1}$ of which 75 to 90% are suspended solids. Alum sludges tend to have neutral pH values. These sludges are readily settlable. But they are very difficult to dewater, have low solid content and large sludge volumes, which make them difficult to handle and subsequently place in a landfill. They tend to accumulate at the site of disposal and may be of concern to the public in relation with Alzheimer's disease [Stauber *et. al.*, 1999].

## 1.17 Necessity for developing new flocculants

From the facts discussed above it is apparent that better flocculants have to be developed, which should have several or all of the following desirable characteristics:

1. They must be easily available locally or manufactured from cheap raw materials.
2. They should be cost effective for continuous application on a large scale.

3. They should be very effective at a very low dosage and should not alter the pH of water considerably from the neutral, so that further corrective treatment is not necessary
4. The residual concentrations of the flocculant in the treated water must be very low or negligible.
5. If at all present, the residual must be non-toxic to man and to aquatic organisms.
6. They should be biodegradable and should not accumulate at the site of disposal.
7. They should be able to remove most or all of the undesirable substances present in the water.
8. They should initiate flocs that grow very rapidly to a large size. This will make processing faster.
9. The sludge produced should be very dense and settle rapidly. This will lead to smaller treatment plants and lower operating costs.
10. The sludge collected should be easy to dewater, have a high solid content and low sludge volume. This makes storage, transport and disposal of the sludge economically viable.

# Chapter 2

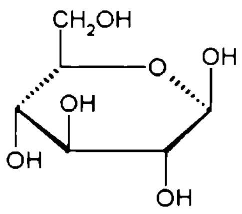
# Chitosan

## 2.1 Introduction

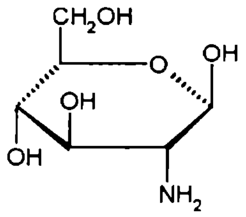
Since the principal material used as flocculant in this study is chitosan, an overview of the physical and chemical nature of chitosan, its availability and suitability for use in water treatment is necessary.

Chitin is a biopolymer widely distributed in nature. In abundance, it is second only to cellulose [Muzzarelli, 1977; Shahidi *et. al.*, 1999]. It was first described by Braconnot in 1811, who obtained it from fungi and called it 'fungine' Chitin is widely distributed as a major component in the tough exoskeleton of arthropods (insects like beetles, millipedes, bees, cockroaches and spiders), crustaceans (aquatic organisms such as crabs, lobsters and shrimps) and in the cell walls of fungi, yeast, and bacteria.

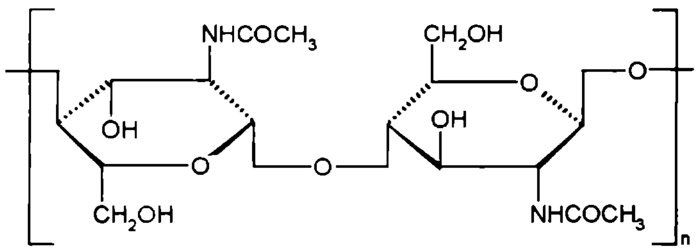
It is the structural material in insects, providing rigidity similar to what cellulose does in plants. It was Odier who coined the word 'chitin' (Gk, meaning 'envelope') in 1823. The chemical structure of chitin as a polymer of N-acetyl glucosamine was conclusively proved only by 1950 [Muzzarelli, 1977]. Chitosan is obtained by deacetylation of chitin using alkali, and is therefore poly(β-D-glucosamine).It is structurally very similar to cellulose, which is poly(β-D-glucose), as shown in Figure 2.1.



(a) β-D-Glucose

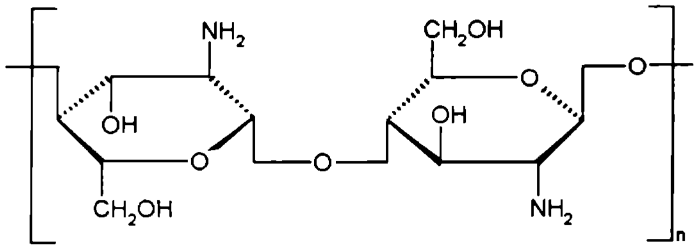


(b) β-D-Glucosamine



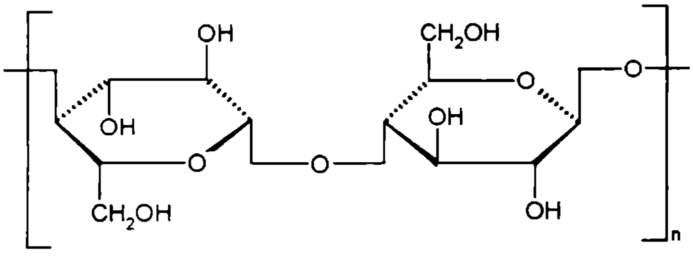
(c) Chitin

poly(N-acetyl- β-D-Glucosamine)



(d) Chitosan

poly( β-D-Glucosamine)



(e) Cellulose

poly( β-D-Glucose)

Figure 2.1
Structural relationship between chitin, chitosan and cellulose

## 2.2 Manufacture of chitosan

Chitosan is available as a commercial byproduct in those parts of the world where fishing and seafood processing are major industries. It is manufactured from the waste shells of crabs, lobsters and prawns. The composition of these waste shells is as follows [Muzzarelli, 1977]:

| | |
|---|---|
| Water | 60 to 70% |
| Calcium carbonate | 50% of dry weight |
| Proteins | 35% of dry weight |
| Chitin | 15% of dry weight |

Extraction of chitin from the shells consist of the following steps:

1. Demineralisation by treating with dilute hydrochloric acid.
2. Removal of proteins by prolonged treatment with caustic soda.
3. Washing and drying of the residual material.
4. Grinding to the required particle size.

Chitin is then deacetylated by autoclaving with 40% caustic soda. The product chitosan is washed free of alkali, dried, powdered and sieved. It is to be noted that the raw material is a throwaway industrial waste, the processing involves only simple steps and no expensive chemicals are involved. Therefore the market price of chitosan will be dictated by demand, the price coming down with increasing demand.

## 2.3 Chemical properties of chitosan:

The extent of deacetylation to produce free amino groups can vary much depending on the strength of the caustic soda used and with the time and temperature of treatment. Commercial chitosan is therefore described as a partially deacetylated chitin, with the extent of deacetylation varying between 60 to 80% [Muzzarelli, 1977]. A brief outline of the chemical nature of chitosan is described below:

1. Appearance: brownish-yellow flakes.
2. Average molecular mass of the order of $10^5$
3. Polymer structure similar to that of cellulose.
4. β-D-glucosamine monomer units. Prolonged boiling with mineral acid gives D-glucosamine.
5. Degree of polymerisation (dp): 600 to 800 monomer units.
6. Decomposes on heating above 150°C (423 K).
7. Insoluble in water and all common organic solvents.
8. Soluble in very dilute acids (0.1 M HCl or 1% acetic acid).
9. Does not react with alkali.
10. Very high metal-binding ability, especially towards $Cu^{2+}$, $Pb^{2+}$ and $Hg^{2+}$
11. Has proven antibacterial activity.
12. Bio-compatible and bio-degradable.

Chitosan is presently being used in the manufacture of cosmetics such as hair sprays, nail polishes, moisturising creams and sunscreen lotions. It is used in medicine as wound dressings, self-absorbing surgical sutures, slimming diets, artificial ligaments, diluent and binder in tablets etc. Chitosan also finds application as viscosity builder in foods and beverages, as animal and fish feeds, in the manufacture of adhesives etc. High affinity for metal ions makes it a good medium for the separation of metal ions in analytical chemistry.

## 2.4 Chitosan as a cationic polyelectrolyte:

Polymers containing ionisable groups on the monomer units are called polyelectrolytes. Since a basic primary amino group is present on the second carbon of each glucosamine unit in chitosan, it dissolves in dilute acids as a salt. If the polymer chain is represented by 'R', the change may be depicted as follows:

$$\text{R-NH}_2\,(s) + \text{HCl}\,(aq) \longrightarrow \text{R-NH}_3^+\,\text{Cl}^-\,(aq)$$

Since the cation $NH_3^+$ is fixed to the polymer chain, chitosan is classified as a cationic polyelectrolyte, and should be capable of binding to the negatively charged surfaces of suspended particles in water and bring about flocculation by the bridging mechanism. Thus, chitosan is expected to act as a cationic flocculant in dilute or neutral medium. In basic medium, chitosan will be insoluble.

## 2.5 Chitosan as a polymeric chelating ligand

The amino groups present in chitosan also make it a good chelating ligand capable of strongly binding to a variety of metal cations. The lone pairs of electrons on the nitrogen atoms and oxygen atoms are donated to the metal ion to form

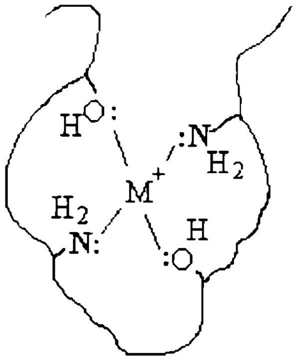


Figure 2.2.
Chelating action of chitosan

coordinate bonds. Since several amino groups and hydroxyl groups are present on the long polymeric chain, the chain can wrap around the metal ion and adopt configurations such that several amino groups are bonded to the metal atom at the same time. This type of chelation leads to the formation of very stable metal complexes. This property makes them useful for concentration of trace metals, removal of radioactive and other harmful heavy metal contaminants, and in chromatographic separation of mixtures of metal ions [Bassi *et. al.* (2000)].

## 2.6 Source of chitosan for the present study

Chitosan used in the studies described in this thesis was obtained from the Central Institute of Fisheries Technology (CIFT), Kochi, and from National Sea Foods, Kochi. The samples were manufactured from waste prawn shells. The material was in the form of a pale brown powder, insoluble in water. It dissolved in 0.1 molar HCl or 1% aqueous acetic acid. A 1% solution of chitosan (w/v) was thick, syrupy and almost colourless.

## 2.7 Characterisation of chitosan used in the present study

The chitosan sample obtained was characterised by (1) estimating the ash content, (2) by comparing the IR spectrum of the chitosan sample with that reported in the literature, (3) by estimating the degree of polymerisation by viscosity measurements and (4) determining the degree of deacetylation, which is a measure of the number of available free amino groups which are the binding sites, by conductometric titration, as described in section 2.7.1.

### 2.7.1 Materials and Methods

Experimental details of methods used for characterisation of chitosan are given below:

#### 2.7.1.1 Estimation of ash content in chitosan:

The chitosan used in our experiments was a commercial product, the characteristics of which can vary depending on the source (shells of prawns, crab etc.) and manufacturing process conditions [Muzzarelli (1977)]. The quality of the product used was therefore beyond our control, and the material was used as received. However, since only one sample from a single production batch was procured and used throughout the work, all experiments were done using material of the same quality. Since it is customary to determine the ash content for products of natural origin, the ash content of a chitosan sample obtained from CIFT was determined gravimetrically by way of providing a characterisation. Since only one sample was used throughout the work, the ash content was determined only once (in duplicate).

About 1 g of the chitosan sample was accurately weighed into a tared silica crucible and heated cautiously using an open Bunsen flame till charring was complete. The residue was then heated strongly for about an hour on an electric Bunsen at red heat. The crucible was then cooled to ambient in a desiccator and weighed. The percentage of ash obtained was calculated based on the chitosan sample taken. Another aliquot of the same chitosan sample was soaked in an ammoniacal solution of ethylenediamine tetraacetic acid disodium salt (EDTA) for 24 hours, filtered, washed with distilled water and dried. The ash content was then determined again as before.

Since the ashes usually consist of inorganic material such as metal oxides and carbonates, a sample of the ash was subjected to inorganic qualitative analysis as given in Vogel [Svehla G., (1979)] to determine the metals present. The results are given in section 2.7.2.1 under results and discussions. No attempts were made for a quantitative estimation of these metals.

### 2.7.1.2 Infrared spectrum of chitosan:

A very dilute (0.1% w/v) solution of chitosan was prepared in 1% aqueous acetic acid. 10 mL of this solution was spread evenly on a horizontal glass plate so as to form a square of side 10 cm.. It was allowed to dry for two days under ambient conditions. The plate was then soaked in a dilute NaOH solution for a few hours to

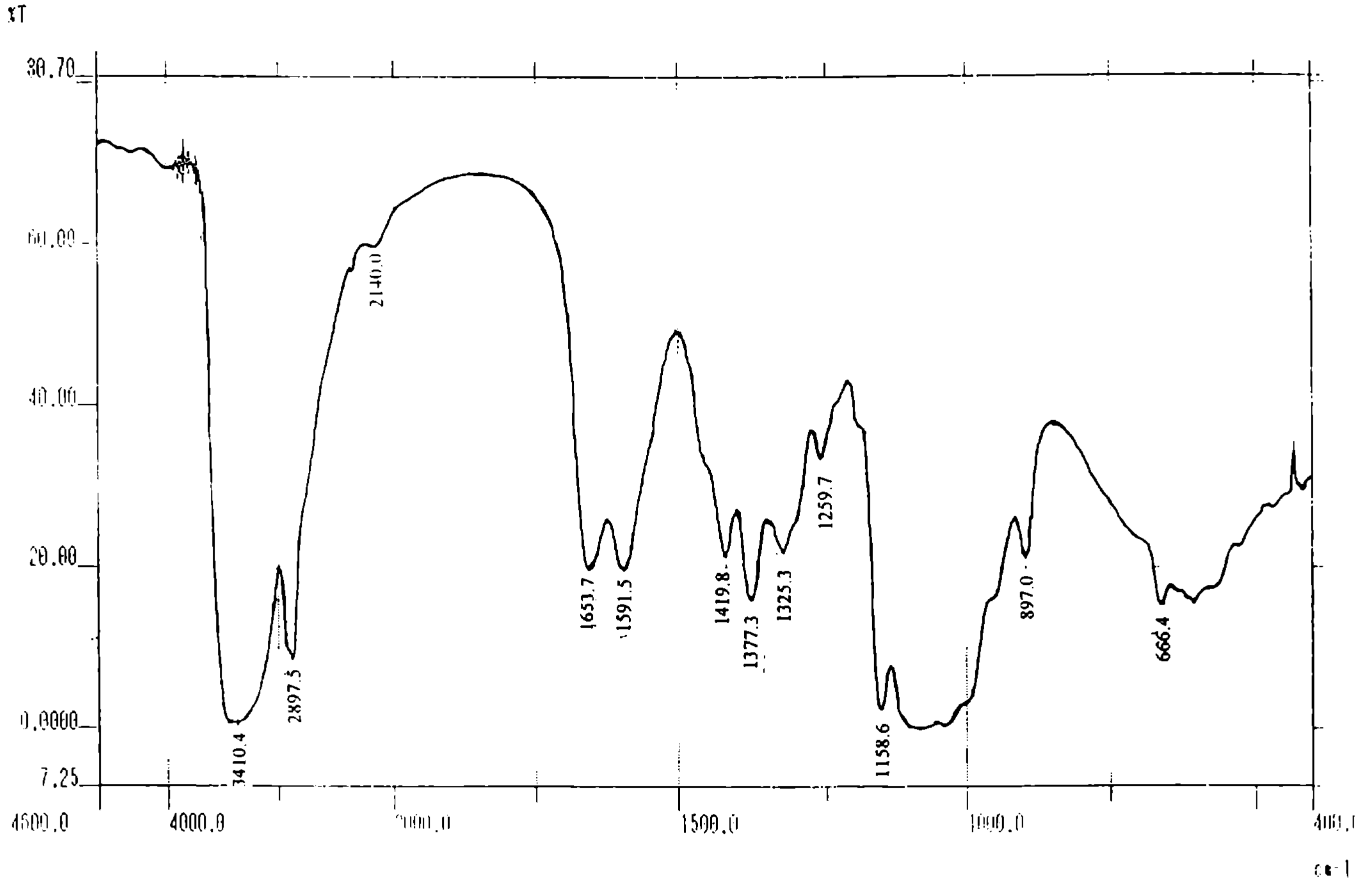


Figure 2.3

Infrared spectrum of chitosan (10 μm film, %T) remove the excess acid and precipitate the chitosan. The plate was then washed repeatedly with distilled water till the washings were neutral. A very thin, perfectly transparent film of chitosan could be easily lifted off the glass plate. It was stretched on a wire mesh and allowed to dry. IR spectral scan of the film was obtained using a Shimadzu model 8101 FT-IR spectrophotometer. This is reproduced in Figure 2.3. Spectrum was also obtained using chitosan powder in the form of a KBr pellet.

### 2.7.1.3 Determination of molecular mass and degree of polymerisation

The bridging mechanism for flocculation described in the previous chapter depends on the chain length of the polymer; longer chains are expected to give better results. Therefore knowledge of the degree of polymerisation of the material being tested for flocculating power is of concern.

For polymeric substances, the number of monomer units present in any individual chain or strand (known as degree of polymerisation, dp) may vary to a large extent from strand to strand. Therefore, only an average estimate of the molecular mass can be given.

The number - average molecular mass $\overline{M}_n$

The weight - average molecular mass $\overline{M}_w$ and

The viscosity - average molecular mass $\overline{M}_v$

are some values usually determined [Rabek, 1980]. It is usually observed that

$$\overline{M}_n < \overline{M}_v < \overline{M}_w$$

Since such polymeric substances are non-volatile and often difficult to dissolve, methods used to obtain molecular masses of small molecules are not applicable. Because of the various conformational changes possible for the chain, and due to the complicated interactions among these chains and of chains with the solvent, even colligative methods such as determination of osmotic pressure or constitutive methods like viscosity measurements can be applied only with some reservation.

In the present case, the viscosity - average molecular mass $\overline{M}_v$ was determined for the chitosan sample used and its degree of polymerisation calculated.

Table 2.1. Important terms used in connection with viscosity measurments.

| Official names | Common names | Quantity |
|---|---|---|
| Viscosity coefficient | Viscosity | $\eta$ |
| Viscosity ratio | Relative viscosity | $\eta_{rel} = \frac{\eta}{\eta_0}$ |
| | Specific viscosity | $\eta_{sp} = \eta_{rel} - 1$ |
| Viscosity number | Reduced viscosity | $\eta_{red} = \frac{\eta_{sp}}{c}$ |
| Limiting viscosity number | Intrinsic viscosity | $[\eta] = \lim_{c \to 0} (\eta_{red})$ |

Viscosity is a measure of the resistance to laminar flow exhibited by a liquid or solution, and represented by the Greek letter '$\eta$' It is defined as the shear stress per unit velocity gradient. If $\eta$ is independent of velocity gradient, the liquid is called a 'Newtonian liquid', and if $\eta$ varies with velocity gradient, it is called 'non-Newtonian' Chitosan solutions in acid behave as non-Newtonian liquids. Some common terms used in connection with the measurement of viscosity of polymer solutions are explained in Table 2.1.

Relative viscosity is the ratio of the viscosity '$\eta$' of a solution having concentration 'c', to that of the solvent or medium, '$\eta_0$' It is therefore a dimensionless quantity. This is easily determined with the help of an Ostwald viscometer. It makes use of the capillary flow method in which the same volume of solution and solvent are allowed to flow the same distance through the same capillary tube. Under these conditions, if both measurements are done at the same temperature, the times of flow depend only on the densities of the solutions and their viscosities. Then we have the relationship:

$$\frac{\eta}{\eta_0} = \frac{\rho t}{\rho_0 t_0}$$

where '$\rho$' and '$\rho_0$' represent the densities of the solution and solvent respectively and 't' and '$t_0$' their times of flow. The limiting viscosity number [$\eta$] is a hypothetical quantity (see Table 2.1) which will be the viscosity of the solution if it had a concentration 'zero' It can be obtained by measuring the viscosity number for solutions of different concentrations in the same solvent, plotting the values against concentration on the x-axis and extrapolating to meet the y-axis (zero c). The y-intercept gives the limiting viscosity number $[\eta]$. The sample concentrations should not be too large because additional effects may arise from intermolecular forces and entanglement of polymer chains.

The viscosity - average molecular mass $\overline{M}_v$ and the limiting viscosity number $[\eta]$ are related by the Mark-Houwink-Sakurada equation [Rabek, 1980]:

$$[\eta] = K \overline{M}_v^{\alpha}$$

where 'K' and '$\alpha$' are constants for a given polymer at a given temperature in a given solvent. '$\alpha$' depends on the thermodynamic interactions between polymer segments and the solvent molecules and is related to the solvent power and expansion factor. The value of '$\alpha$' is unity for a long molecule kinked in random fashion and approaches zero for a chain coiled into a ball [Lee, 1974].

The Mark-Houwink-Sakurada equation may also be written in the form:

$$\log [\eta] = \log K + \alpha \log M$$

which is also referred to as the Staudinger equation [Muzzarelli, 1977]. Therefore a plot of log $[\eta]$ against log M will give a straight line, the slope of which gives '$\alpha$' and the y-intercept is log K. This allows one to determine the values of 'K' and '$\alpha$' for a particular polymer by measuring the limiting viscosity numbers of various samples of it having known uniform molecular mass M (monodisperse samples).

This also requires determination of the molecular mass by some other method such as light scattering or equilibrium sedimentation. Monodisperse polymer samples are seldom available and so carefully selected fractions of the polymer are normally used. Thus Wang *et. al.* reported the values of 'K' and 'α' for samples of chitosan with various degrees of deacetylation in aqueous solutions of 0.2 M acetic acid and 0.1 M sodium acetate at 30°C [Wang *et. al.*, 1991]. The values determined by them for a 69% deacetylated sample of chitosan were as follows:

$$K = 0.104 \times 10^{-3} \qquad \text{and} \qquad \alpha = 1.12$$

Chitosan in acid solutions exhibits the polyelectrolyte effect. There is an abnormal increase in the viscosity of the more dilute solutions because of an enlarged effective volume due to charge repulsion and stretching out of the molecules. When sufficient salt is added to neutralise this charge effect, the viscosity behaviour becomes normal [Muzzarelli, 1977]. For this reason, aqueous medium containing 0.2 M acetic acid + 0.1 M sodium acetate is preferred for viscosity measurements.

***Experimental:***

An Ostwald viscometer was cleaned thoroughly by passing chromic acid through it followed by rinsing several times with distilled water. It was then clamped vertically such that all of the capillary and the bulbs dipped in a water bath maintained at 30 ± 1°C.

Twelve millilitres of glacial acetic acid and 13.6 g sodium acetate trihydrate were dissolved in distilled water and made up to 1 L to get the 0.2 M acetic acid +

0.1 M sodium acetate solvent system. The bottom bulb of the viscometer was filled with this solution. The liquid was then carefully drawn by suction into the upper bulb and into the stem above the upper mark. It was then allowed to flow down freely. The time rquired for the meniscus to pass from the upper mark to the lower mark was measured. The measurement was replicated and the average time was taken as $t_0$ in calculations. The solution was then taken in a 10 mL density bottle and weighed accurately to determine its density $\rho_0$.

A 0.2% solution of chitosan was prepared by dissolving 400 mg of it in 200 mL of the above solvent system. The mixture was kept for some time to dissolve completely. Solutions having concentrations of 0.15, 0.1, 0.075, 0.05, 0.025, 0.0125 and 0.00625% were prepared from this by serial dilution using the solvent system. The time of flow 't' and the density '$\rho$' were determined for each of these solutions in the same manner as described above for the solvent system. All measurements were done within 2 hours of preparing the stock solution. Another set of similar readings were obtained after keeping the solution for about 18 hours.

Since the densities of the solutions and that of the solvent system were found to be the same within the number of significant figures considered for measurement, $\rho/\rho_0$ was unity for all the solutions. Therefore the relative viscosities $\eta_{rel}$ for each solution was calculated using the formula

$$\frac{\eta}{\eta_0} = \frac{\rho t}{\rho_0 t_0} = \frac{t}{t_0}$$

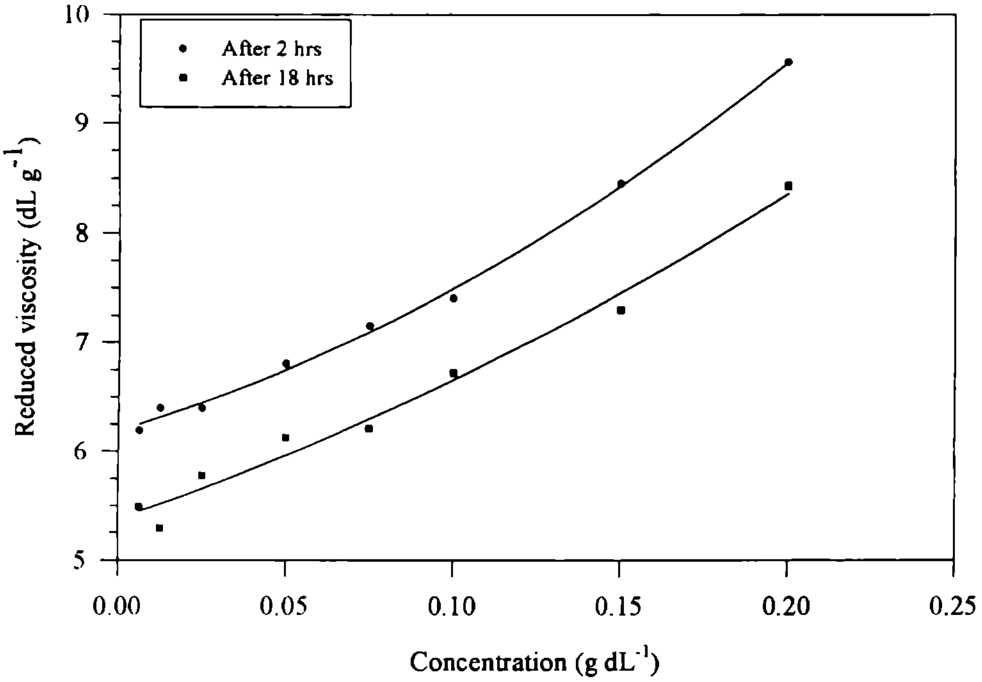


Figure 2.4.

Plot of reduced viscosities of chitosan solutions

against their concentrations

The following values were also calculated for each solution:

$$\eta_{sp} = \eta_{rel} - 1 \qquad \text{and} \qquad \eta_{red} = \eta_{sp}/c$$

The values of reduced specific viscosity '$\eta_{red}$' were then plotted against the concentration 'c' A similar plot was made using values obtained after 18 hours also. Extrapolating the curves to meet the y-axis, the limiting viscosity number $[\eta]$ for the 2-hour sample and for the 18-hour sample were read as 6.2 and 5.4 dL $g^{-1}$ respectively (see Figure 2.4).

The molar mass M was then calculated by substituting the values of K and $\alpha$ determined by Wang *et. al.* and the value of $[\eta]$ determined as above in the Staudinger equation. The degree of polymerisation was obtained by dividing the molar mass of chitosan by 161, which is the molar mass of one glucosamine unit.

### 2.7.1.4 Determination of degree of deacetylation

Two different methods involving conductometric titration were used to determine degree of deacetylation, namely direct acidimetric titration and back titration.

All reagents were prepared from analytical grade chemicals. Finely powdered chitosan was used in the experiment. A digital conductometer, Century model CC 601-P with calibrated platinum sheet electrodes was used. Conductometric titration was carried out under constant speed slow stirring.

In the direct titration method, about 0.25 g of accurately weighed chitosan was added to 25 mL of distilled water in a 100 mL beaker. The suspension was stirred using a magnetic stirrer and slowly titrated using 0.1 M HCl. To get a clearly defined titration curve, the titrant was added in 1 mL increments and conductivity measured after each addition.

In the back-titration method, about 0.25 g of accurately weighed chitosan was added to 25 mL of 0.1 M HCl in a 100 mL beaker and magnetically stirred till dissolved. The contents of the beaker were titrated using standard 0.1 M NaOH solution. Conductivity was measured after stirring for a minute after the addition of each 1 mL increment.

In both cases the measured conductivity was corrected for dilution using the equation:

$$C_{corr} = \frac{C_{obs}\ (V + \Delta V)}{V}$$

where $C_{corr}$ is the corrected conductivity corresponding to the addition of titrant, $C_{obs}$ is the observed conductivity value, V is the initial volume of titrand and $\Delta V$ is the cumulative volume of titrant added at the point of measurement.

In each case, the corrected conductivity values were plotted against the volume of titrant added to determine the end points of the titrations. The plot obtained for direct-titration method is given in Figure 2.5 and that for back-titration method is given in Figure 2.6. Degree of deacetylation was then calculated in each case using the formula:

$$\text{Degree of deacetylation (\%)} = \frac{V_t\, N_t}{V_{in}} \times \frac{M_G}{m_C} \times 100$$

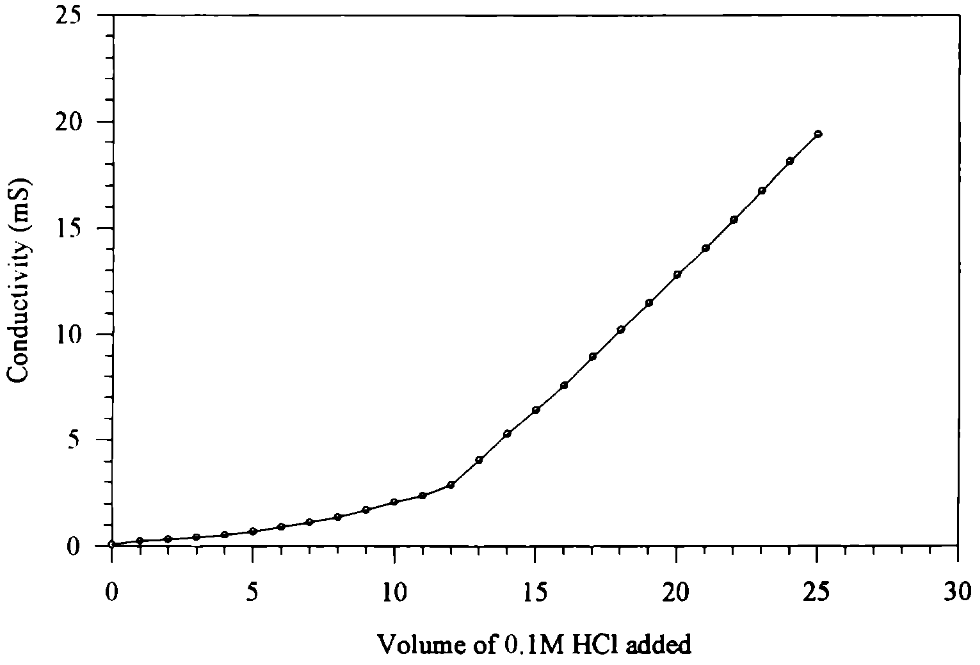


Figure 2.5.

Titration curve for the direct method.

Volume of HCl taken up by chitosan = 12 mL

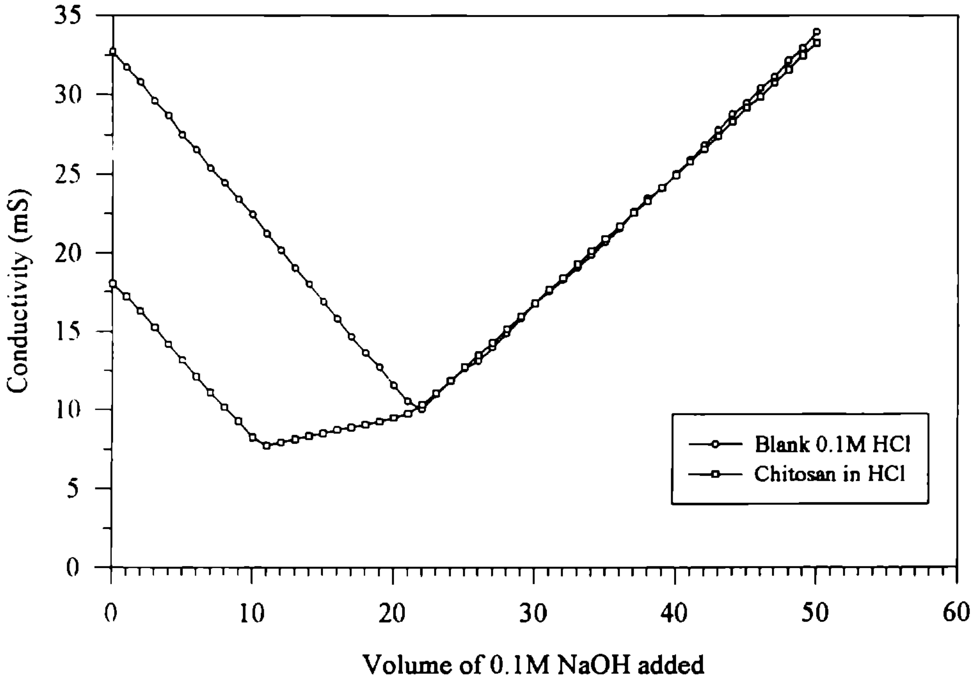


Figure 2.6.

Curve for the back-titration method.

Volume of NaOH equivalent to acid taken up by chitosan = 10.5 mL where $V_t$ is the volume of titrant equivalent to HCl absorbed by chitosan, $N_t$ its normality, $V_{in}$ is the initial volume of chitosan solution, $M_G$ is the molar mass of glucosamine monomer unit and $m_C$ is the mass of chitosan per litre of the solution.

### 2.7.2 Results and discussion

#### 2.7.2.1 Ash content

The original sample of chitosan was found to contain 1.2% ash consisting of magnesium, calcium, aluminium and nickel. Extraction with ammoniacal EDTA reduced the ash content to 0.27%, and the ash consisted of aluminium and nickel only. Thus calcium and magnesium were the major metal ions present, and these were preferentially removed by washing with EDTA solution. However, these results are of significance only when the chitosan is to be used as a complexing agent for metal ions or when it is ingested. Although the presence of traces of these metal ions in chitosan may block a few binding sites in connection with its flocculation properties, complete removal of these metal ions from the commercial product will be a very costly affair; application of such highly purified material will be uneconomic for its routine use as a flocculant in treatment plants. The metals present and their quantities may also vary depending on the source and manufacturing process of the chitosan. A sample analysis of the trace metals usually present, using emission spectrographic and neutron activation methods is provided by Muzzarelli (1977).

### 2.7.2.2 Infrared spectrum

The IR spectrum of chitosan taken as a thin film gave more defined absorption bands than that in the form of KBr pellet (Figure 2.3). The pattern and positions of the absorption bands in the fingerprint region were in good agreement with the one reported for chitosan by Muzzarelli [(Muzzarelli, 1977], but the spectrum obtained here is better resolved and with sharper bands. There is a report that new sharp bands appear with increasing degree of deacetylation [Mima *et. al.*, 1983].

### 2.7.2.3 Molar mass and degree of polymerisation

The molar mass calculations of the chitosan sample gave a value of $1.8 \times 10^4$ daltons for the 2-hour sample and $1.6 \times 10^4$ daltons for the 18-hour sample respectively. This translates into a degree of polymerisation of about 114 glucosamine units for the sample kept for 2 h after dissolution and 100 units for the one kept for 18 h. The degree of polymerization of the chitosan sample will depend very much on the concentration of the acid used and time of treatment during the manufacturing process since the polymeric chain can be randomly ruptured by acid treatment [Rege and Block (1999)]. There also appears to be great variation in the solution properties of chitosan and the Mark–Houwink constants reported by different workers [Knaul *et. al.* (1998), Tsaih and Chen (1999), Jiang and Han (1999), Rinaudo *et. al.* (1999), Sashiwa and Shigemasa (1999) Signini and Filho

(1999), Chen and Tsaih (2000) and Kasaai *et. al.* (2000)]. Molar masses of upto $10^6$ daltons have been reported in literature for chitosan by workers who have isolated the material carefully using mild conditions to avoid chain degradation [Muzzarelli, 1977]. Compared to these values, it appears that the commercial sample obtained by us has undergone considerable chain degradation during the manufacturing process.

However, the results also indicate that degradation of chitosan takes place even when stored as acid solution and the degree of polymerisation decreases slowly with time of storage. Similar observations on the degradation of acid solutions of chitosan have also been reported by others (Chen *et. al.*, 1997; Vincendon, 1997). As the degree of polymerisation increases, dissolution of chitosan in the acid is slower, thus preventing immediate and reliable measurement of viscosity

As far as application of chitosan as a flocculant in water treatment is concerned, it is desirable to have as high a value for the degree of polymerisation, considering the bridging mechanism. But it is also essential that it be possible for the chitosan to be made into a solution that can be effeciently and rapidly mixed with the bulk of the water to be treated. Since good flocculation characteristics were observed even with low molar mass samples used in this study, the performance is expected to be even better if higher molar mass samples are used. But there is also a study which reports that chitosan chains of smaller molar mass are more extended than chains of larger molar mass [Tsaih and Chen, 1999]. Other morphological factors may also play a role (see section 2.7.2.5).

It was also noted that there was considerable variation in the degree of polymerisation among various samples of chitosan procured. One sample obtained from CIFT would dissolve in dilute acid only very slowly to give a thin gel which would not flow in the Ostwald viscometer. This sample obviously had a much higher degree of polymerisation than that for which the above calculations were made. This sample also showed a better flocculation efficiency in experiments described in the coming chapters.

#### 2.7.2.4 Degree of deacetylation

Calculation based on the direct titration method gave a degree of deacetylation of 70.6% for the sample examined and that using the back-titration method gave a value of 69.6%. The results obtained using the two methods are in good agreement within limits of experimental error. Thus about 70% of the amino groups in the sample of chitosan are free.

#### 2.7.2.5 Microscopic examination of precipitated chitosan gels

Microscopic examination of chitosan precipitated from a 0.1 M hydrochloric acid solution immediately and after storage for about 3 months also indicated some change in its characteristics. These are shown in Plates 1 and 2 respectively.

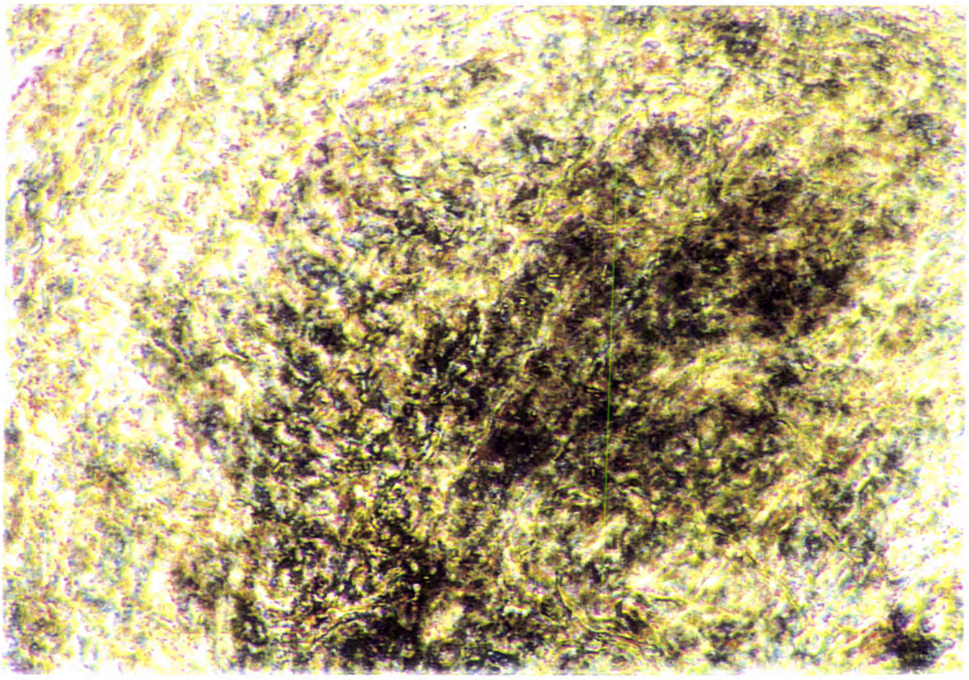


Plate 2.1. Chitosan precipitated from fresh solution.

(seen under the microscope with phase contrast at ×400 magnification)

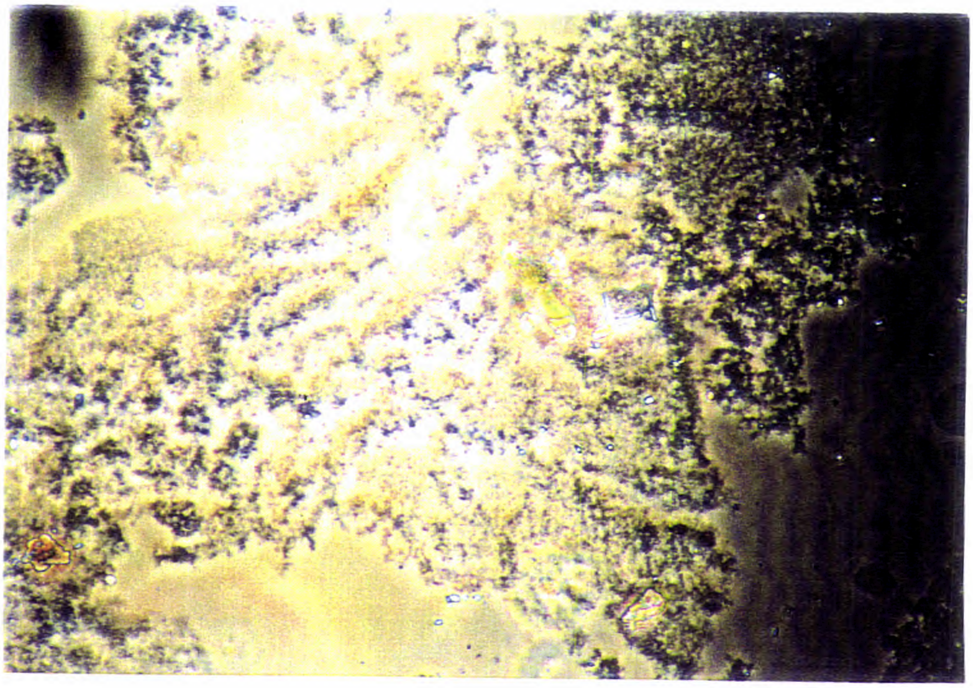


Plate 2.2. Chitosan precipitated from an aged solution.

(seen under the microscope with phase contrast at ×400 magnification)

A Nikon Optiphot AFX-IIA light microscope at a magnification of ×400 was used in this study.

In Plate 2.1, the strands of chitosan appear to be very long and closely packed in the form of a continuous mat extending in all directions beyond the field of view. But in Plate 2.2, they appear to be fragmented and aggregated into smaller clusters or loose strands.

## Chapter 3

# Flocculation of kaolinite suspensions using chitosan

### 3.1 Introduction

Removal of kaolinite turbidity from the effluents before discharging into the environment is a problem faced by the clay processing industry. Faust and Aly (1983) quote extensive application of kaolinite suspensions as a model for natural turbidity of raw surface water in Jar Test studies of coagulation and flocculation in the laboratory Inorganic coagulants such as alum in combination with lime have been conventionally used for the removal of clay particles in the effluents from clay processing industry as well as from raw water The sludge formed from such treatment poses disposal problems because of its aluminium content and tends to accumulate in the environment

Studies on the use of chitosan, either alone or in conjunction with inorganic coagulants such as alum and ferric chloride for the treatment of wastewater from pig and poultry farms, distilleries and food processing industries have been reported [Bough (1975), Bough (1975a), Bough *et al.* (1975), No and Meyers (1989), Kawamura (1991), Pinotti *et al.* (1997), Chen *et al.* (1998), Lalov *et al.* (2000)]. Application of chitosan in the flocculation of montmorillonite, bentonite and kaolinite suspensions have been reported earlier by Peniston and Johnson (1970), Huang and Chen (1996), Pan *et al.* (1999), Gidas *et al.* (1999) and several others.

Huang and Chen (1996) reported that chitosan, when used alone, fails to flocculate kaolinite suspensions and that the effect of pH on its coagulation efficiency is insignificant. Huang and Chen (1996) also failed to notice any appreciable flocculation of kaolinite suspensions in water by chitosan and concluded that the properties of suspended particles have a significant effect on coagulation using chitosan. Gidas *et al.* (1999) reported that flocculation ability of chitosan depends on the physico-chemical characteristics of raw water

However, attempted flocculation of fine kaolinite clay dispersed in tap water using chitosan in our laboratory proved to be very successful. Therefore a detailed investigation was initiated. In this study, the effectiveness of chitosan as a flocculant, when used alone, on the removal of kaolinite turbidity was examined using various concentrations of suspended kaolinite particles and chitosan, and at different pH values.

## 3.2 Initial experiments using tap water as the dispersion medium

### 3.2.1 Materials and methods

#### 3.2.1.1 Kaolinite suspensions

Highly refined kaolinite powder of very fine particle size, recommended for use in the coating of specialty papers, manufacture of paint, electronic ceramics and as filler for toilet soaps, was obtained directly from English India Clays Limited, Thiruvananthapuram, Kerala. The physical and chemical characteristics of the material (data provided by the manufacturer) are as follows: particle size: 2 microns (83 ± 2.0% w/w); particles larger than 50 microns: 0.03% maximum; specific surface area: 16.5 $m^2$ $g^{-1}$ (BET); bulk density 0.35 kg $dm^{-3}$; moisture 2% maximum; silica: 45%; alumina: 38%; ferric oxide 0.5%, titania: 0.45%; loss on ignition 15%.

The kaolinite used was processed kaolinite from industry. Waste water from this industry contains the same material in suspended form from the process stream. This does not contain any material from natural environment deposited on the surface. Natural suspended matter containing clay minerals is more heterogeneous in character and invariably contains organic materials adsorbed from the water body. However, kaolinite is a representative clay mineral frequently used in coagulation research [Faust and Aly (1983) p. 304].

Kochi city in Kerala receives its drinking water supply from Periyar River after conventional treatment in the water treatment plants maintained by the Kerala Water Authority at Aluva. The plants have an installed capacity for treating approximately 300 million litres per day. The treatment involves removal of debris from the raw river water, skimming and aeration, removal of suspended particulate matter by alum flocculation and settling, filtration through sand beds and disinfection by chlorination. Kerala receives intense rains during June – July and October – November. The River is flooded during this period and the water contains much suspended matter. At other times, especially during the dry season in March – April, the River is but a stream with fairly clear water. Being almost at sea level, some salinity also appears at times. Due to these reasons, the raw water quality is not constant and fluctuates very much. Because other sources are not available, and because the supply is far below demand, the contaminant levels may overshoot the treatment capacity of the plants occassionally. In spite of all this, the quality of the supplied tap water during the period of this research remained fairly constant, and typically had the following properties as determined by procedures given in Standard Methods (1995): specific gravity 0.9997, pH 7.2 to 7.5, turbidity $< 1.0$ NTU, total acidity = 0, total alkalinity equivalent to 18 mg $CaCO_3$ per litre, total hardness equivalent to 12 mg $CaCO_3$ per litre. The organic matter was below the levels detectable by UV method.

Approximately 2 g of kaolinite powder was thoroughly mixed with 1 L of tap water (of quality described above) and allowed to settle undisturbed for about 30 minutes. The turbid supernatant was withdrawn and diluted as necessary with tap water to obtain the turbidity required for the experiments. The suspensions were prepared from kaolin powder immediately before each set of experiments. The mass of suspended kaolinite at each turbidity value was determined gravimetrically.

#### 3.2.1.2 Gravimetric determination of kaolinite concentrations

The concentration of kaolinite in suspensions of measured turbidities were determined gravimetrically by evaporating 250 mL of each suspension to dryness in tared beakers on a water bath. The beakers were then dried in an air oven at 110°C for 30 minutes, cooled to ambient temperature in a desiccator and quickly weighed on an electronic balance since the solids were very hygroscopic. A blank determination using 250 mL of the tap water used for preparing the suspensions was also carried out to find the mass of dissolved solids in it. This mass was then subtracted from the mass of kaolinite in each estimation to get the actual mass of suspended solids at each turbidity. All estimations were conducted in duplicate and the average values were plotted.

#### 3.2.1.3 Chitosan solution

Chitosan prepared from prawn shells was obtained from Central Institute of Fisheries Technology, Kochi. This material was a pale brown powder soluble in dilute acetic and hydrochloric acids. One hundred milligrams of chitosan powder was accurately weighed into a glass beaker and mixed with 10 mL of 0.1 M HCl solution and kept aside for about an hour to dissolve. The dissolution was slow, and some amount of chitosan remained in the form of a thin gel even after this time. It was then diluted to 100 mL with tap water to obtain a solution containing one mg chitosan per mL of solution. The solutions were prepared fresh before each set of experiments.

#### 3.2.1.4 Jar tests

Turbidity of kaolinite suspensions were measured on a Systronics nephelo-turbiditymeter model 131 calibrated using standard formazine suspension as recommended in Standard Methods (1995). pH values of the suspensions were measured using a Systronics digital pH meter model 335. Flocculation was carried out on a six-spindle multiple stirrer unit with stainless steel paddles.

pH of the kaolinite suspensions were adjusted by adding the required amount of either 0.1 M HCl solution or 0.1 M sodium carbonate solution before adding the flocculant. It was thus ascertained that flocculation took place at the desired pH value. The required volume of chitosan solution was added to each test suspension and mixed thoroughly by stirring rapidly for five seconds. Stirring was then continued at a steady rate of 60 rpm for 30 minutes since rapid growth of the flocs was observed for this stirring rate and no further growth was observed after this time during pilot studies. The stirrer was then switched off and the flocs allowed to settle undisturbed for 30 minutes, the time taken to obtain maximum clarity in most of our experiments. The sample for turbidity measurement was withdrawn using a pipette from a height of 5 cm below the surface in each beaker.

Six replications of each test were carried out. The mean value obtained for residual turbidity and standard deviation from the six replications were computed.

### 3.2.2 Results and discussion

#### 3.2.2.1 Relationship between kaolinite concentration and turbidity

Determination of suspended kaolinite concentration gravimetrically gives a linear relationship with turbidity. The plot of mass of suspended kaolinite (in mg $L^{-1}$) against the turbidity produced by it is shown in Figure 3.1.

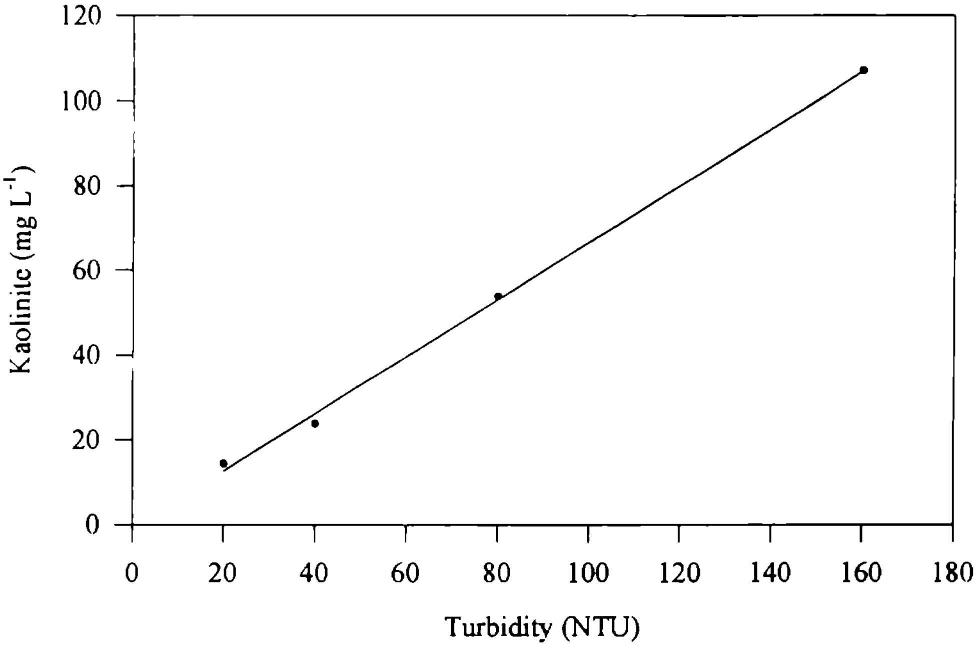


Figure 3.1.

Plot of mass of suspended kaolinite against turbidity of the suspension.

#### 3.2.2.2 Effect of pH on the flocculation of kaolinite using chitosan

Tests were conducted as described, initially at pH values of 5.0, 6.0, 7.0, 8.0 and 9.0 ± 0.1 using a kaolinite suspension of turbidity 40 NTU and a flocculant concentration of 1 mg $L^{-1}$ As the lowest residual turbidities were found between pH 6 to 8, further tests were conducted at pH 6.5, 7.2 and 7.5. The results are presented in Figure 3.2. From the figure, it is seen that chitosan produces an appreciable removal of turbidity only between pH 6.5 and 7.5. The maximum efficiency is observed at a pH of 7.5. About 90% removal of turbidity is achieved at this pH, with the residual turbidity dropping below 5 NTU. The flocs obtained were very coarse and settled almost completely in a few minutes.

#### 3.2.2.3 Determination of optimum dosage of chitosan

As maximum efficiency of flocculation was seen at pH 7.5, tests were conducted by varying the dosage of chitosan at this pH. Tests were conducted using 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0 mg $L^{-1}$ of chitosan. It was observed that chitosan is not very effective at dosages less than 0.75 mg $L^{-1}$, but very effective at the higher dosages examined. The lowest dosage with maximal efficiency was found to be 1 mg $L^{-1}$ after which no advantage was obtained by increasing the dosage. Plots of percentages of turbidity removed against dosages of chitosan used as flocculant (in mg $L^{-1}$) at a pH of 7.5 for various initial turbidity values is given in Figure 3.3.

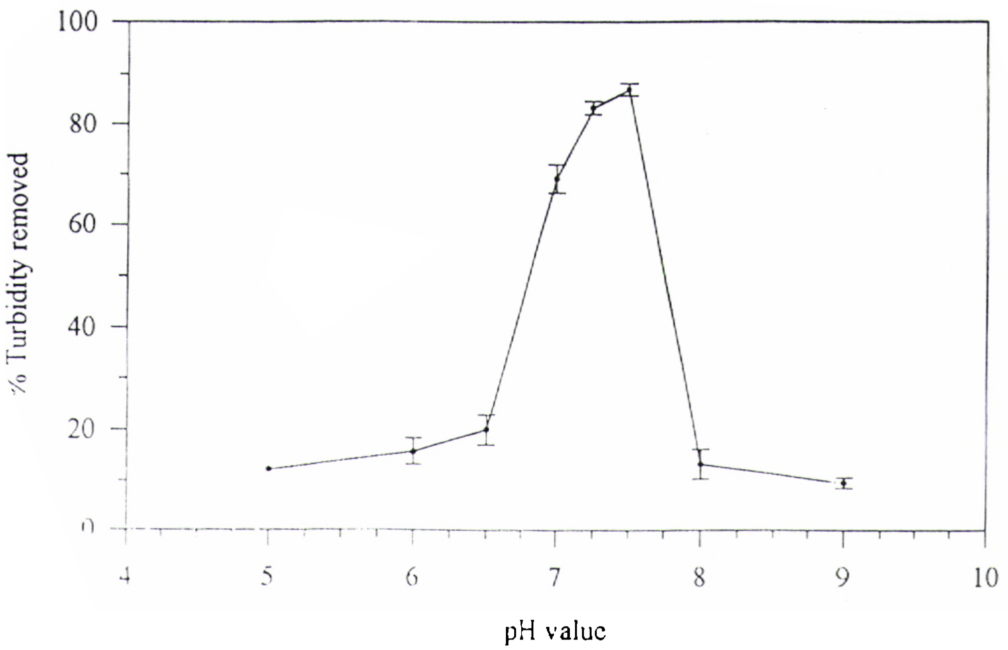


Figure 3.2.

Effect of pH on the removal of kaolinite turbidity using chitosan. Whiskers indicate one standard deviation from six replications.

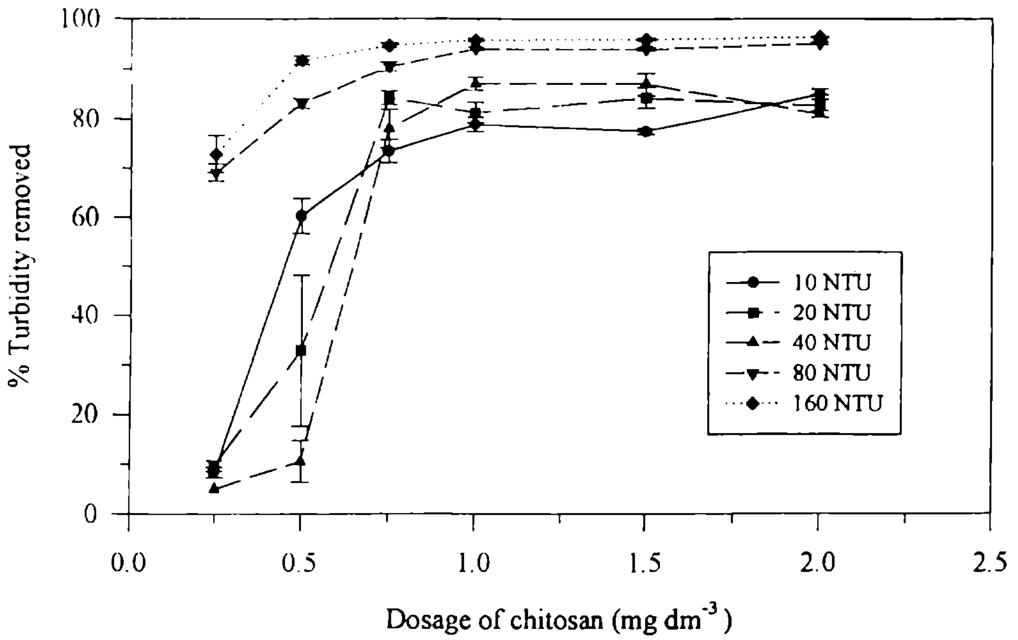


Figure 3.3.

Effect of varying chitosan dosage on kaolinite turbidity removal at pH 7.5.

Whiskers indicate one standard deviation from six replications.

### 3.2.2.4 Relationship between turbidity and dosage of chitosan

To determine the effect of various initial turbidity values, tests were conducted as described under section 3.2.2.3 above at initial turbidity values of 10, 20, 40, 80 and 160 NTU, using various concentrations of chitosan. The results of these studies are also presented in Figure 3.3. Results indicate that chitosan is very efficient in removing kaolinite turbidity in the entire turbidity range examined. It is also seen that irrespective of initial turbidity, a chitosan dosage of 1.0 mg $L^{-1}$ produces maximum clarification, with no noticeable advantage at higher dosage levels. Further, the optimal dosage of 1 0 mg $L^{-1}$ chitosan is also much less than that described in earlier studies by other workers [Huang and Chen (1996), Pan *et al.* (1999)].

However, "the percentage of turbidity removed" does not indicate the final turbidity present in the treated water. From the point of view of water treatment professionals, the residual turbidity is of more concern. Therefore, the residual turbidity after treatment is indicated against chitosan dosages in Figure 3.4. In the present study, we found that irrespective of initial turbidity, chitosan leaves a residual turbidity of only less than 10 NTU, without any filtration, under the test conditions. The residual turbidities reported earlier by Huang and Chen (1996) using chitosan alone as flocculant were much higher. The residual turbidity was found to decrease with decreasing initial turbidities. Starting with a low turbidity value of 10 NTU, which is usually very difficult to clarify in conventional treatment plants using alum, a residual turbidity of about 2 NTU was obtained after

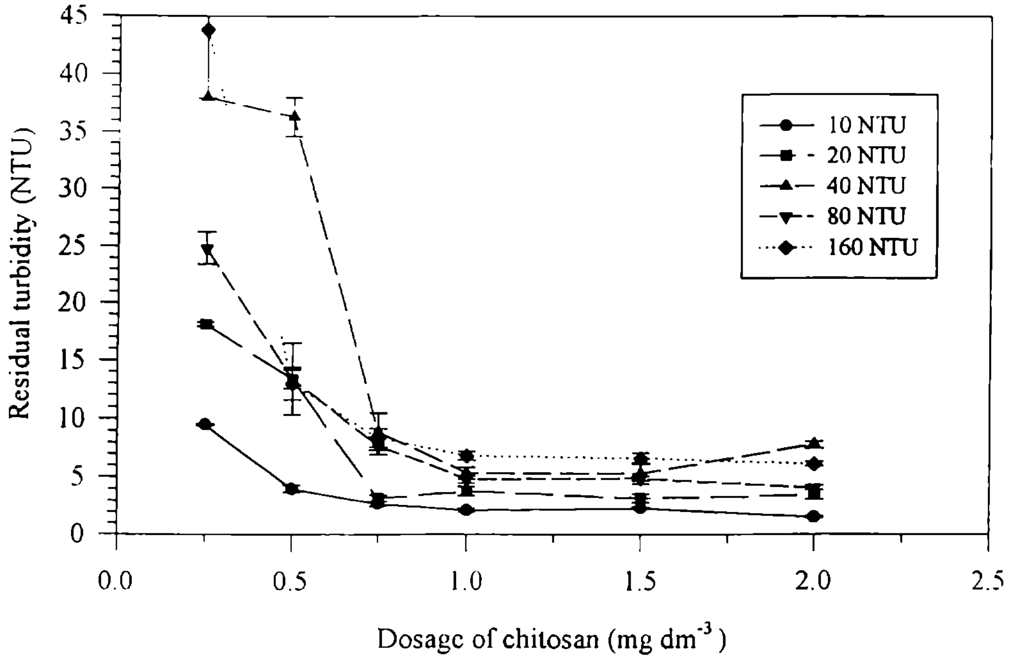


Figure 3.4.

Effect of chitosan dosage on residual kaolinite turbidity at pH 7.5. Whiskers indicate one standard deviation from six replications.

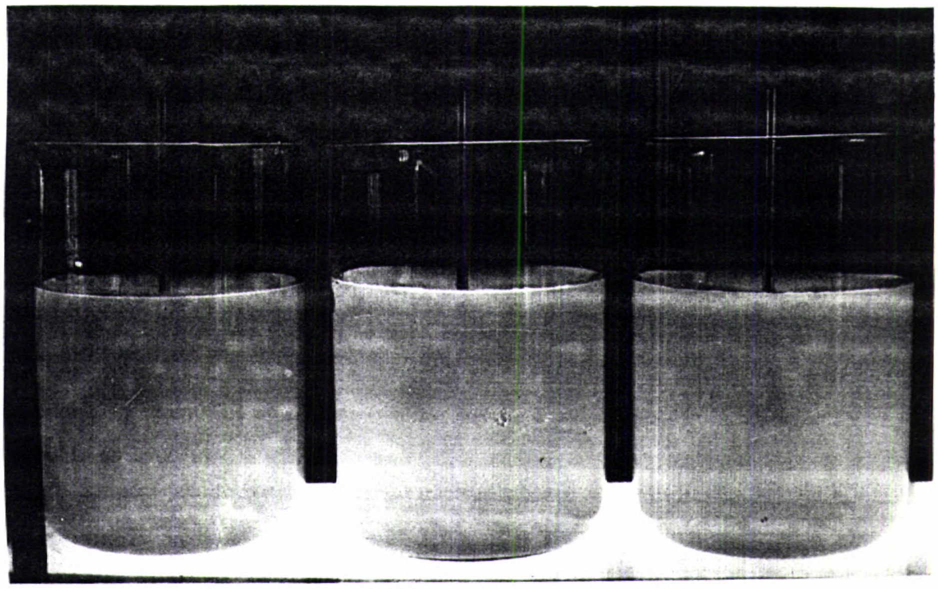


Plate 3.1.

Kaolinite suspension of turbidity 55 NTU, before flocculation.

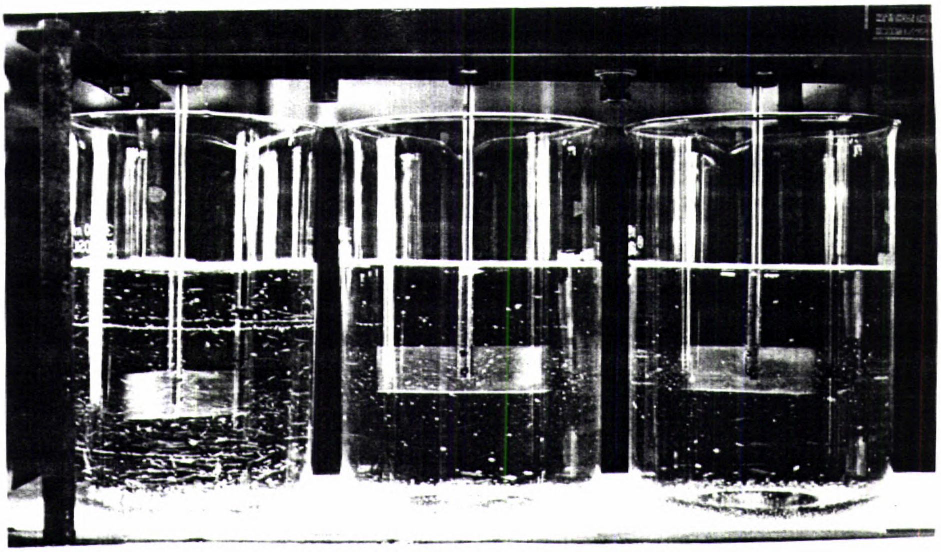


Plate 3.2.

Flocculation of kaolinite suspension using chitosan, in progress.

flocculation using chitosan. It was also observed that at higher initial concentrations of suspended solid, the flocs appear in a shorter time and grow to a larger size. The flocs are fibrous and stick to the stirrer or form large entangled matter resembling cobwebs.

### 3.3 Experiments using distilled water as the dispersion medium

Thus having observed from experiments described above that chitosan is quite effective in the flocculation of kaolinite suspensions in tap water, we decided to establish the effectiveness of the technique conclusively and systematically using distilled water as the suspension medium. Contrary to expectations, chitosan failed to flocculate kaolinite suspensions in distilled water!

Earlier reports by other workers indicated that chitosan was very effective as a coagulant aid but failed in the case of kaolinite when used alone as a flocculant. The first doubt to cross our minds was that the presence of some inorganic cation like $Ca^{2+}$ or $Mg^{2-}$ in the tap water used might be assisting chitosan in the flocculation of kaolinite particles. But it can be seen from the analysis provided in section 3.2.1.1 that the tap water was very soft and did not have an appreciable mineral content. Therefore the influences of all cations, anions and even chlorine that may be found in tap water were checked by adding calculated amounts of these into distilled water used as the suspension medium. The amounts were calculated so as to match the range reported or probable for the source water and tap water.

Table 3.1. Chemical species tested for assisting flocculation of kaolinite using chitosan in distilled water.

| Species tested | Concentration (mol $L^{-1}$) | | Effect |
|---|---|---|---|
| *Cations* | | | |
| $Ca^{2+}$ | $10^{-4} - 10^{-3}$ | $= 10$ to 100 ppm $CaCO_3$ | No effect |
| Na | $10^{-3} - 10^{-2}$ | $= 58$ to 580 ppm NaCl | No effect |
| $NH_4$ | $10^{-7} - 10^{-4}$ | $= 1.7\times10^{-3}$ to 1.7 ppm $NH_3$ | No effect |
| $Fe^{2-}$ | $10^{-7} - 10^{-4}$ | $= 5.6\times10^{-3}$ to 5.6 ppm Fe | Flocculation by Fe above $10^{-5}$ mol $L^{-1}$ |
| $Mg^{2+}$ | $10^{-4}$ | $= 10$ ppm $CaCO_3$ | No effect |
| $Pb^{2+}$ | $10^{-5} - 10^{-4}$ | $= 2.7$ to 27 ppm as Pb | No effect |
| *Anions* | | | |
| $Cl^-$ | $10^{-4} - 10^{-2}$ | $= 5.8$ to 580 ppm NaCl | No effect |
| $SO_4^{2-}$ | $10^{-7} - 10^{-4}$ | $= 9.6\times10^{-3}$ to 9.6 ppm | No effect |
| $HCO_3$ | $10^{-4}$ | $= 10$ ppm $HCO_3^-$ | No effect |
| $SiO_3^{2-}$ | $10^{-5} - 10^{-3}$ | $= 0.75$ to 75 ppm | No effect |
| $H_2PO_4^-$ | $10^{-5} - 10^{-3}$ | $= 0.97$ to 97 ppm | No effect |
| Acetate | $10^{-3}$ | $= 60$ ppm | No effect |
| *Combinations* | | | |
| $NH_4^+ + HCO_3^-$ | $10^{-7} - 10^{-4}$ each | | No effect |
| $Fe^{2+} + HCO_3$ | $10^{-7} - 10^{-4}$ each | | Flocculation by Fe above $10^{-5}$ mol $L^{-1}$ |
| $SO_4^{2-} + HCO_3$ | $10^{-7} - 10^{-4}$ each | | No effect |
| $Ca^{2-} + HCO_3$ | $10^{-4}$ each | | No effect |
| $Mg^{2+} + HCO_3^-$ | $10^{-4}$ each | | No effect |
| $Cl^- + HCO_3^-$ | $10^{-4}$ each | | No effect |
| $Ca^{2+} + Mg^{2-} + Cl^- + HCO_3^-$ | $10^{-4}$ each | | No effect |
| *Others* | | | |
| Glycine | $10^{-3} - 10^{-2}$ each | | No effect |
| Alum | 0.1 – 1 mg $L^{-1}$ | | No effect |
| $CaCO_3$ | Saturated | | No effect |
| Iron | Residual after precipitation | | No effect |
| Sea water | 25 – 75% of total volume | | No effect |
| Bleaching powder | to get below 0.2 mg $L^{-1}$ of $Cl_2$ | | No effect |
| Boiled and cooled tap water | 100% of total volume | | Very effective |

A summary of the analysis, listing the species or combinations examined, their concentrations and the observed effects, is provided in Table 3.1.

Concentration of the tap water by evaporating off up to 5% of the water showed appreciable improvement in the flocculation of kaolinite suspensions in it using chitosan. But the effect was certainly not due to any cations, anions, glycine or chlorine that may be present in the tap water, as can be seen from Table 3.1. A possibility that remained was that natural organic matter (NOM) might be assisting the flocculation. (NOM is not present in potable water, after usual treatment procedures, in any concentration detectable by commonly employed techniques without preconcentration). To check this, some soil from the laboratory premises was extracted with distilled water. This soil extract was filtered and aliquots were added to the distilled water used as medium for preparing kaolinite suspensions. Focculation characteristics very similar to that obtained when tap water was used as medium were observed. Aqueous extract of humus rich soil containing much organic detritus, such as that found near trees, showed better results.

During these experiments, it was also noted that suspensions of kaolinite prepared in the tap water, or in distilled water to which some soil extract was added, was considerably more stable than those prepared in distilled water. Suspensions of considerably higher turbidities could be prepared by stirring 1 g of the kaolinite powder with 1 L of these media and allowing the larger particles to settle down, than could be obtained when distilled water alone was used. These suspensions also took a much longer time to settle when left undisturbed. Thus, small amounts of humic substances present in water can stabilise kaolinite suspensions. This also points to the possibility of an interaction of these dissolved humic substances with kaolinite particles, which modifies the surface of the latter in such a manner that flocculation with chitosan becomes possible. The humic material present may be fulvic acid in varying molecular weight fractions [Gjessing, 1976]. A plausible interaction is adsorption, which takes place for humic materials on clay minerals [Huang and Shiu (1996); Arias *et al.* (1996); Malekani *et al.* (1997)].

### 3.3.1 Chemical nature of humic substances

The available knowledge on the chemical nature of humus has been compiled by Gjessing and Orlov in their books [Gjessing, 1976; Orlov, 1985]. It is well known that humic substances adsorb to the surface of minerals in soil. Studies using montmorillonite show that adsorption increases with decrease in pH. The chemical composition of humic acids vary a great deal depending on the type of soil, the type of biomass that has been decomposed and the products of decomposition.

Carbon and oxygen form more than 90% of the humic material, along with hydrogen and nitrogen. Traces of sulphur, phosphorus and minerals have also been detected. Elemental analysis of humic acids from various soils gives an average composition of $C = 54.5\%$, $H = 4.8\%$, $O = 36.7\%$ and $N = 4.0\%$. This translates into an average empirical formula of $C_{16}H_{17}O_8N$. The hydrolysates of humic acids have indicated the presence of a wide variety of amino acids, amino sugars, carbohydrates, phenols, aldehydes, carboxylic acids, quinones, polynuclear aromatic compounds and nitrogen-containing heterocycles.

The water-soluble fraction of humic acids is called fulvic acids. Fulvic acids generally have a lower average molecular mass compared to the insoluble humic acids, but the compositions are otherwise comparable. The mean molecular masses of fulvic acid particles are estimated to be in the range of 4 000 to 10 000 while that of the insoluble humic acids can be as high as 300 000 to 600 000. The sizes indicate that much of these material may be in the form of colloidal aggregates. The ashes from the gray humic acids consist chiefly of clay minerals.

It has been shown by many different experiments that humus has a net negative charge. More than 50% of humic substances are electrically neutral at a pH of 1 to 2. In addition to much –OH and –COOH groups, free radicals are also present.

In studies unconnected with the present work, Malekani *et al.* (1997) have examined the interaction of natural organic matter in soil extracts with surfaces of clay minerals such as kaolinite. They have concluded that the mineral matter has smooth surfaces and it is the organic matter coatings that are responsible for their surface roughness. Data on microscopic examination of kaolinite in mineralogy also describe them as flakes with smooth surfaces and a greasy feel [Winchell and Winchell, 1968]. Other workers [Huang and Shiu (1996); Arias *et. al.* (1996), Rebhun *et al.*, (1998)] have also reported adsorptive associations between humic substances and mineral matter. In yet another study, Popa *et. al.* (2000) discusses the strong interaction between chitosan and polyphenols from wood below pH 9.

### 3.3.2 A plausible mechanism for flocculation of kaolinite by chitosan

Considering all the above observations, we propose the following mechanism for the observed flocculation of kaolinite suspensions by chitosan in water containing traces of dissolved humic substances:

The dissolved humic particles, in general, contain much more of phenolic and carboxylic acid groups than there are amino groups [Gjessing, 1976]. Since there are both acidic and basic groups present, the particles exhibit multiple Zwitter ionic character. Some of the –OH and –COOH groups exist in the ionised form as $-O^-$ and $-COO^-$, and some of the $-NH_2$ groups are in the $-NH_3^+$ form at pH close to neutral (see Figure 3.5). Since the number of negative charges is more than the number of positive charges, the particles are observed to exhibit a net negative character, and are themselves stabilised in aqueous suspensions.

At low pH of the medium, more of the amino groups in the humic particles become protonated while the ionisation of acidic and phenolic groups are suppressed. Thus they have much more positive charges than negative, which makes them strongly adsorbed on the smooth negative surfaces of refined kaolinite particles. Since most of the positive groups on the humic particles now point inside towards the negative kaolinite particle at the centre, the outer portions again have more of negative charges (cf. hydration shells of aqueous anions). This again tends

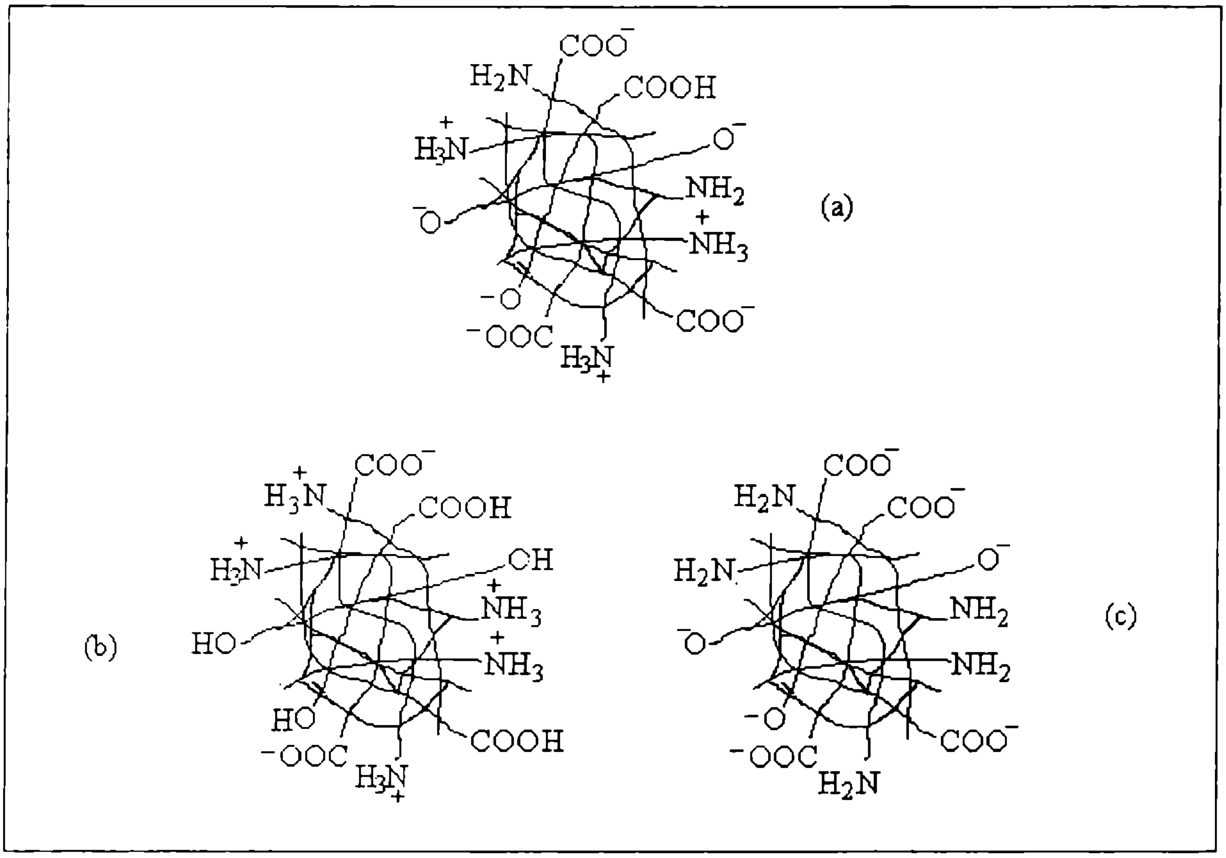


Figure 3.5.

The chemical nature of dissolved humic substances.

**Note:** Only the groups present on the surface are shown for clarity. Similar groups are present also within the porous body of the particles, but these are not important for stabilisation or adsorption to other external surfaces. (a) At neutral pH, some of the groups are ionised, but the number of negative charges is more than the number of positive charges. (b) At low pH, there are more positive charges than negative charges. (c) At high pH, the number of negative charges is more than the number of positive charges.

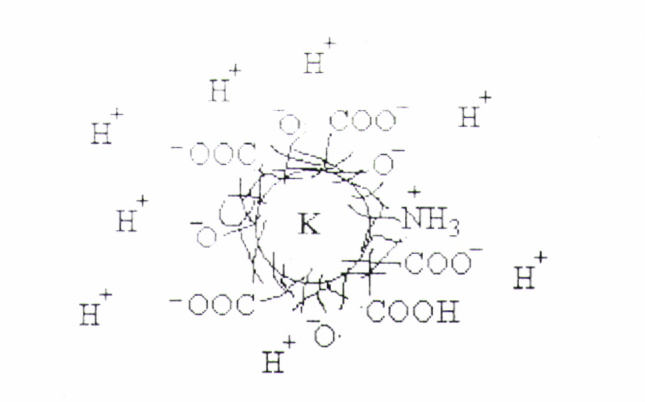


Figure 3.6. Humus adsorbed on kaolinite particle(K)

to stabilise the suspension. The adsorbed humus also provides a rough outer covering to the kaolinite particles. This also explains the observation that adsorption of humus to mineral particles increases with decrease in pH since the number of $-NH_3^+$ groups increases. Suspensions of such "conglomerate" particles are expected to be more stable in weakly acidic solutions than in strongly acidic solutions since the negative groups will be more ionised in the former. In basic medium, the humus will be in the salt form, which is highly solubilised and has no $-NH_3^+$ groups. Therefore the humus desorbs from the surface of the kaolinite particles, making them 'naked' again.

Assuming that cationic chitosan chains adsorb better on a humus-covered kaolinite particle than on a 'naked' one, and considering the fact that chitosan is very soluble in highly acidic medium, all observations can now be explained:

(1) Suspensions of kaolinite particles in water containing traces of humic substances are more stable than those in distilled water since the particles are covered by a sheath of humus with more surface negative charges;

(2) Chitosan solutions start precipitating at pH values close to neutral and flocculates such humus-covered kaolinite particles (see the effect of pH on flocculation in Figure 3.2) by forming bridges between them.

(3) At very low pH values, chitosan is soluble and therefore incapable of producing flocs (see Figure 3.2).

(4) In alkaline medium the effectiveness of chitosan disappears totally since (i) the humus covering of kaolinite particles dissolve off and (ii) chitosan itself loses its cationic nature at high pH values (see Figure 3.2).

(5) Thus the pH of the suspension is a very critical factor in flocculation using chitosan; it is highly effective in a narrow range close to pH 7. Traces of humic substances in the water aid flocculation of mineral particles with very smooth surfaces.

(6) Montmorillonite has a surface that is rougher than that of kaolinite and is usually part of humic substances. Hence its flocculation by chitosan has been noted by earlier workers [Huang and Chen (1996), Pan *et al.* (1999)].

## 3.4 Effect of aging of chitosan solution on kaolinite flocculation

A 1% (w/v) solution of chitosan in 0.1 M HCl was prepared, stored in a closed amber-coloured bottle and checked periodically for its effectiveness in flocculating kaolinite suspensions of 40 NTU turbidity in tap water as described in sections 3.2.2.2 and 3.2.2.3. It was observed that aging of the chitosan solution had some effect on its ability to flocculate kaolinite suspensions.

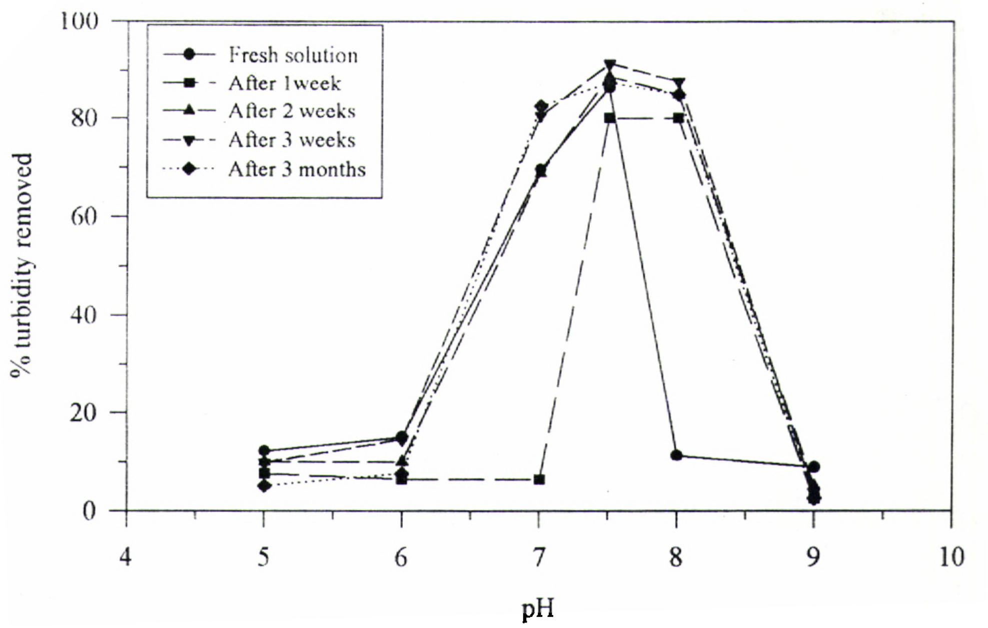


Figure 3.7.

Effect of aging of the chitosan solution on the pH of kaolinite suspension required for effective flocculation. Turbidity = 40 NTU.

Figure 3.7 shows the effect of aging of the chitosan solution on the pH of kaolinite suspension required for effective flocculation. A fresh solution of chitosan is most effective in the narrow pH range 7.0 to 7.5. After storing for one week, this is seen to shift to the pH range 7.5 to 8.0. Thereafter, the effectiveness below pH 7.5 improves and after 3 months of storage, maximum flocculation is obtained in a broader pH range from 7.0 to 8.0.

Figure 3.8 shows the effect of aging of the chitosan solution on the optimal flocculant concentration. The most notable feature is that while a flocculant concentration of 0.5 mg/L using fresh chitosan solution reduces turbidity by only about 10%, the same concentration obtained using a chitosan solution stored for a week or more reduces the turbidity by more than 80%. Higher concentrations of a one-week-old chitosan solution was noted to restabilise the kaolinite suspension, but this effect disappears in the second week, reappears in the third week and again disappears on prolonged storage.

The above changes in the flocculation efficiency of chitosan solution on storage may be a result of the morphologic changes described at the end of the second chapter (See Plates 2.1 and 2.2). After 3 months, fungal growth was noticed in the solution, probably due to extensive degradation of chitosan chains on prolonged storage. It may be noted that chitosan forms a hydrochloride and the pH of the solution rises to a level where fungi can grow. pH values of chitosan solutions were not measured since chitosan had a tendency to adsorb on the glass electrode.

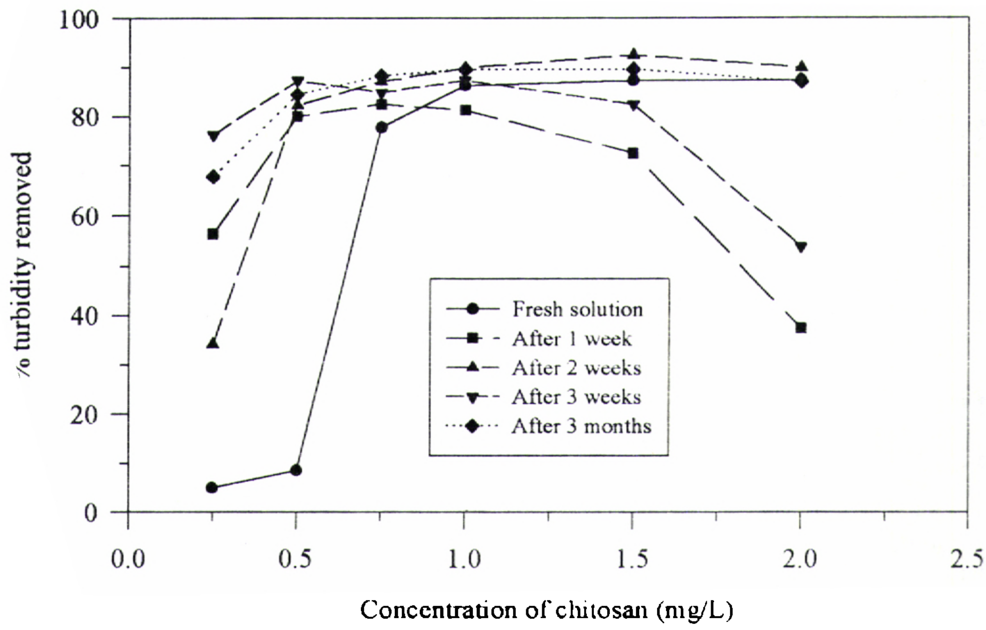


Figure 3.8.

Effect of aging of the chitosan solution on the optimal flocculant concentration.

Kaolinite turbidity = 40 NTU, pH = 7.5.

## 3.5 Flocculation experiments using alum-chitosan combinations

The effect of chitosan as a flocculant aid along with alum on kaolinite suspensions in tap water was examined. The data obtained using a 50 NTU suspension at a pH of 7.5 are given in Figure 3.9.

It can be seen from Figure 3.9 that 1 mg $L^{-1}$ chitosan alone (A) is very effective in removing kaolinite turbidity from tap water whereas 1 mg $L^{-1}$ alum alone (B) is not effective. But additions of small amounts of chitosan to 1 mg $L^{-1}$ alum (C, D & E) greatly enhances the flocculation efficiency and turbidity removal. One milligram of alum combined with 0.25 mg of chitosan per litre of suspension gives the best result and is much better than when chitosan alone is used. The pH of the suspension does not change on adding this flocculant mixture and hence no treatment for correction of pH is required.

More than the quantitative aspect, a great improvement in the quality of flocs formed using this combination was observed. Flocs appear within a minute after the addition of the flocculant mix, and grow to a very large size in less than five minutes. They are so heavy as to circle near the bottom of the beaker even while the suspension is being stirred at the rate of 60 rpm, and the settling times required in the jar tests were less than one minute for maximum clarity. Appearance of the flocs was fluffy and similar to that when chitosan alone is used. When chitosan alone is used as flocculant, flocs appear only after 15-20 minutes of slow stirring. It must also be noted that either 1 mg $L^{-1}$ alum or 0.25 mg $L^{-1}$ chitosan does not produce any flocs when used separately.

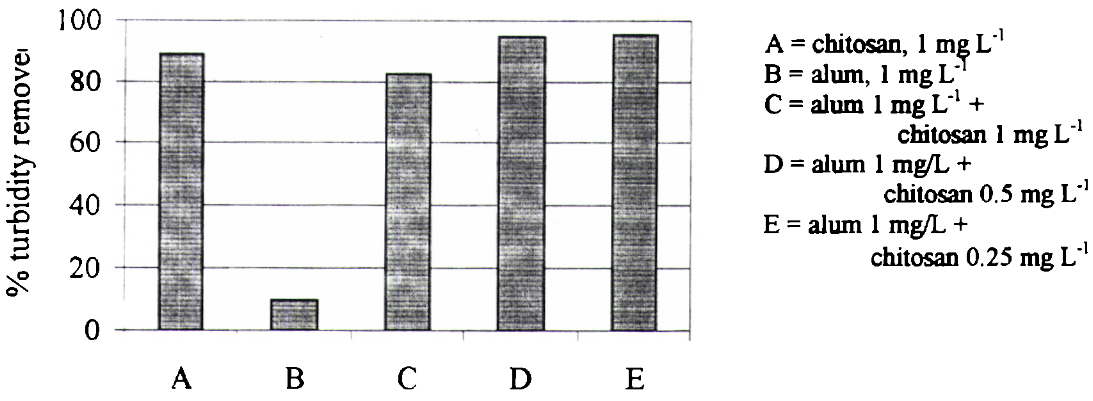


Figure 3.9.

Effect of various combinations of alum and chitosan on removal of turbidity from a kaolinite suspension of 50 NTU in tap water, pH=7.5.

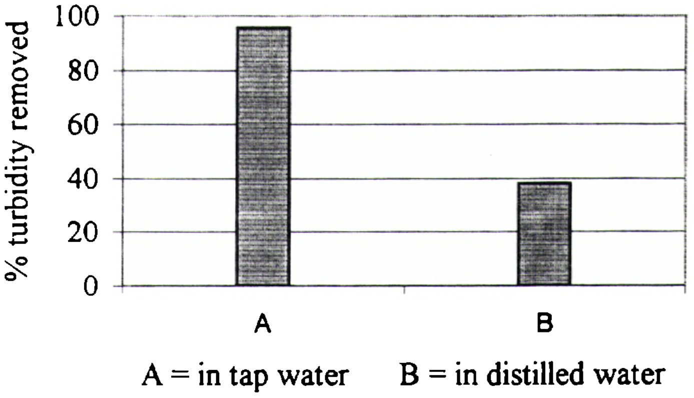


Figure 3.10.

Effect of preparing kaolinite suspension in tap water and in distilled water on flocculation using a mixture of 1 mg L$^{-1}$ alum and 0.25 mg L$^{-1}$ chitosan

Further experiments showed that this combination does not work when suspensions of kaolinite in distilled water are used. This is illustrated using a mixture of 1 mg of alum and 0.25 mg of chitosan as flocculant per litre of kaolinite suspensions of turbidity 50 NTU in tap water and distilled water respectively. The result is shown in Figure 3.10.

### 3.6 Conclusions

Chitosan is able to flocculate and remove turbidity due to suspended kaolinite particles in aqueous medium containing traces of humic substances. The efficacy of chitosan in flocculating kaolinite is strongly dependent on pH of the medium under the test conditions. It is very effective in the pH range 7.0 to 7.5 and the effectiveness falls rapidly at higher or lower pH values. A very low flocculant concentration of 1 mg/L of chitosan effectively reduces the residual turbidity to below 5 NTU irrespective of initial turbidity. A mechanism for flocculation of kaolinite by chitosan in presence of humic substances, which is capable of explaining all the above observations, is proposed. It was also noted that the efficiency of chitosan solution to flocculate kaolinite suspensions improves by storing the solution for two weeks to three months. The flocs produced are coarse and settle rapidly. The total time required for flocculation and settling is less than one hour. The chitosan added is removed as the sludge. Further, chitosan is non-toxic, biodegradable and the sludge produced can be safely disposed off in landfills.

## Chapter 4

# Flocculation of river silt using chitosan

### 4.1 Introduction

Removal of turbidity from river water is a problem confronting water treatment plants for producing potable water, especially during the dry season when silt is sucked into the pumping well. Alum, combined with lime, is commonly being used for clarifying water at present. The experience gained from our studies on the flocculation of kaolinite suspensions using chitosan was applied to this situation. In this study, the effectiveness of chitosan as a flocculant, when used alone, on the removal of turbidity due to river silt was examined at various concentrations of suspended particles and flocculant, and at different pH values. Samples of river silt collected from the *Periyar River* was resuspended and used in the laboratory studies, and the results were confirmed using natural turbid river water during the monsoon.

## 4.2 Materials and methods

### 4.2.1 Silt suspensions

Samples of naturally settled river silt were collected from shallow areas of the *Periyar River* near Aluva (Ernakulam District, Kerala, India). Raw silt was mixed with water and resuspended by rapid mechanical agitation. Portions of this suspension were withdrawn and diluted as necessary with tap water to obtain the turbidity required for the experiments. The tap water used for dilution had the following properties estimated as given in Standard Methods: pH 7.2 to 7.5, turbidity less than 1.0 NTU, total acidity = 0, total alkalinity 18 mg $CaCO_3$ per litre, total hardness 12 mg $CaCO_3$ per litre. The suspensions were prepared immediately before each set of experiments. The mass of suspended solid at each turbidity value was determined gravimetrically. The silt used consisted mainly of silica particles, with some adsorbed iron oxide. The volatile organic matter was estimated by heating the dry silt to 550°C [Standard Methods, 1995] and found to be 17.8 ± 1.0% of the dry mass.

Turbid water was collected from flooded *Periyar River* during heavy monsoon rainfall at the end of June, and at the end of August when monsoon was receding.

### 4.2.2 Chitosan solution

One hundred milligrams of chitosan powder was accurately weighed into a glass beaker and mixed with 10 mL of 0.1 M HCl solution and kept aside for about an hour to dissolve. The dissolution process was slow, and some amount of chitosan remained in the form of a thin gel even after this time. It was then diluted to 100 mL with tap water to obtain a solution containing 1.0 mg chitosan per mL of solution. As it was observed that chitosan solutions in acid medium undergo some change in properties over a period of time (section 3.4), the solutions were prepared fresh before each set of experiments.

### 4.2.3 Jar tests

Turbidity of silt suspensions were measured on a Systronics nephelometer model 131 calibrated according to the procedure recommended in Standard Methods. pH values of the suspensions were measured using a Systronics pH meter model 335. Flocculation was carried out on a six-spindle multiple stirrer unit with stainless-steel paddles. All tests were carried out at an ambient temperature of 28 ± 4°C.

The pH of test solution was adjusted by adding either 0.1 M HCl solution or 0.1 M sodium carbonate solution prior to adding the flocculant. It was thus ascertained that flocculation took place at the desired pH. The desired volume of chitosan solution was added to each test suspension and mixed thoroughly by stirring rapidly for five seconds. It was then stirred at a steady rate of 60 rpm for 30 minutes. The stirrer was then switched off and the flocs were allowed to settle undisturbed for 30 minutes. The samples for turbidity measurements were withdrawn using a pipette from a height of 5 cm below the surface.

Six replications of each test were carried out. The mean value obtained for residual turbidity and standard deviation from the six replications were computed.

## 4.3 Results and discussion

### 4.3.1 Relationship between silt concentration and turbidity

Determination of suspended silt concentration (in mg $L^{-1}$) gravimetrically gives a linear relationship with turbidity in NTU, as shown in Figure 4.1.

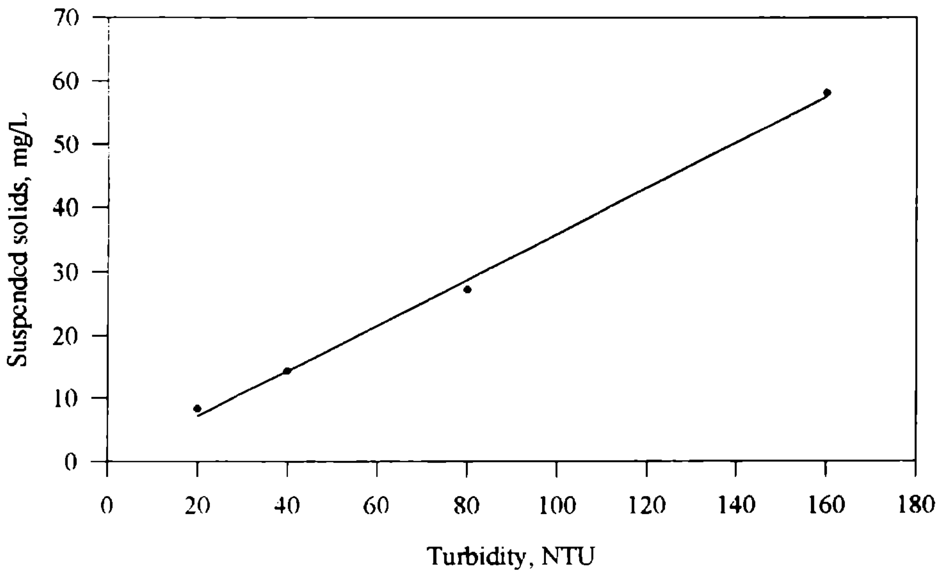


Figure 4.1.

Relationship between mass of suspended river silt (dry mass) and turbidity produced.

### 4.3.2 Effect of pH on flocculation of river silt using chitosan

Tests were conducted as described, at pH values of 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 9.0 ± 0.1 using a flocculant concentration of 1.0 mg $L^{-1}$ The results are presented in Figure 4.2. From the figure, it is seen that chitosan produces appreciable reduction of turbidity only between pH 5 and 7.5. The final turbidities recorded show large variations between experiments at pH values close to 5.5 and 8. The maximum efficiency is observed at pH 7.0. About 90% removal of turbidity is achieved at this pH, without filtration, and the residual turbidity drops below 5 NTU. The flocs obtained are very coarse and settled almost completely in less than 5 minutes.

### 4.3.3 Determination of optimal dosage of chitosan

As maximum efficiency of flocculation was around pH 7, experiments were conducted by varying the dosage of chitosan at this pH on silt suspensions having initial turbidities ranging from 10 to 160 NTU. Tests were run using 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0 mg/L of chitosan. The results are presented in Figure 4.3. The lowest dosage with maximal efficiency was found to be 0.5 mg $L^{-1}$ Above this dosage, the suspensions showed a tendency to restabilise.

### 4.3.4 Relationship between initial turbidity and dosage of chitosan

Results shown in Figure 4.3 indicate that effectiveness of chitosan in removing turbidity due to river silt is very much dependent on flocculant concentration.

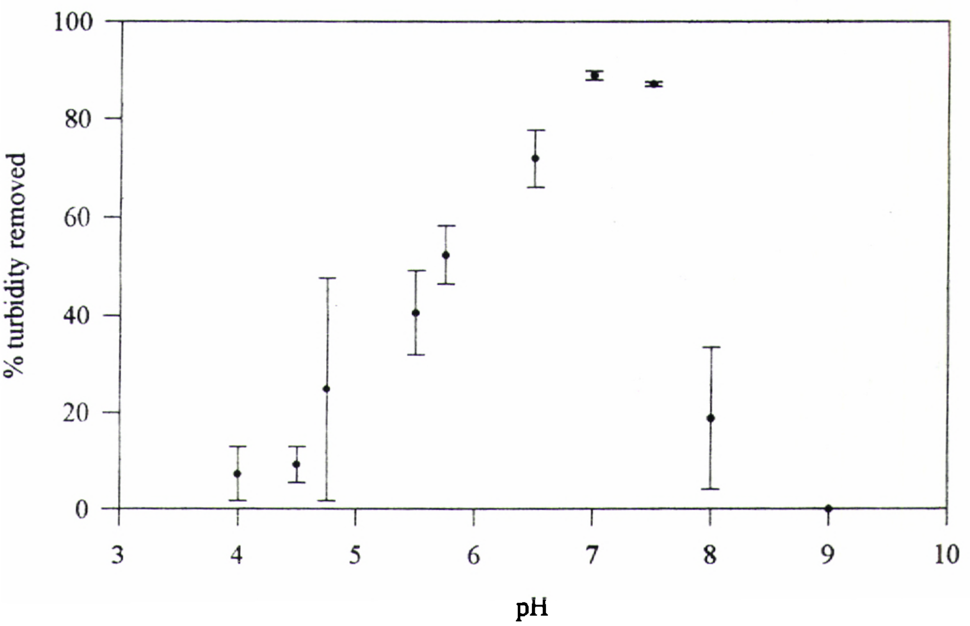


Figure 4.2.

Effect of pH on removal of turbidity due to river silt.

initial turbidity = 40 NTU.

Whiskers indicate one standard deviation from six replications;

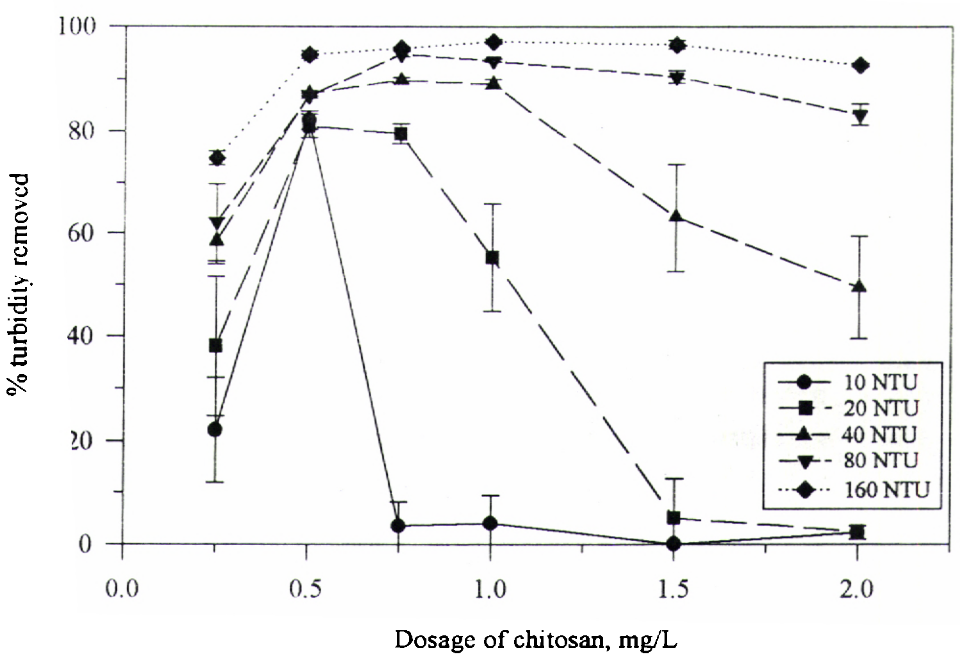


Figure 4.3.

Effect of chitosan concentration on flocculation of river silt.

Whiskers indicate one standard deviation from six replications.

Irrespective of initial turbidity, 0.5 mg $L^{-1}$ of chitosan lowers turbidity below 5 NTU Beyond this dosage turbidity increases, probably due to restabilisation of suspended particles. When flocculant dosage reaches 0.75 mg $L^{-1}$, complete restabilisation occurs for an initial turbidity of 10 NTU. This shifts to 1.5 mg $L^{-1}$ for an initial turbidity of 20 NTU and for higher turbidities, the concentration required to restabilise completely is beyond the range examined here. Thus the dosage of chitosan required to restabilise the silt suspension depends on the concentration of suspended silt particles. Again, in the present study, it is observed that irrespective of initial turbidity, application of 0.5 to1.0 mg $L^{-1}$ of chitosan leaves a residual turbidity of only less than 5 NTU, before filtration, under test conditions (Figure 4.4). The chitosan dosage required to produce the lowest final turbidities increase very slightly with the concentration of suspended silt particles as can be seen from Figure 4.4. Starting with a turbidity value of 10 NTU, which is usually very difficult to clarify in conventional treatment using alum, a residual turbidity of about 2 NTU was obtained after flocculation and settling using chitosan.

It was also observed that at higher initial turbidities, flocs appear rapidly and grow to a larger size. The flocs are fibrous and stick to the stirrer or form large entangled mass resembling cobwebs.

## 4.4 Confirmation of results using naturally turbid river water

Results obtained in the laboratory studies using resuspended river silt were confirmed in experiments carried out using turbid raw water collected from *Periyar*

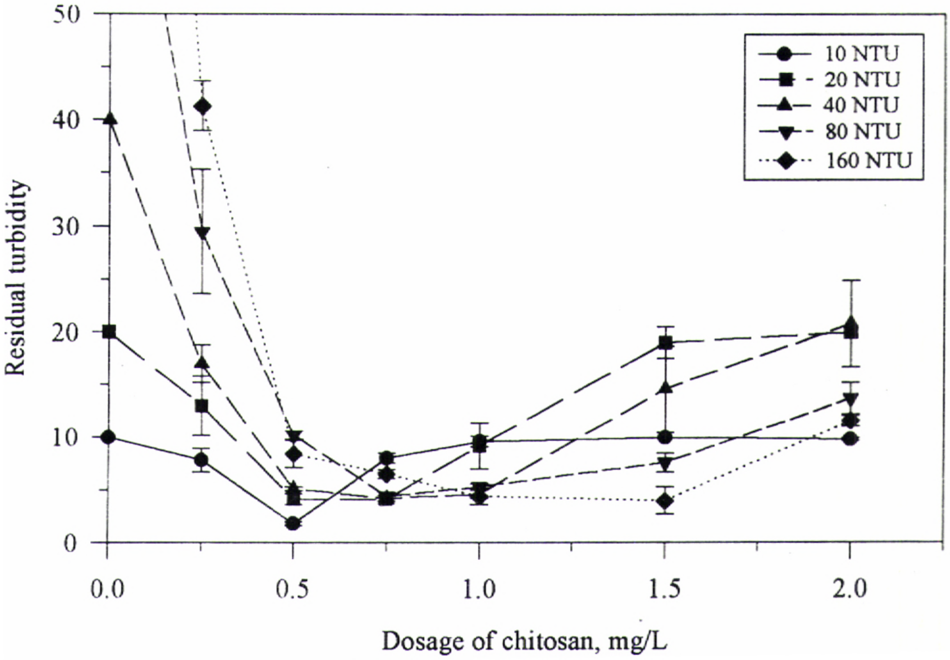


Figure 4.4.

Effect of chitosan concentration on residual turbidity of river silt.

Whiskers indicate one standard deviation from six replications.

Table 4.1.

Characteristics of water collected from *Periyar River.*

| Parameter | June 2000 | August 2000 |
|---|---|---|
| pH | 6.8 | 7.2 |
| Average turbidity | 36 NTU | 26 NTU |
| Methyl orange alkalinity | 4 mg $CaCO_3$ per litre | 4 mg $CaCO_3$ per litre |
| Phenolphthalein alkalinity | nil | nil |
| Total hardness | 14 mg $CaCO_3$ per litre | 10 mg $CaCO_3$ per litre |
| Calcium hardness | 6 mg $CaCO_3$ per litre | 4 mg $CaCO_3$ per litre |

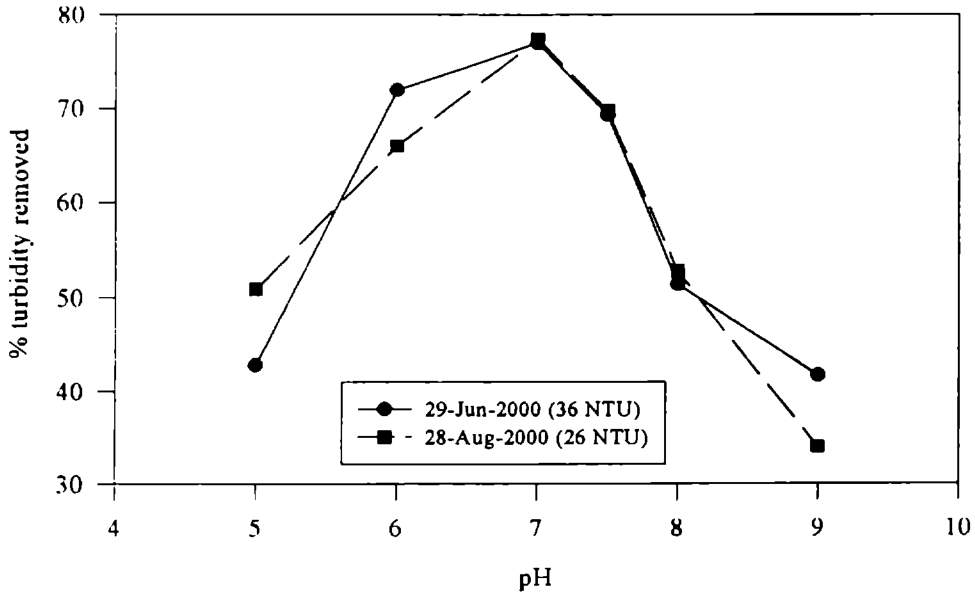


Figure 4.5.

Effect of pH on flocculation of turbid river water

using 1 mg $L^{-1}$ chitosan

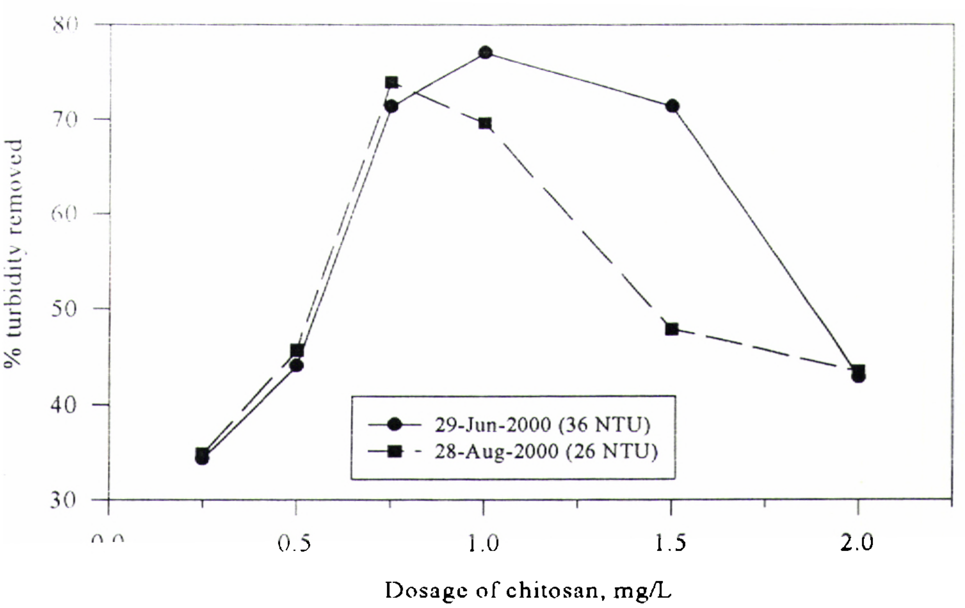


Figure 4.6.

Effect of chitosan concentration on flocculation

of turbid river water at pH = 7.0

*River* during flooding. Important parameters for the collected water are presented in Table 4.1. The effect of varying pH while keeping the flocculant concentration at 1 mg $L^{-1}$ is shown in Figure 4.5. Effect of increasing flocculant dosage keeping the pH at 7 is shown in Figure 4.6. These agree well with the results for resuspended river silt shown in Figures 4.2 and 4.3.

## 4.5 Conclusion

About 90% of the turbidity due to river silt can be removed by chitosan at a pH of 7.0. Any addition of other chemicals for pH correction was not necessary The final turbidity dropped below 5 NTU without filtration. The flocs were coarse and settled almost completely in less than 5 minutes. The optimum concentration of chitosan was found to be 0.5 to 1.0 mg per litre. In fact, the effectiveness decreased at higher concentrations due to restabilisation.

## Chapter 5

# Flocculation of algae using chitosan

### 5.1 Introduction

Algae are constituents of the microscopic phytoplankton which give blue, green or sometimes reddish-brown colour to water bodies and impart a grassy, musty or even fishy odour to the water [Faust and Aly, 1983]. The algae also act as food for zooplankton such as Daphnia and other organisms like small fishes higher up in the food chain. In water bodies or wastewater rich in organic compounds and nutrients like nitrogen and phosphorus, the algae grow much faster than they are consumed, leading to the formation of the so-called "blooms" which float on the water.

The dead algae settle to the bottom and decay, producing malodorous and poisonous gases like ammonia and hydrogen sulphide, and also lead to the rapid depletion of dissolved oxygen in the water. Intracellular and extracellular products released by microalgae in water suppress the growth of harmless bacteria like *E. coli*, but stimulate the growth of harmful pathogens like *V. cholerae* [Oufdou *et al.*, 1998]. Several other problems faced by water quality managers and water suppliers due to the presence of algae have been described [Whitehead *et al.*, 1997; Steynberg *et al.*, 1998; Pan *et al.*, 1999a]. Thus their removal by cost-effective means is very important to treatment plants for the production of potable water as well as water for recreational purposes.

Gravity sedimentation is usually the first method considered for removal of algae in wastewater treatment systems, but the required overflow rates in conventional clarifiers are too low [Nurdogan and Oswald, 1996]. The surface charge of algal particulate matter also makes possible their flocculation by conventional flocculants such as alum and polyelectrolytes. But observations indicate that flocculation of algae using alum is not always very effective [Hejzlar *et al.*, 1998].

Chitosan was used as an aid to assist in the removal of algae by dispersed air flotation process using surfactants [Liu *et al.*, 1999]. This method may be suitable for harvesting the algae, but cannot be used to produce potable water from water bodies infested by algae, due to the presence of the added surfactants. The removal of algae by flocculation and settling using chitosan alone has not been previously reported.

In this study, the effectiveness of chitosan as a flocculant, when used alone, on the removal of algal turbidity due to three different freshwater species of algae, namely *Spirulina*, *Oscillatoria* and *Chlorella* and one brackish water species, namely *Synechocystis*, were examined.

## 5.2 Materials and methods

### 5.2.1 Algal suspensions

Water containing suspended algal cells of *Spirulina* and *Oscillatoria* were obtained from aquaculture ponds maintained by the University, during periods of "bloom" when algae of a particular species were present in high concentrations. The aqueous suspensions had the following characteristics: no methyl orange or phenolphthalein acidity, methyl orange alkalinity 18 mg $CaCO_3$ per litre, no phenolphthalein alkalinity, murexide hardness 60 mg $CaCO_3$ per litre, total hardness 76 mg $CaCO_3$ per litre and conductivity 161 µmho. *Chlorella* and *Synechocystis* were cultured in the laboratory in suitable nutrient media. All the algal suspensions had an original pH of 8.5 to 9.0. These suspensions were then diluted with tapwater of hardness 14 mg $CaCO_3$ per litre and pH 7.4 to 7.6 to obtain the turbidities or concentrations required for the study

### 5.2.2 Chitosan solution

One hundred milligrams of chitosan powder was accurately weighed into a glass beaker and mixed with 10 mL of 0.1M HCl solution and kept aside for about an hour to dissolve completely It was then diluted to 100 mL with tapwater to obtain a solution containing 1.0 mg chitosan per mL of solution.

### 5.2.3 Jar tests

Jar tests were used to determine the parameters for flocculation and settling. Turbidities of algal suspensions were measured on a Systronics nephelometer model 131 calibrated according to the procedure specified in Standard Methods (1998). Chlorophyll content of the suspensions were estimated before and after flocculation using the trichromatic method (method 10200H of Standard Methods,

1998) by measuring the absorbance of aqueous acetone extracts at 630, 645, 663 and 750 nm on a Varian Cary 50 Probe Spectrophotometer. pH values of the suspensions were measured using a Systronics digital pH meter model 335. Flocculation was carried out on a six-spindle multiple stirrer unit with stainless steel paddles. All tests were carried out at an ambient temperature of 28 ± 2°C.

The pH of test solution was adjusted by adding the necessary amount of 0.1 M HCl solution prior to addition of the flocculant. It was thus ascertained that flocculation took place at the desired pH. The required volume of chitosan solution was added to each test suspension and mixed thoroughly by stirring rapidly for five seconds. It was then stirred at a steady rate of 60 rpm for 30 minutes. The stirrer was then switched off and the flocs were allowed to settle undisturbed for 30 minutes. The samples for turbidity measurements were withdrawn using a pipette from a height of 5 cm below the surface.

Six replications of each test were carried out. The mean value obtained for residual turbidity and standard deviation from six replications were computed.

## 5.3 Results and discussion

### 5.3.1 Relationship between chlorophyll-*a* content of the water and turbidity

The algal contents of the tested suspensions were measured before and after flocculation and settling using turbidimetry as well as by measuring chlorophyll-*a* concentration spectrophotometrically. The chlorophyll-*a* concentration (in mg $m^{-3}$)

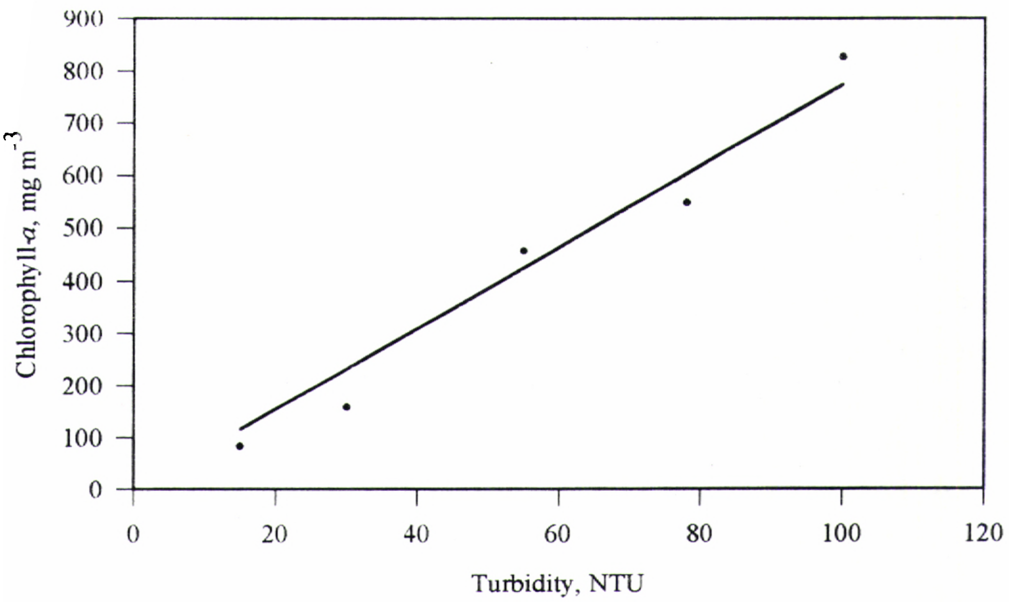


Figure 5.1.

Plot of algal chlorophyll content of water against measured turbidity.

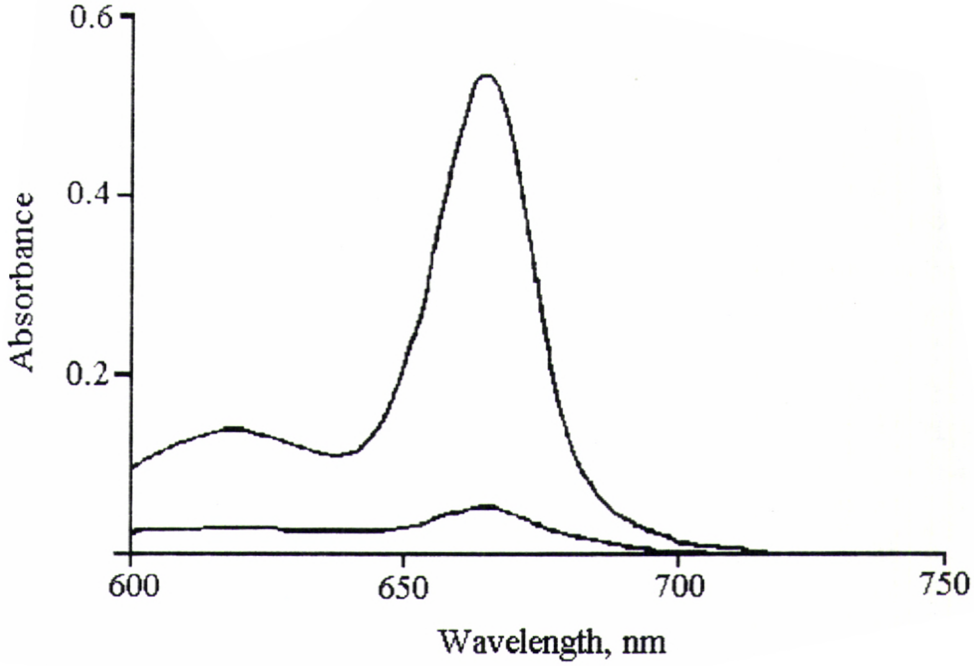


Figure 5.2.

UV absorption spectrum of chlorophyll extracted from water before (upper trace) and after (lower trace) removal of algae by flocculation using chitosan gives a linear relationship with turbidity in NTU as shown in Figure 5.1. This ensures that the decrease in turbidity obtained in the experiments truly reflect a decrease in the concentration of algal cells. A sample UV scan of the chlorophyll extract from an algal suspension of turbidity 55 NTU, in the range 600 to 750 nm, both before and after flocculation is shown in Figure 5.2.

### 5.3.2 Effect of pH on the flocculation of algal cells using chitosan

Tests were conducted as described, at pH values of 5.0, 6.0, 7.0, 7.5, 8.0 and 9.0 ± 0.1 using a flocculant concentration of 5.0 mg $L^{-1}$ The results are presented in Figure 5.3. From the figure, it is seen that chitosan produces maximum reduction of turbidity at pH 7.0. About 90% removal of algal turbidity is achieved at this pH, with the residual turbidity dropping below 5 NTU without filtration. The effect rapidly decreases at higher pH. The flocs produced from low initial algal concentrations are not very dense and show a tendency to float, giving rise to some final turbidity and large standard deviations for the readings obtained. But with high algal concentrations initially, the flocs obtained were very coarse and settled almost completely in a short time.

In the case of the unicellular marine alga *Synechocystis*, the high density of seawater obstructed rapid settling. Therefore dilution of the raw suspension was necessary. The pH had to be brought down to about 5 before adding chitosan to induce flocculation. On keeping this suspension undisturbed, the algal mass slowly

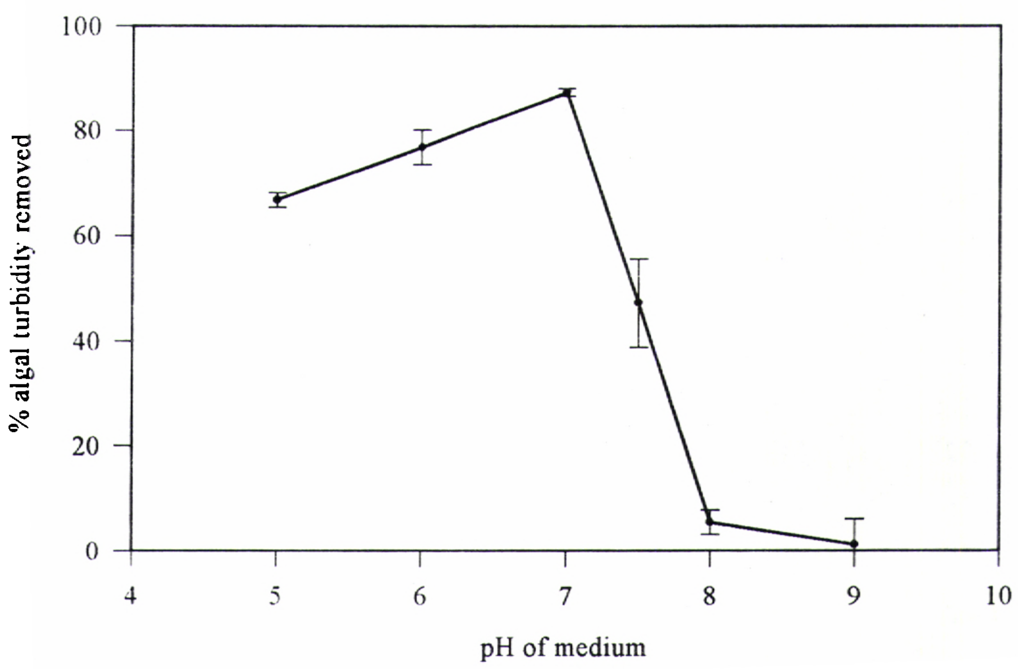


Figure 5.3.

Effect of pH on removal of algae by flocculation using chitosan.

Whiskers indicate one standard deviation from six replications.

fell down in the pattern of zone settling. In this case, stirring was found to fragment the flocs and resuspend it, and then it took a longer time to resettle.

### 5.3.3 Determination of optimal dosage of chitosan

As maximum efficiency of flocculation was around pH 7, experiments were conducted by varying the dosage of chitosan at this pH. Experiments were run using 1.25, 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 mg $L^{-1}$ of chitosan. The results are presented in Figure 5.4. Depending on the concentration of suspended algae, higher doses of flocculant were required to obtain maximum removal, but no definite relationship between these quantities could be established within the range studied. A maximum of 15 mg $L^{-1}$ of chitosan was found to be sufficient to clarify all the suspensions examined. The final turbidities obtained are presented in Figure 5.5.

### 5.3.4 Nature of flocculated algal cells

In all cases, observation under the microscope showed that the algal cells were intact after flocculation, but stuck together in massive clumps. Culturing of these clumps in fresh media showed growth as usual, indicating that the cells were alive even after flocculation and capable of reproduction and growth. But since the clumps tend to remain settled, the fresh cells took longer to become suspended. The decanted supernatant, after removal of the remaining algal flocs by filtration, was also examined for its ability to sustain growth by seeding with fresh cultures of algae. Multiplication of the algal cells was observed, showing that the medium can

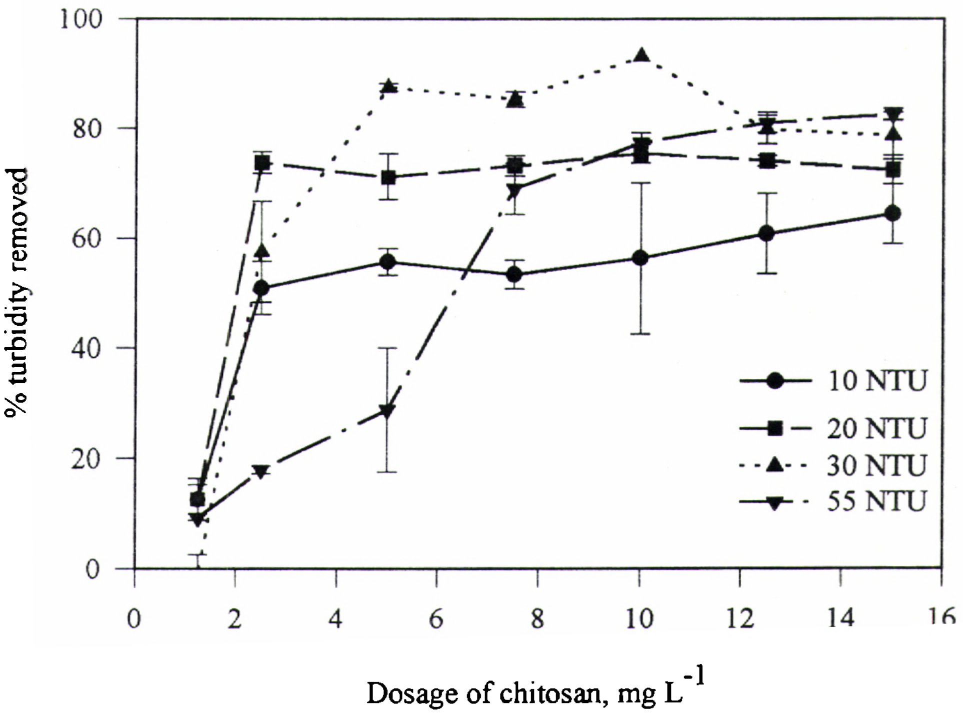


Figure 5.4.

Effect of chitosan dosage on removal of algal turbidity.

Whiskers indicate one standard deviation from six replications.

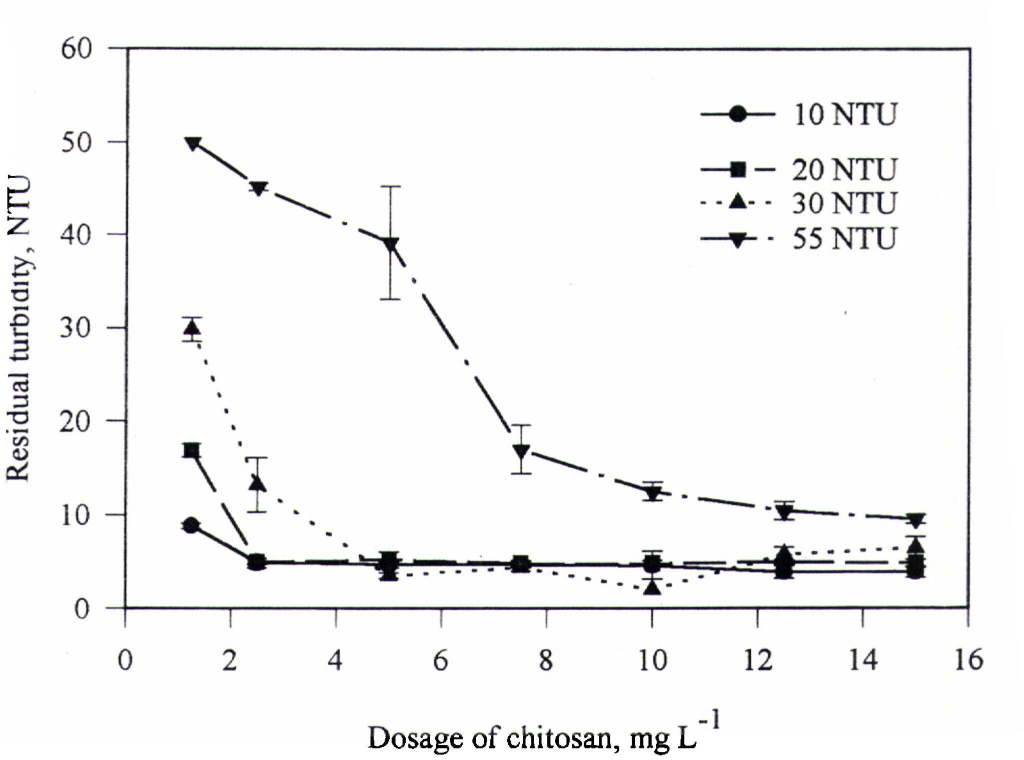


Figure 5.5.

Effect of chitosan dosage on residual turbidity due to algae.

Whiskers indicate one standard deviation from six replications.

still sustain algal growth. But the cells grew as a layer at the bottom of the glass beaker, showing no tendency to become suspended even on mild agitation. This may probably be due to the influence of any residual chitosan present in the medium from the earlier flocculation process. The fact that the algal cells are intact even after chitosan flocculation is very important since release of large amounts of taste and odour compounds usually contained within the algal cells into the water can be prevented.

### 5.3.5 Application in areas other than water treatment

Algae are cultured on a large scale to be used as feed in fish farms and in aquaculture. They are used as "health food", in skin-care preparations, beverages, medicines and plastics [Yang and Zhou (1998), Golz-Berner and Zastrow (1998), Wang (1998), Wadström and Aletung (1998), Berlowitz-Tarrant *et al.* (1998)]. For these purposes, algae are separated from the medium by microfiltration or high-speed centrifugation, which involve poor recovery rates. Chitosan is also a material widely used in foods and medicines, and therefore concentration of algae for the above purposes by flocculation using chitosan offers a fast and safe alternative to these industries. Further, the flocculated algal cells are intact and alive. This is an added advantage in applications in which useful chemicals and drugs produced by suitably bioengineered algae have to be recovered. High-speed centrifugation usually ruptures the cells resulting in the passage of the contents into the medium.

### 5.3.6 Economic feasibility of using chitosan

Chitosan is available as a commercial product, manufactured from crab and prawn shells that are discarded by seafood processing industries. Countries like Japan, China, Taiwan, India and the US, having long coastlines, with large numbers of fishing and seafood processing industries are major producers. The availability, manufacturing processes, production economics, chemistry and possible applications of chitosan are all well documented (Muzzarelli, 1977) and are periodically reviewed in publications related to fisheries. The estimated cost of production is below two US dollars per kg, which is sufficient to treat 50 000 to 500 000 litres of water depending on the algal concentration. Chitosan is nontoxic and biodegradable. Therefore sludge disposal after water treatment may not be a problem. In aquaculture, chitosan has been used for a long time as an immuno-stimulant for protection against bacterial diseases in fish, for controlled release of vaccines and as a diet supplement. Thus, apart from the use in water treatment, the technique is expected to have valuable application in aquaculture, cosmetics, food and pharmaceutical industries, where concentration of algal cells from water using non-toxic, bio-compatible and food-grade material is required.

## 5.4 Conclusion

This study assessed the effectiveness of chitosan in removing turbidity in water due to suspended algal cells belonging to four different genera. Chitosan was found to be very effective in flocculating all these varieties of algae. The efficacy of chitosan in flocculating algal suspensions depends on pH of the medium and the concentration of chitosan under test conditions. There is an earlier report noting that in the coagulation of algal cells, pH values are more important than the differences between various species of algae [Clasen *et al.*, 2000]. Our own observations point to the same conclusion.

Chitosan is most effective at a pH of 7.0 for freshwater algae studied. The effectiveness falls off rapidly at higher pH. Stirring the suspension at a slow rate of about 30 to 60 rpm was also necessary to bring about flocculation in the case of freshwater algae. The mechanism of flocculation is charge neutralization and bridging between algal cells by chitosan chains as in the case of other polyelectrolytes.

For flocculation of the brackish water cyanobacterial species *Synechocystis*, dilution of the medium with fresh water was required to reduce density and facilitate settling. In this case, the pH had to be reduced to 5.1 before adding chitosan, and only the initial rapid mixing for a few seconds was necessary. Fast zone settling occurred in the undisturbed suspension. The flocculated algal cells appear intact on examining under the microscope.

## Chapter 6

# Flocculation of titanium dioxide using chitosan

### 6.1 Introduction

Titanium dioxide is a refractory oxide, insoluble in water and biologically inert. Aqueous suspensions of $TiO_2$ are very difficult to settle even when centrifuged. These properties provide it with a wide variety of applications in the manufacture of paints, ceramics, cosmetics, medicines, surgical prostheses, catalysts, glass etc. It can enter water from all these industrial sources apart from a large quantity directly from the mineral (rutile) processing industries. Study of literature indicates that till about 1995, all research had been directed towards developing methods for producing stable suspensions of the compound for application in paints, cosmetics and medicines [for example, Bauer *et al.*, 1999]. Microfiltration using ceramic membranes [Li and Zhao (1999), Tong *et al.* (1999), Xing *et al* (1999)] hydrogravimetric and magnetic separation methods [Belardi and Piga, 1998] have been used earlier for the recovery of $TiO_2$ from process streams. There are only a few reports available on the removal of $TiO_2$ by flocculation and settling, in which sodium sulphate [Fernandez-Nieves *et al.* (1998)], basic aluminium chloride [Kagaya et al., 1999] and synthetic cationic polyelectrolytes [Subramanian *et al.* (1999), Li *et al.* (1999)] have been used. Floatation using surfactants has also been reported [Shen, 1998]. There is no report on the use of chitosan for this purpose. Recently, there is a spurt in the number of papers appearing on the application of $TiO_2$ as a catalyst for the photochemical mineralization of refractory organic contaminants in wastewater [Bekbolet (1996), Duczmal and Sobczynski (1999), Goncalves *et al.* (1999), Rao *et al.* (1999), Wu *et al.* (1999) to cite a few]. Since $TiO_2$/UV treatment is turning out to be a preferred technology for the destruction of refractory organic pollutants in industrial wastewater, this is expected to be a major source of the compound in treated wastewater, which necessitates treatment for its removal.

In this chapter, the results of our studies using chitosan for removal of $TiO_2$ turbidity from water by flocculation and settling are presented.

## 6.2 Materials and methods

### 6.2.1 $TiO_2$ suspensions

Titanium(IV) oxide supplied by Merck (Batch No. TG5 27816) as laboratory reagent was used in these studies. It was obtained in the form of a very fine, white powder, which forms a milky suspension in water. Five milligrams of the powder dispersed in one litre of tap water containing traces of humic substances (not added, see chapter 3 for details) gave a stable suspension of 70 to 80 NTU turbidity, which did not settle on standing.

### 6.2.2 Chitosan solution

Chitosan solution was prepared as given under section 3.2.1.3 in chapter 3.

### 6.2.3 Solution for adjusting calcium hardness

Solutions were prepared by dissolving 2.5 g of $NaHCO_3$ and 2.2 g $CaCl_2.2H_2O$ (both Qualigens Laboratory Reagent grade) separately in 100 mL each of distilled water. Addition of 1 mL each of these solutions to 1 L distilled water will produce in it a hardness equivalent to 15 mg $L^{-1}$ of $CaCO_3$, which is similar to that in tap water (see section 3.2.1.1 in chapter 3) but without the natural organic matter.

### 6.2.4 Jar tests

Jar tests were carried out as described in section 3.2.1.4 in chapter 3, using $TiO_2$ suspensions. The stirring period at 60 rpm was extended to 1 h since formation of flocs took a longer time.

## 6.3 Results and discussion

### 6.3.1 Effect of pH on flocculation of $TiO_2$ using chitosan

The effect of pH was studied by flocculating a suspension containing 5 mg $L^{-1}$ of $TiO_2$ having a turbidity of about 80 NTU using a dosage of 1 mg $L^{-1}$ of chitosan. Jar tests were conducted at pH values of 3.0, 4.0, 5.0, 6.0, 7.0, 7.5, 8.0, 9.0, 10.0 and 11.0. Plot of the percentage of turbidity removed against pH of the suspension is shown in Figure 6.1. Chitosan flocculates $TiO_2$ suspensions only between pH 7.0 and 8.0, with maximum removal of turbidity at pH 7.5.

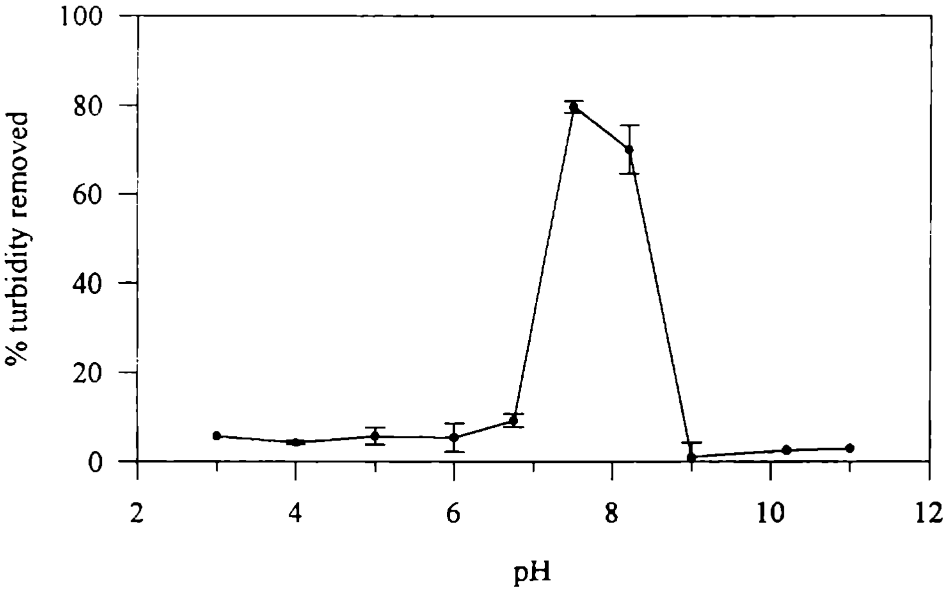


Figure 6.1.

Effect of pH on flocculation of $TiO_2$ suspension

(turbidity = 80 NTU) using 1 mg/L of chitosan.

Whiskers indicate one standard deviation from six replications.

### 6.3.2 Effect of chitosan dosage on flocculation of $TiO_2$

It is seen from Figure 6.2 that 0.75 to 1.0 mg/L chitosan is the most effective dosage for an 80 NTU suspension of $TiO_2$. But the residual turbidity attained is somewhat high as can be seen from Figure 6.3. The turbidity does not fall below 15 NTU.

### 6.3.3 Studies using suspensions in distilled water

Similar to the observations in experiments discussed in chapter 3, chitosan failed to produce any flocs with $TiO_2$ suspensions in distilled water. As this could be due to the absence of adsorbed humic matter, flocculation studies were carried out after adding various amounts of soil extract to the distilled water used as suspension medium. The results are presented in Figure 6.4. The soil extract was prepared by extracting 1 kg of a dark surface soil containing much decomposed organic matter with 1 L of distilled water followed by filtration. This gave a golden yellow solution. The humic material was not further characterised.

It was observed that even the addition of humic substances to the distilled water failed to produce sufficient flocs in the case of $TiO_2$ suspensions. Suspensions of 88 NTU prepared in distilled water media containing varying amounts of soil extract all resulted in almost similar but high residual turbidity of around 40 NTU, indicating that the medium is still not equivalent to the tap water used in these studies. On the other hand, addition of sufficient amounts of a synthetic hardness solution, prepared as described in section 6.2.3, in addition to

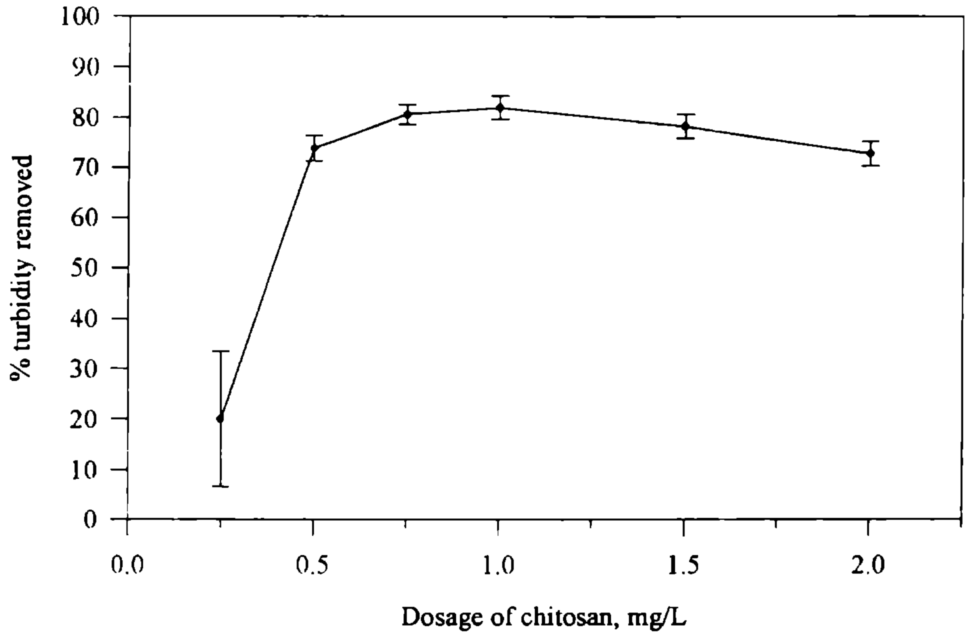


Figure 6.2.

Effect of chitosan dosage on removal of $TiO_2$ turbidity by flocculation and settling. 5 mg/L suspension in tap water. Turbidity = 80 NTU, pH = 7.5. Whiskers indicate one standard deviation from six replications.

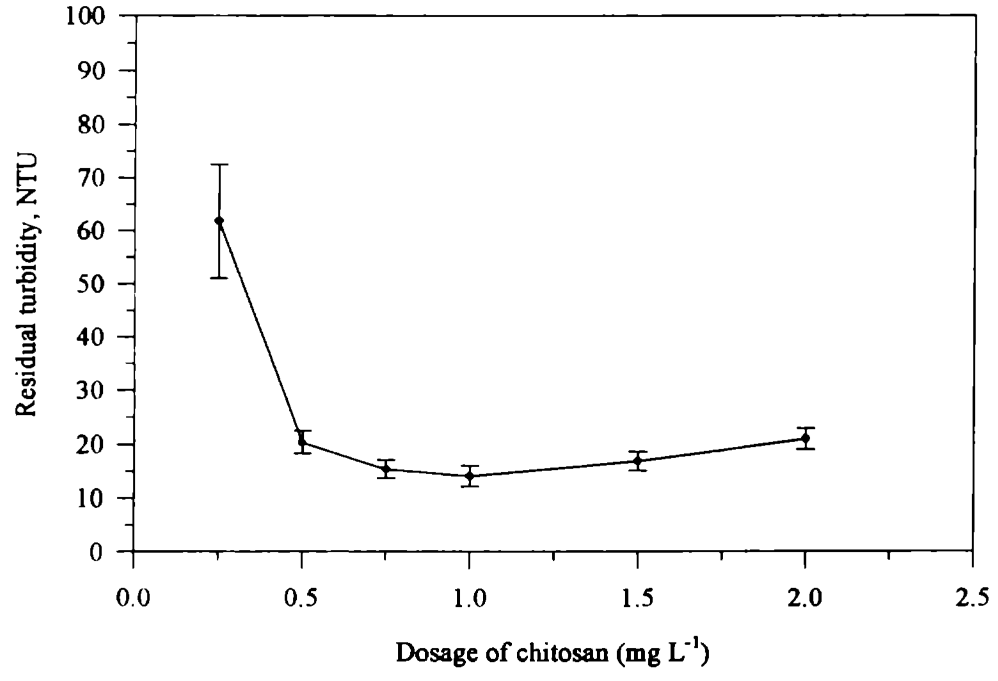


Figure 6.3.

Effect of chitosan dosage on residual turbidity after flocculation and settling of a 5 mg/L suspension of $TiO_2$ in tap water, Turbidity = 80 NTU, pH = 7.5. Whiskers indicate one standard deviation from six replications.

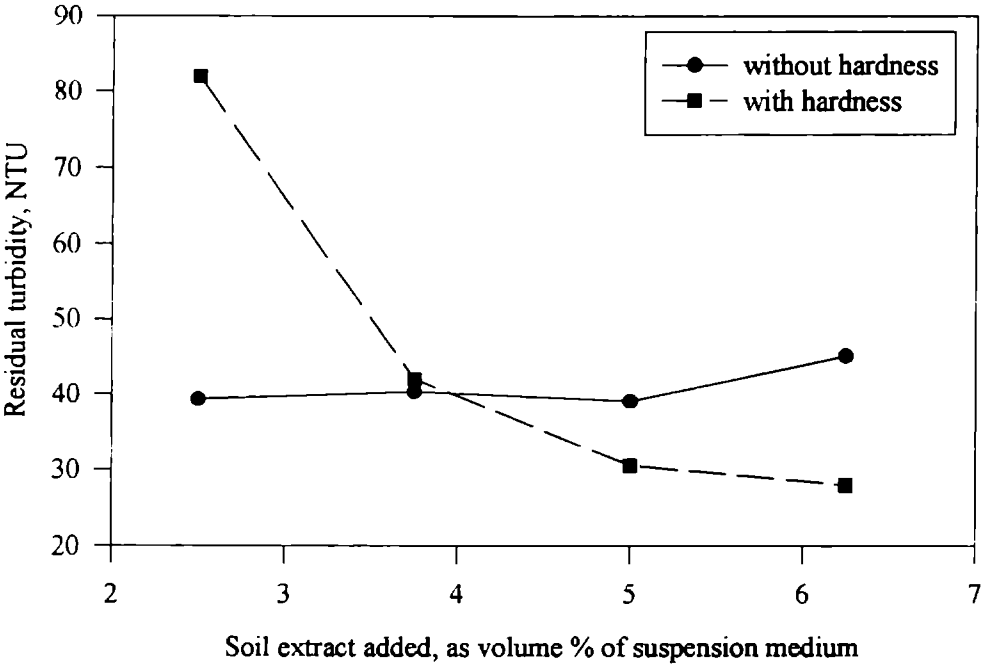


Figure 6.4.

Effect of adding soil extract and hardness to the distilled water used as suspension medium for $TiO_2$ on flocculation using chitosan.

Turbidity = 88 NTU, pH = 7.5

the soil extract, produces a clarity that increases with increasing humic content. Thus in the case of $TiO_2$, the presence of a small amount of hardness is necessary in addition to humic substances in order to produce good flocs with chitosan.

## 6.4 Conclusion

Suspensions of 5 mg of $TiO_2$ per litre of tap water containing traces of humic substances can be clarified by flocculation and settling using chitosan. Suspensions in distilled water did not respond to the treatment. The presence of a small amount of calcium hardness in addition to humic substances in small concentrations was found necessary to induce flocculation of $TiO_2$ suspensions using chitosan. Flocculation of $TiO_2$ suspensions was more difficult compared to the other suspensions used in our studies. It was even more sensitive to pH than the other suspensions studied, and the best results were obtained at a pH of 7.5. A prolonged period of slow stirring (about 45 minutes) was necessary to initiate flocculation. A long kinetic delay in the onset of flocculation of $TiO_2$ using synthetic polyelectrolytes was also noticed by Li *et al.* (1999) who reported that this delay increases with polymer dosage. A concentration of about 1 mg per litre of chitosan gave the best results. Flocs of $TiO_2$ containing less chitosan were seen to adhere strongly to the bottom of the beaker on settling and could be washed off only with difficulty while flocs with a higher chitosan content washed off easily. This probably indicates incomplete coverage of the surface of $TiO_2$ particles when low dosages of chitosan are used and active sites are still present on the surface. At higher dosages of chitosan, these sites are also covered.

## Chapter 7

# Reducing the colour of tea decoction using chitosan

### 7.1 Introduction

Tea is a mildly stimulating drink liked by people all over the world. It ranks as the most popular refreshing drink in more countries than any other beverage. India has always played a dominant role in world tea production. Today, India produces about 720 million kilograms each year. China is the second largest producer with about 525 million kilograms annually. Other tea-producing countries include Indonesia, Kenya and Sri Lanka. In 1990, the global production of dried tea was about 2.5 billion kilograms.

Botanically, the tea plant is *Camellia sinensis*, a member of the family Theaceae. The plant matures in three to five years and produces a growth of new shoots called *flush*. Each shoot consists of several leaves and a bud. These are plucked and processed. There are three main kinds of tea: (1) black, (2) green, and (3) oolong. They differ in the method used to process the leaves. Black tea is the most common variety. It is used in various forms such as black tea, lemon tea, with added milk and sugar, instant tea etc. Teas grown in different countries, or even in different parts of the same country, vary in taste, flavour, and quality.

The quality of tea is decided by properties like flavour, colour and briskness or strength. The flavour and aroma depend on the aldehydes and terpenoids such as geraniol, linalool and β-ionene that are present. The colour is due to the polyphenols extracted into the (boiling) water during preparation of the decoction and the strength is contributed by the caffeine content.

Caffeine, $C_8H_{10}O_2N_4$, is a nerve stimulant and a diuretic. It is weakly basic and its salts, usually with citric acid, are used in medicine.

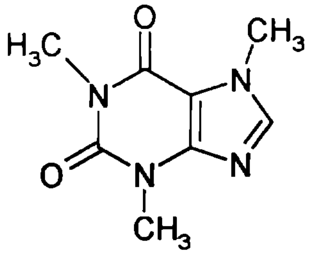


Figure 7.1. Caffeine

About 2.5 to 5% of caffeine is present in processed dry tea leaves. It is the active principle, and is extracted into water along with polyphenolic materials when the decoction is prepared. The latter gives a dark colour to the extract and contributes to the bitter taste. There have been several attempts, especially by the tea industry, to improve the taste, colour, shelf-life etc. of the extract and to produce instant or bottled tea. Several diverse and exotic chemical substances and enzymes like cyclodextrins and tannases [Ito *et al.* (1991), Shibata *et al.* (1993)], oligosaccharides [Takahara and Igai (1991)], fatty acid esters of glucose and glycerin [Tanaka, 1991a], alkali hydroxides [Tanaka, 1991b], calcium salts [Imura and Sakurai, 1991] and alanine [Osada *et al.* (1992)] have been tried for clarification of tea decoctions.

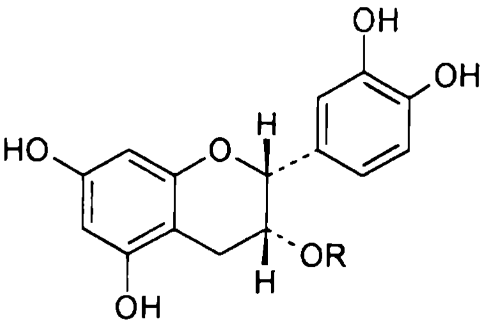
Chitosan being an edible and safe substance with proven flocculating ability, we checked its effect on tea extract as an example of colour removal.

## 7.2 The chemical nature of colouring matter in tea decoction

The polyphenolic compounds present in raw tea leaves are a small group of flavanols belonging to the group known as catechins. These are colourless, water-soluble substances with an astringent taste. They constitute 20 to 30% of the dried leaves. Chemical structures of a few of the more important compounds belonging to this class are given in Figures 7.2 and 7.3.

Black tea is manufactured by promoting the oxidation of catechins in fresh tea leaves by atmospheric oxygen catalysed by tea polyphenolase enzymes through processes like withering, rolling, fermentation and taring [Kirk-Othmer, 1983]. During these processes, several chemical changes take place within the leaves, building up the colour, flavour and strength acceptable to a discerning user. A brief sketch of these changes are given in Figures 7.4 and 7.5. Theaflavins, theaflavic acids and bisflavanols are produced by the condensation of oxidation products of catechins.

Theaflavins exhibit a bright orange-red colour in solution and are important in the determination of brightness, a desirable attribute in tea beverages. They are distinctive enough in colour to be determined spectrophotometrically in tea brew. Theaflavic acids are bright red, acidic substances present only in very small quantities. Bisflavanols are also present only in small quantities.

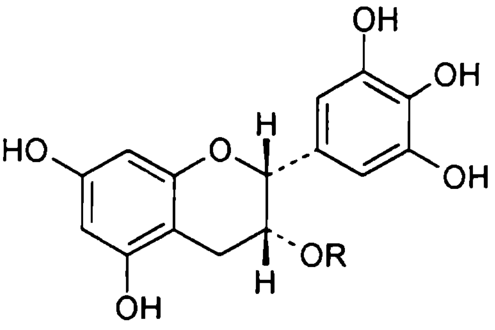


[1] (-)-epicatechin

R = H; 1 to 3% dry wt.

[2] (-)-epicatechin gallate

R = 3,4,5-trihydroxybenzoyl
3 to 6% dry wt.

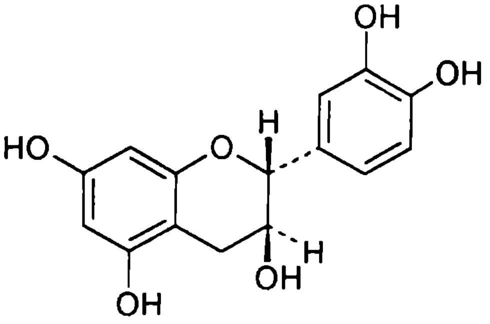


[3] (-)-epigallocatechin

R = H; 3 to 6% dry wt.

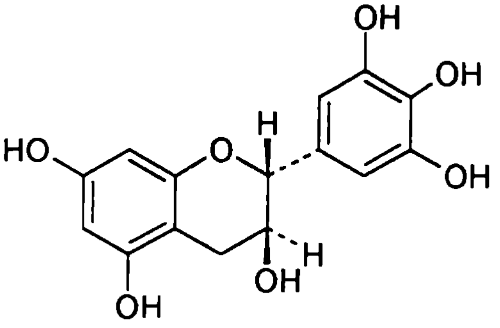
[4] (-)-epigallocatechin gallate

R = 3,4,5-trihydroxybenzoyl
9 to 13% dry wt.



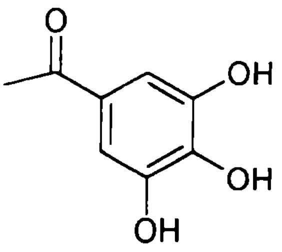
[5] (+)-catechin

1 to 2% dry wt.



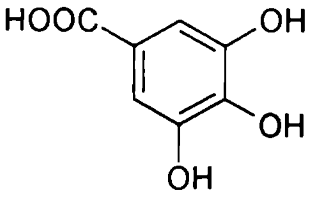
[6] (+)-gallocatechin

3 to 4% dry wt.

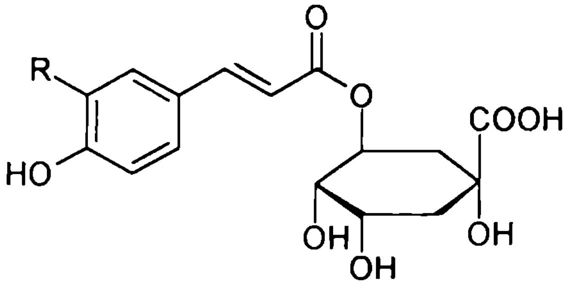


The 3,4,5-trihydroxybenzoyl group

Figure 7.2. Flavanols present in tea leaves.

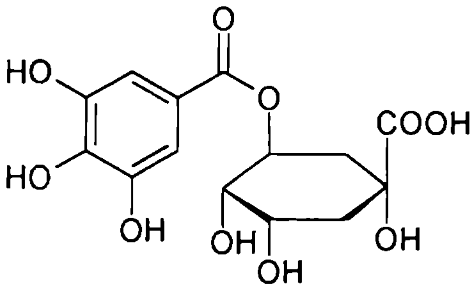


[7] gallic acid

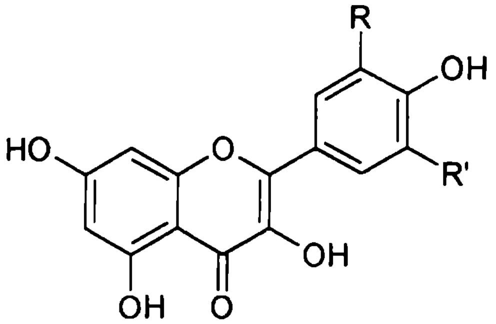


[8] chlorgenic acid; R = OH

[9] p-coumaryl quinic acid; R = H



[10] theogallin



[11] kaempferol; R = R' = H

[12] quercetin; R = OH, R' = H

[13] myricetin; R = R' = OH

Figure 7.3.

Polyphenols other than flavanols present in raw tea leaves.

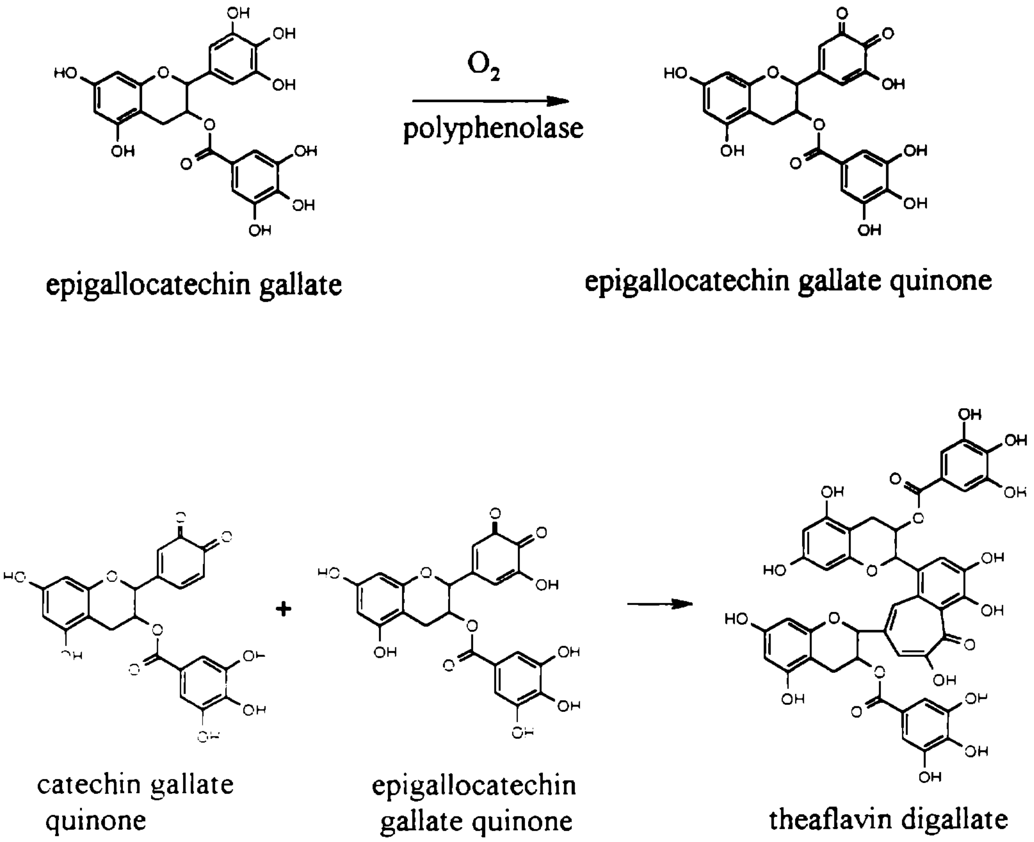


Figure 7.4.

Formation of theaflavins from catechins

during processing of tea

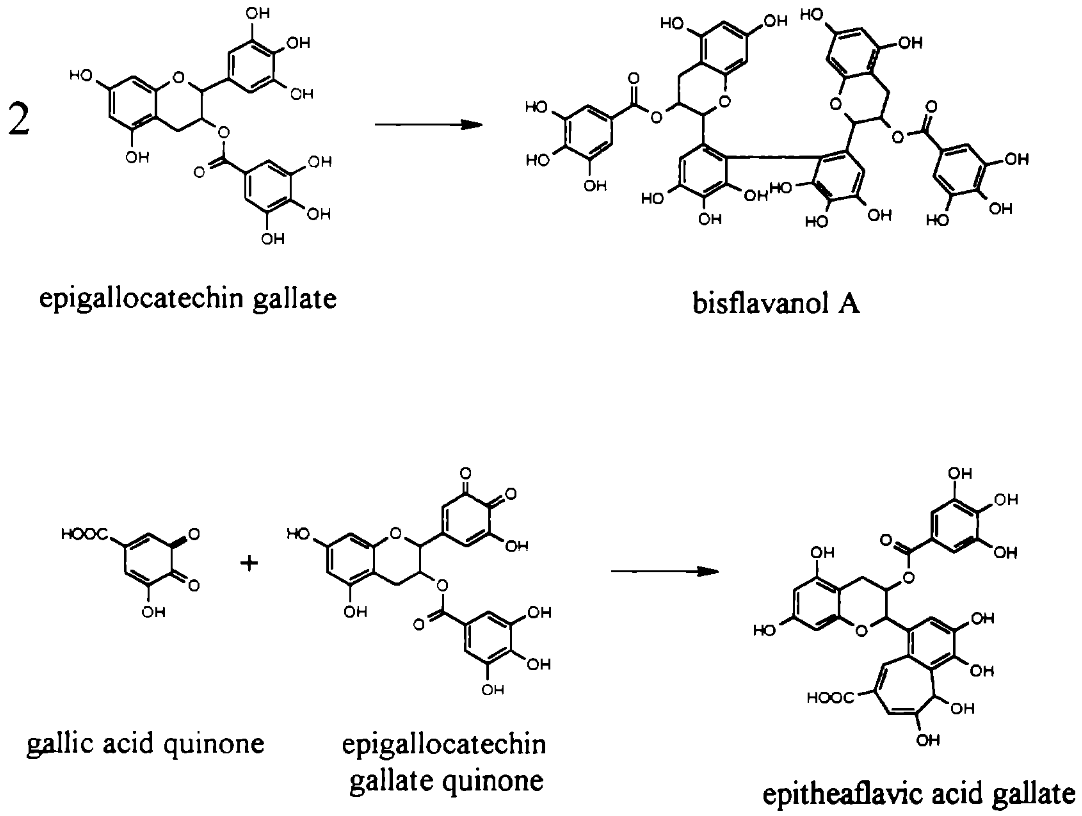


Figure 7.5.

Formation of bisflavanols and theaflavic acids from catechins during processing of tea

In the finished product, about 15% of the original catechins remain unchanged and about 10% are converted to the compounds mentioned above. The remaining 75% undergo further condensation resulting in complex, poorly defined incompletely separated group of substances known as thearubigens because of their red-brown colour. Investigations of thearubigen chemistry have resulted in conflicting data and conclusions, indicating the complexity of the mixture. However, examination of the structure of simpler intermediates provided in figures 7.4 and 7.5 may provide some insight into what is to be expected of the final structures of thearubigens. Molecular weight determinations indicate a range between 700 and 40 000.

## 7.3 Materials and methods

### 7.3.1 Tea

AVT Premium Garden Fresh CTC tea purchased from the local market was used in this study.

### 7.3.2 Chloroform solvent

Qualigens LR-grade Chloroform was distilled and the middle fraction boiling at 59-60°C was used. It did not show any UV absorption in the 250-350 nm range.

### 7.3.3 Chitosan solution

One gram of chitosan powder was dissolved in 100 mL of 0.1 M HCl solution to get a 1% (w/v) solution of chitosan.

### 7.3.4 Preparation of pure caffeine

Method given in Chawla and Prakash (1979) was used. About 100 g of the tea leaves were boiled with 1 L of distilled water and filtered by squeezing through fine cloth. The filtrate was treated with lead acetate to precipitate tannins. Unreacted lead is precipitated by adding dilute sulphuric acid and filtered. Caffeine is then extracted into chloroform. The extract is washed with dilute KOH solution and then with water. The solvent is distilled off and the residue washed with a little petroleum ether to remove oily matter. The residue was recrystallised twice from hot water to get white needle-shaped crystals. The sample gave a single spot on TLC (silica gel/acetone), m. p. 234°C, UV $\lambda_{max}$ = 276 nm.

### 7.3.5 Tea decoction

Ten grams of tea leaves were added to one litre of boiling distilled water, stirred to mix and kept for 5 min. Decanted the clear supernatant and allowed to cool to room temperature.

### 7.3.6 Determination of total solids in tea decoctions

Clean 250 mL glass beakers were dried in the air oven at 110 ± 5°C for two hours, cooled to ambient in a dessicator and quickly weighed on an electronic balance. Exactly 200 mL each of tea decoction were taken in these beakers and evaporated to dryness on a water bath. The beakers with their contents were then dried in the air oven for 2 h and weighed as before. Total solids were estimated from the difference in weights, and its percentage (w/v) calculated.

### 7.3.7 Calibration curve for caffeine estimation

About 5 mg of pure caffeine (see section 7.3.4), were accurately weighed, dissolved and made up to 100 mL using chloroform. Aliquots of 5, 10, 15, 20 and 25 mL were withdrawn from this solution and separately made up to 50 mL using chloroform to get standard solutions of varying caffeine content. The absorbance of each solution was measured on a Varian Cary 50 Probe UV-Visible spectrophotometer using quartz cell of 1 cm path length. The calibration curve obtained is shown in Figure 7.6.

### 7.3.8 Estimation of caffeine in tea decoctions

Method specified in Official Methods of Analysis of AOAC International (16th edition) recommends purification of the chloroform extract by passing through acidic and basic celite-545 columns. Instead, washing the extract with dilute acid and alkali solutions is adopted in the procedure used here.

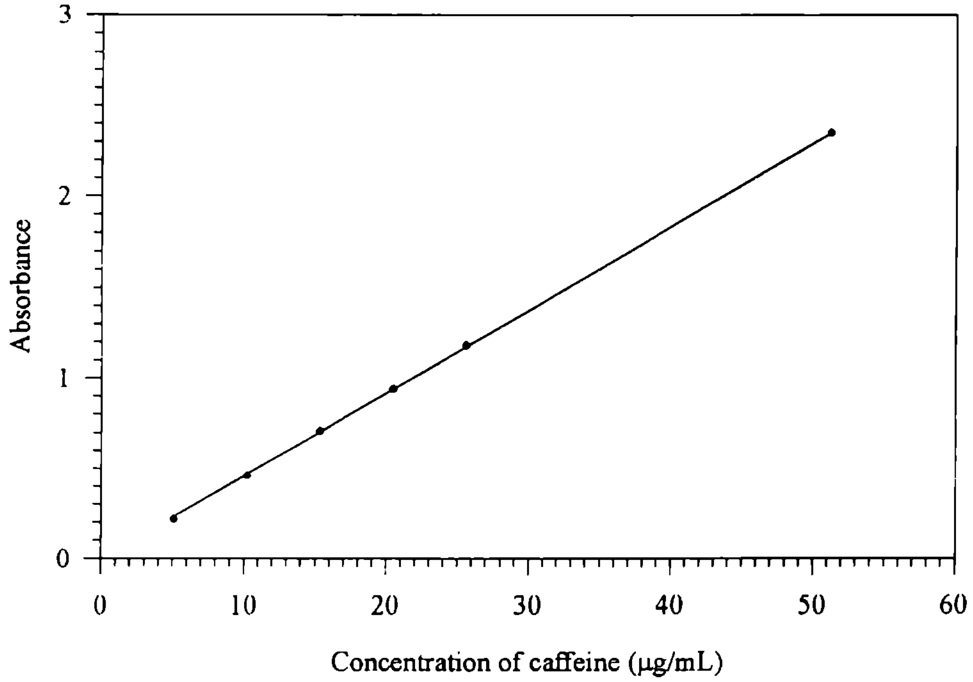


Figure 7.6.

Calibration curve for caffeine solutions in chloroform. $\lambda_{max}$ = 276 nm.

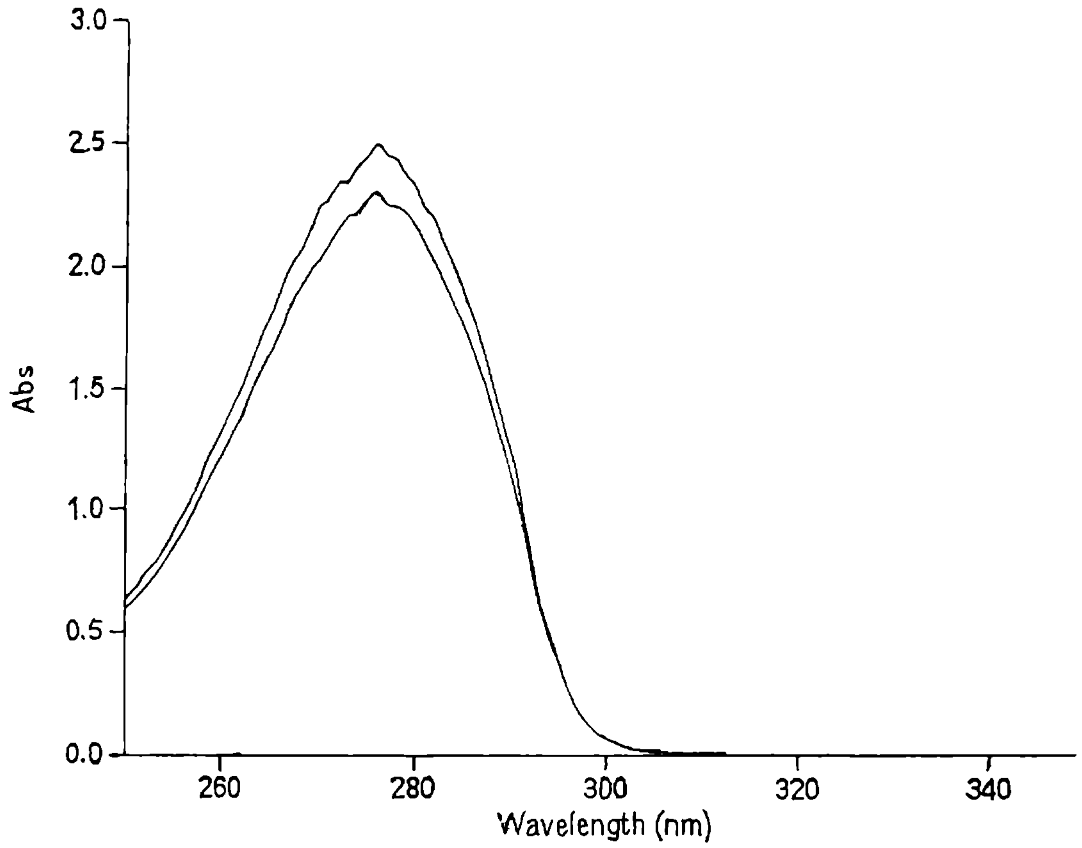


Figure 7.7.

UV absorption bands of caffeine extracted from tea decoctions.

Upper trace = raw decoction; lower trace = after treatment with chitosan.

Fifty millilitres of the tea decoction was treated with 10 mL of dilute sulphuric acid (1 10). This was extracted three times with 10 mL, 5 mL and 5 mL quantities of chloroform. The combined extract was washed with 5 mL of 0.5% KOH solution followed by 5 mL distilled water. The extract was made up to 50 mL using chloroform. One millilitre of this solution was again diluted to 5 mL using chloroform and the absorption at 276 nm was measured on a Varian Cary 50 Probe UV-Visible spectrophotometer and compared against the calibration curve to read the concentration. Since the calibration plot is linear, a single point calibration was used in routine measurements.

### 7.3.9 Treatment of tea decoction using chitosan

Tea decoction (450 mL) was taken in a 1 L Erlenmeyer flask and small quantities of chitosan solution were added to it with swirling. A bulky brown mass precipitated, and the process was continued till no more precipitation occurred. The contents of the flask were allowed to settle and the clear supernatant was decanted. Total solids in the decanted decoction were estimated as in section 7.3.6 and caffeine content estimated as in section 7.3.8.

### 7.3.10 Spectrophotometric method for colour determination

In order to avoid subjective judgements, the tristimulus spectroscopic method of colour measurement [method 2120c in Standard Methods] was adopted for comparing colour of tea decoctions. This method attempts to describe the

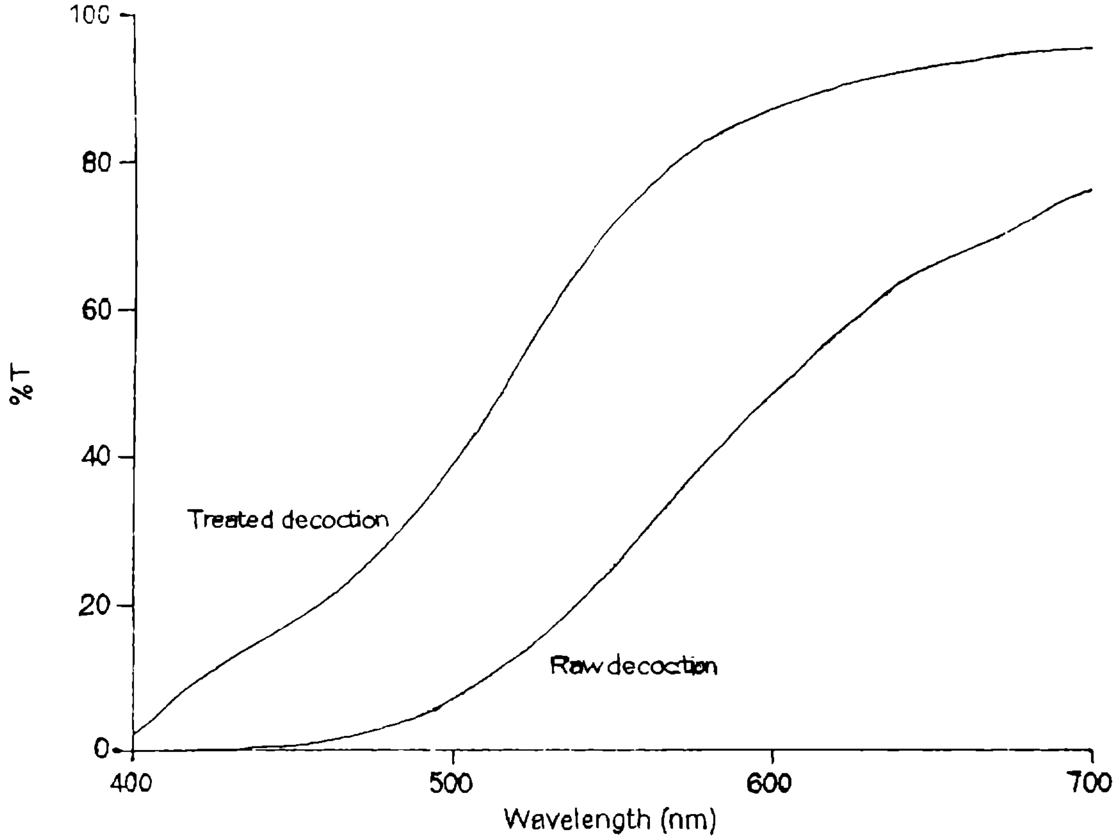


Figure 7.8.

UV scan of tea decoctions in the visible region.

Lower trace = raw decoction, upper trace = chitosan-treated decoction.

sensation realised when the sample is actually viewed. The 'hue' (red, green, yellow etc.) is designated by the term "dominant wavelength", the 'degree of brightness' by "luminance" and the 'saturation' (pale, dark etc.) by "purity" These values are determined from the light transmission characteristics of the sample recorded by means of a spectrophotometer.

The sample, the colour of which is to be determined, is taken in a clean glass cuvette and scanned in the visible range from 400 to 700 nm. The %T values corresponding to three different sets (X, Y and Z) of ten specified wavelengths are recorded and tabulated (Tables 7.1 and 7.2). The sum of the %T values in each set is calculated and multiplied by a specified factor to obtain representative values of $X_c$, $Y_c$ and $Z_c$. The $X_c$ and $Y_c$ values are then separately divided by the sum $X_c+Y_c+Z_c$ to get trichromatic coefficients "x" and "y" The value of $Y_c$ gives the percent "luminance" The "dominant wavelength" and "purity" can be read directly from a chart known as "chromaticity diagram" (Figure 7.9) by locating the point (x, y) on it. The hue corresponding to the "dominant wavelength" can be obtained from Table 7.3. The 'hue' gives the colour of the sample. Luminance is specified on a scale measuring from zero to hundred; proximity to zero indicates a very dull colour while100 indicates a sparkling bright appearance. "Purity" is also specified on a zero to hundred scale, where zero indicates a colour so pale as to be barely perceptible, with higher values indicating progressively deeper shades.

Table 7 1.

Calculation of tristimulus values for raw tea decoction.

(*Factor = recommended multiplication factor; see text for details)

| X | | Y | | Z | | |
|---|---|---|---|---|---|---|
| λ | %T | λ | %T | λ | %T | |
| 424.4 | 0.13 | 465.9 | 1.78 | 414.1 | 0.05 | |
| 435.5 | 0.27 | 489.5 | 4.94 | 422.2 | 0.11 | |
| 443.9 | 0.50 | 500.4 | 7.02 | 426.3 | 0.14 | |
| 452.1 | 0.84 | 508.7 | 9.44 | 429.4 | 0.18 | |
| 461.2 | 1.38 | 515.2 | 11.22 | 432.0 | 0.22 | Xc + Yc + Zc = 67.1570 |
| 474.0 | 2.57 | 520.6 | 13.19 | 434.3 | 0.25 | |
| 531.2 | 16.94 | 525.4 | 14.63 | 436.5 | 0.27 | x = Xc/(Xc+Yc+Zc) = 0.53 |
| 544.3 | 22.60 | 529.8 | 16.53 | 438.6 | 0.37 | |
| 552.4 | 26.11 | 533.9 | 18.21 | 440.6 | 0.42 | y = Yc/(Xc+Yc+Zc) = 0.44 |
| 558.7 | 29.37 | 537.7 | 19.87 | 442.5 | 0.49 | |
| 564.1 | 31.82 | 541.4 | 21.16 | 444.4 | 0.50 | Dominant λ = 585 nm |
| 568.9 | 34.32 | 544.9 | 23.02 | 446.3 | 0.57 | |
| 573.2 | 36.29 | 548.4 | 24.34 | 448.2 | 0.66 | Hue = yellowish orange |
| 577.4 | 38.13 | 551.8 | 26.11 | 450.1 | 0.73 | |
| 581.3 | 40.06 | 555.1 | 27.48 | 452.1 | 0.84 | Purity = 90% (dark) |
| 585.0 | 41.89 | 558.5 | 29.37 | 454.0 | 0.95 | |
| 588.7 | 43.76 | 561.9 | 30.95 | 455.9 | 1.06 | Luminance = 29.7% (dull) |
| 592.4 | 45.13 | 565.3 | 32.31 | 457.9 | 1.16 | |
| 596.0 | 46.80 | 568.9 | 34.32 | 459.9 | 1.31 | |
| 599.6 | 48.48 | 572.5 | 36.29 | 462.0 | 1.46 | |
| 603.3 | 49.67 | 576.4 | 37.71 | 464.1 | 1.60 | |
| 607.0 | 51.24 | 580.4 | 39.60 | 466.3 | 1.78 | |
| 610.9 | 52.90 | 584.8 | 41.89 | 468.7 | 2.04 | |
| 615.0 | 54.43 | 589.6 | 44.27 | 471.4 | 2.23 | |
| 619.4 | 55.98 | 594.8 | 46.35 | 474.3 | 2.57 | |
| 624.2 | 57.91 | 600.8 | 48.91 | 477.7 | 3.06 | |
| 629.8 | 60.19 | 607.7 | 51.67 | 481.8 | 3.64 | |
| 636.6 | 62.58 | 616.1 | 54.81 | 487.2 | 4.42 | |
| 645.9 | 65.17 | 627.3 | 59.14 | 495.2 | 5.92 | |
| 663.0 | 68.55 | 647.4 | 65.38 | 511.2 | 9.95 | |
| Sum = | 1086.01 | Sum = | 891.92 | Sum = | 48.95 | |
| *Factor = | 0.03269 | *Factor = | 0.03333 | *Factor = | 0.03938 | |
| Xc = | 35.5017 | Yc = | 29.7277 | Zc = | 1.92765 | |

Table 7.2.

Calculation of tristimulus values for chitosan-treated tea decoction.

(*Factor = recommended multiplication factor; see text for details)

| X | | Y | | Z | | |
|---|---|---|---|---|---|---|
| λ | %T | λ | %T | λ | %T | |
| 424.4 | 10.49 | 465.9 | 22.70 | 414.1 | 7.12 | |
| 435.5 | 13.78 | 489.5 | 33.36 | 422.2 | 9.91 | |
| 443.9 | 15.92 | 500.4 | 39.05 | 426.3 | 11.02 | |
| 452.1 | 18.17 | 508.7 | 44.77 | 429.4 | 11.93 | |
| 461.2 | 20.92 | 515.2 | 48.93 | 432.0 | 12.75 | Xc + Yc + Zc = 163.0559 |
| 474.0 | 25.72 | 520.6 | 52.96 | 434.3 | 13.28 | |
| 531.2 | 60.05 | 525.4 | 55.92 | 436.5 | 13.78 | x = Xc/(Xc+Yc+Zc) = 0.43 |
| 544.3 | 68.57 | 529.8 | 59.31 | 438.6 | 14.61 | |
| 552.4 | 72.75 | 533.9 | 62.21 | 440.6 | 15.17 | y = Yc/(Xc+Yc+Zc) = 0.43 |
| 558.7 | 75.98 | 537.7 | 64.84 | 442.5 | 15.68 | |
| 564.1 | 77.97 | 541.4 | 66.65 | 444.4 | 15.91 | Dominant λ = 578 nm |
| 568.9 | 79.83 | 544.9 | 69.12 | 446.3 | 16.45 | |
| 573.2 | 81.20 | 548.4 | 70.77 | 448.2 | 17.04 | Hue = yellow |
| 577.4 | 82.33 | 551.8 | 72.75 | 450.1 | 17.57 | |
| 581.3 | 83.40 | 555.1 | 74.22 | 452.1 | 18.17 | Purity = 62% (medium) |
| 585.0 | 84.43 | 558.5 | 75.98 | 454.0 | 18.80 | |
| 588.7 | 85.23 | 561.9 | 77.22 | 455.9 | 19.34 | Luminance = 70% (bright) |
| 592.4 | 85.94 | 565.3 | 78.37 | 457.9 | 19.97 | |
| 596.0 | 86.62 | 568.9 | 79.83 | 459.9 | 20.68 | |
| 599.6 | 87.39 | 572.5 | 81.20 | 462.0 | 21.28 | |
| 603.3 | 87.83 | 576.4 | 82.05 | 464.1 | 21.98 | |
| 607.0 | 88.47 | 580.4 | 83.17 | 466.3 | 22.70 | |
| 610.9 | 89.11 | 584.8 | 84.43 | 468.7 | 23.76 | |
| 615.0 | 89.55 | 589.6 | 85.53 | 471.4 | 24.53 | |
| 619.4 | 90.06 | 594.8 | 86.48 | 474.3 | 25.72 | |
| 624.2 | 90.72 | 600.8 | 87.51 | 477.7 | 27.40 | |
| 629.8 | 91.42 | 607.7 | 88.57 | 481.8 | 29.44 | |
| 636.6 | 92.10 | 616.1 | 89.77 | 487.2 | 31.83 | |
| 645.9 | 92.83 | 627.3 | 91.01 | 495.2 | 36.11 | |
| 663.0 | 93.77 | 647.4 | 92.84 | 511.2 | 46.02 | |
| Sum = | 2122.55 | Sum = | 2101.52 | Sum = | 599.95 | |
| *Factor = | 0.03269 | *Factor = | 0.03333 | *Factor = | 0.03938 | |
| Xc = | 69.3862 | Yc = | 70.0437 | Zc = | 23.626 | |

## 7.4 Results and discussion

### 7.4.1 Effect of chitosan on total solids in tea decoction

Addition of chitosan solution to raw tea decoction produces a bulky brown precipitate immediately, which settles rapidly on standing. This is accompanied by a lightening in the colour of the supernatant. An average of three estimations showed that the total solids decreased from 3.04 g per litre to 2.46 g per litre. This amounts to a difference of 0.58 g per litre or a reduction of total solids by 19%. Approximately 20 mL of the chitosan solution, containing 400 mg of chitosan, was required to complete precipitation of colouring matter from 1 L of decoction.

### 7.4.2 Effect of chitosan on caffeine content of tea decoction

Estimations show a slight decrease in the caffeine content after treatment with chitosan solution (Figure 7.3). Average of three estimations showed a decrease from 24.8 mg caffeine per 100 mL of decoction to 23.2 mg per 100 mL. This amounts to a decrease of 2.5% in the caffeine content.

### 7.4.3 Effect of chitosan on the colour of tea decoction

After decanting from the brown precipitate produced, the supernatant becomes lighter in colour and improves in clarity (compare Tables 7.1 and 7.2).The tristimulus description changes from a "dull dark yellowish orange colour" to a "bright medium yellow colour" as a result of chitosan treatment.

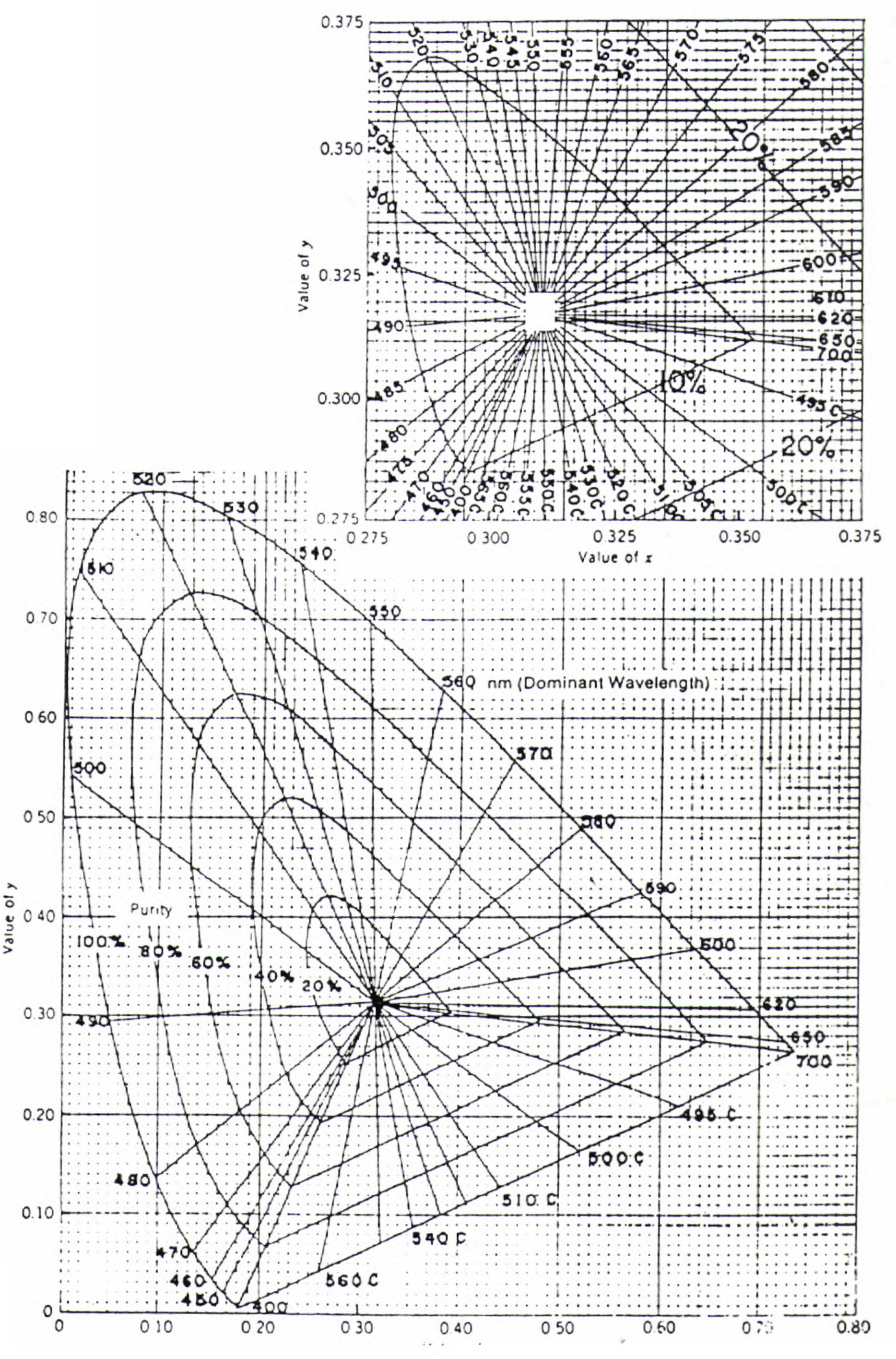


Figure 7.9.

Chromaticity diagram

(Reproduced from Standard Methods, APHA, 1995)

Table 7.3.

Relationship between dominant wavelength and hue.

(Reproduced from Standard Methods, APHA, 1995)

| **Dominant Wavelength** (nm) | **Hue** |
|---|---|
| 400 - 465 | Violet |
| 465 - 482 | Blue |
| 482 - 497 | Blue green |
| 497 - 530 | Green |
| 530 - 575 | Greenish yellow |
| 575 - 580 | Yellow |
| 580 - 587 | Yellowish orange |
| 587 - 598 | Orange |
| 598 - 620 | Orange red |
| 620 - 700 | Red |
| 400 – 530c | Blue purple |
| 530c - 700 | Red purple |

### 7.4.4 Subjective assessment of tea before and after treatment

Tea prepared using raw and chitosan-treated decoctions were given to four people to compare the taste and appearance. All were of the opinion that tea treated with chitosan had a better taste and was visually more appealing.

### 7.4.5 Effect of chitosan solutions prepared using organic acids

Chitosan solutions of a similar concentration prepared in 0.1 M acetic acid or 0.1 M citric acid were also seen to be quite effective and gave similar results, except that it tastes like lemon tea when citric acid is used. 0.1 M solutions of HCl, acetic and citric acids alone also produced a scanty brown precipitate very slowly when added to raw tea decoction, but it was so fine as to not settle on standing.

## 7.5 Conclusion

Our studies have shown that much of the dark-coloured matter from the tea decoction is precipitated and removed by chitosan. The supernatant, which is of a lighter colour, may be more appealing to tea users or can be introduced as a new beverage. The colour difference is also confirmed by tristimulus analysis of samples before and after treatment. Analysis showed that the caffeine content was only marginally reduced as a result of the treatment. This also has implications in the commercial extraction and purification of caffeine.

## Chapter 8

# Studies on flocculation and colour removal using PASS

### 8.1 Introduction

Poly aluminum silicate sulphate (PASS) is a patented product [Haase *et al.*, 1991] recently introduced in Canada and Europe for water treatment [Arnold-Smith *et al.*, 1992]. It is produced by a combination of aluminum sulphate, sodium aluminate and sodium silicate, all materials traditionally used in water purification. Since it is a partially hydrolysed polymeric product of aluminium in the liquid form, it is expected to produce dense flocs very fast. A few trials using the material described in literature have demonstrated its applicability in European countries in water below 16°C, the effectiveness reportedly decreasing at higher temperatures. But there are no reports on its efficacy in water from countries with much higher ambient temperatures like India. Several other composite flocculants have also been developed with the hope of obtaining a better performance compared to any single, conventionally used flocculant [Nishizava and Fukui (1996), Hu and Zhou (1999)].

PASS is described in literature as having the average composition

$$Al_A(OH)_B(SO_4)_C(SiO_X)_D(H_2O)_E$$

where A = 1.0, B = 0.75-2.0, C = 0.3-1.12, D = 0.005-0.1, X = 2.0-4.0 and E is larger than 4. It is an aqueous solution containing about 9% Al estimated as $Al_2O_3$ and a density of 1.25 to 1.30 g $cm^{-3}$

Cochin Minerals and Rutiles Limited (CMRL) is a rutile processing industry in Edayar near Cochin. A waste slurry containing titanium dioxide fines, ferric chloride and HCl is generated in the process and poses a disposal problem to the industry. The fines settle on keeping and the supernatant is an acidic, yellowish solution containing about 4% iron (w/v as $Fe_2O_3$).This liquid shall be called "Fe-solution" in all subsequent discussions in this chapter. Ferric chloride is also a flocculant conventionally used in water treatment plants.

PASS and the Fe-solution described above were examined for their flocculation efficiency using kaolinite suspensions, and for removal of colour due to polyphenols in water that has been used for coir retting (see section 1.6). Efficacy of the materials in removing kaolinite turbidity and colour due to polyphenolics in water was compared with that of alum under similar conditions.

## 8.2 Materials and methods

### 8.2.1 Polyaluminium silicate sulphate (PASS)

PASS was prepared in our laboratory using commercial alum used in water treatment, sodium metasilicate, scrap aluminium turnings and NaOH flakes. The method of preparation was as follows:

(1) About 100 g of powdered alum was stirred with 200 mL of water in a beaker and kept for two days. The supernatant was filtered to obtain a saturated solution of alum, coloured light brown due to the presence of traces of iron.

(2) A saturated solution of sodium metasilicate in 30 mL of water was prepared and filtered.

(3) About 13 g of NaOH flakes was dissolved in 130 mL water and cooled to ambient temperature in a suitable round-bottomed flask. Approximately 7 g of aluminium turnings was added into it. Vigorous reaction ensues with liberation of hydrogen, and the flask is kept cooled in running tap water till the reaction subsides. The solution of sodium aluminate is filtered immediately through a fluted filter paper. As this solution will gel on keeping, it has to be prepared immediately before mixing the components.

About 102 mL of solution (1) was taken in a 1 L beaker and stirred mechanically while cooling in an ice bath. Solution (2) was added to it, dropwise, till about 21 mL was consumed. Stirring the mixture was continued and 65 mL of solution (3) was added to it dropwise over a period of 40 min. The liquid mixture containing PASS was allowed to reach ambient temperature and stored in a glass bottle. Density = 1.3 g $cm^{-3}$, pH = 3.5, $Al_2O_3$ = 8%. Since some sediment slowly forms on standing and increases on keeping, the shelf life of the product is limited.

### 8.2.2 Fe-solution

Slurry containing $TiO_2$ fines, iron and HCl was obtained from Cochin Minerals and Metals Limited, Edayar. It was kept undisturbed for two days, the supernatant decanted out and stored in a glass bottle. Density = 1.0 g $cm^{-3}$, pH = 1.7, $Fe_2O_3$ = 4% (w/v).

### 8.2.3 Kaolinite suspensions

Prepared as under section 3.2.1.1 in chapter 3.

### 8.2.4 Model coloured water

Coconut husks from ripe coconuts were soaked in water in a fibreglass tank for two months. The water coloured reddish brown due to the polypheolic materials leached out from the husks was used in the experiments.

### 8.2.5 Jar tests

Tap water as obtained was used as suspension medium and no attempts to adjust the pH of the suspensions were made during these tests. The suspensions were mixed rapidly for 5 min after adding the flocculant solutions using a syringe, followed by slow stirring at 60 rpm for 15 min. Stirring was then stopped and allowed to settle for 60 min. The supernatant was withdrawn from 5 cm below the surface using a pipette for taking measurements. pH was measured before and after the jar tests to note any change. In the case of kaolinite, suspensions with an initial turbidity of about 65 NTU on an average was used; the initial and final turbidities were measured as described under section 3.2.1.4 in chapter 3. In the case of coloured water, the colour was measured using a spectrophotometer by noting the absorbance at 430 nm where it showed maximum absorption; a higher value for absorbance indicating a deeper colour. The coloured water was diluted with tap

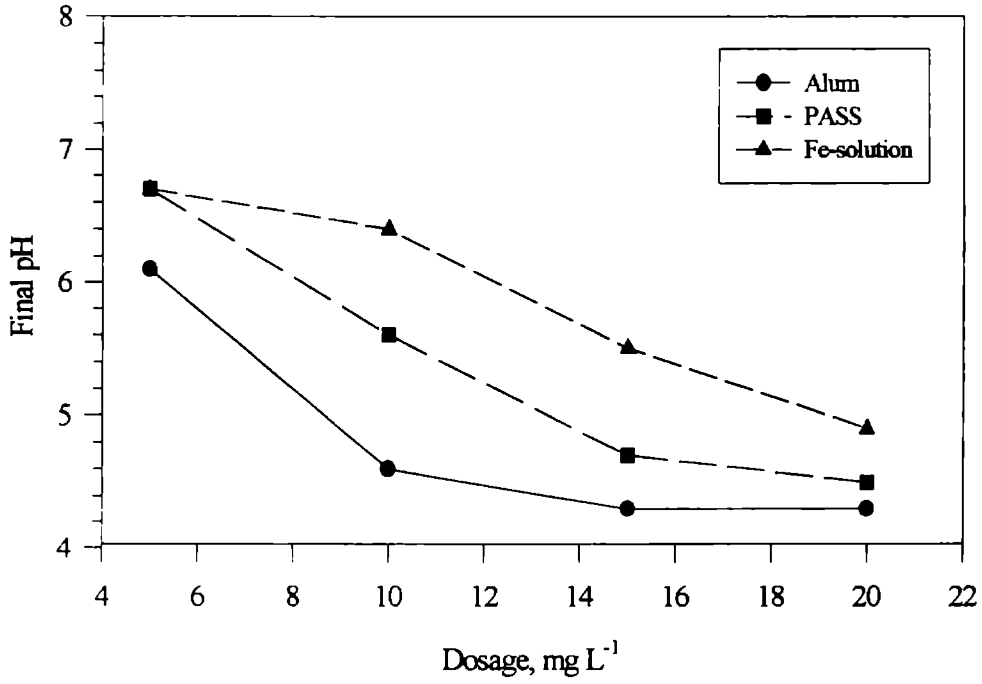


Figure 8.1.

Change in pH of kaolinite suspension of initial pH 7.2

produced by adding various amounts of test substances.

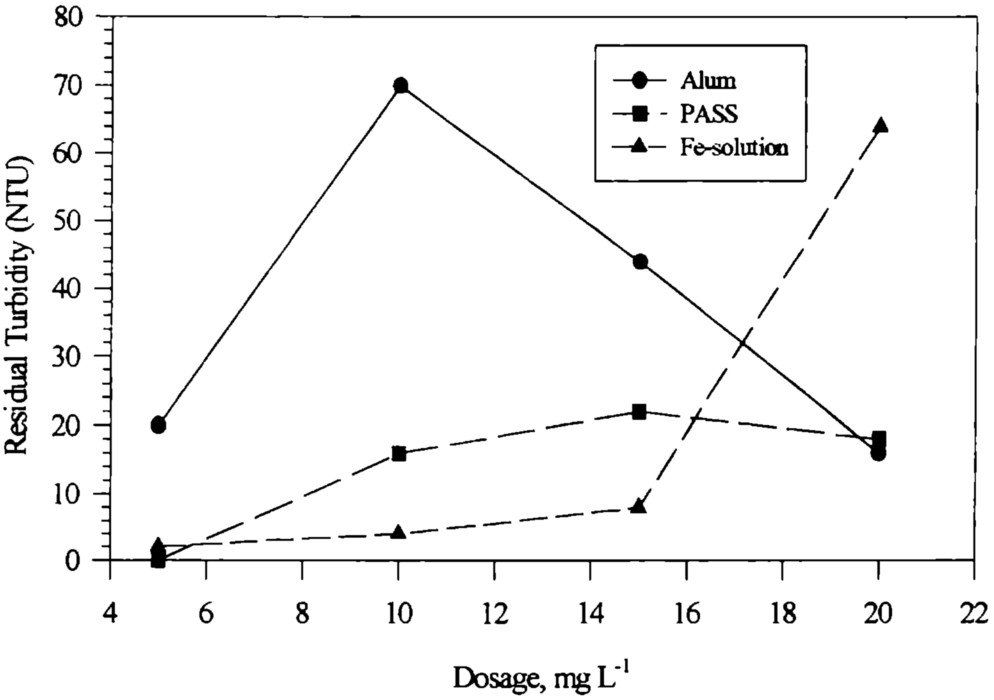


Figure 8.2.

Effect of various dosages of test substances on the residual turbidity

(Kaolinite suspensions of 65 NTU initial turbidity, pH = 7.2) produces the lowest residual turbidity at dosages below 15 mg $L^{-1}$, but loses its effectiveness totally at higher dosages. The effectiveness was also noted to decrease below 5 mg $L^{-1}$ Since the Fe-solution produces the lowest residual turbidity at a very low dosage of 5 mg $L^{-1}$ with the least change in pH of the water (see Figure 8.1), and being an industrial waste material, considerable savings can be obtained by its application in clarifying water or waste water.

### 8.3.3 Removal of colour due to polyphenolics

The effect of various dosages of the test substances on the colour of water due to dissolved polyphenolics is summarised in Figure 8.3. At dosages up to 15 mg $L^{-1}$, all the substances tested showed a tendency to deepen the colour. But there was a big difference among their behaviour above this dosage. At a dosage of 30 mg $L^{-1}$, both alum and PASS produced flocs very quickly. But on settling, the water treated with alum had lost all its colour while there was almost no change in the colour of water treated with PASS. On the other hand, there were no flocs in water treated with Fe-solution. The colour intensified and changed to a dark blue probably due to oxidation and complex formation between iron and the polyphenolic compounds.

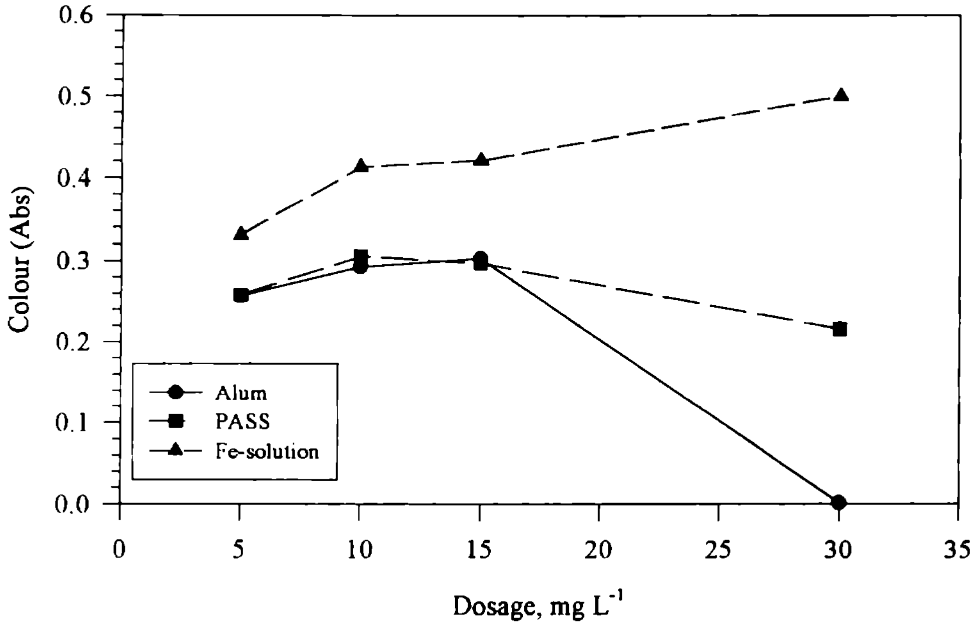


Figure 8.3.

Change in colour of water used for retting coir when treated with various dosages of test substances

## 8.4 Conclusion

The results show that both PASS and the byproduct ferric chloride were more effective than alum in removing kaolinite turbidity. PASS produced a denser floc with a lesser sludge volume. The byproduct gave the lowest residual turbidity using a lesser concentration of the flocculant. Both PASS and the byproduct produced treated water with pH closer to neutral than when alum was used. This can lead to a reduction in treatment costs (1) by using an industrial byproduct at comparatively lower dosages instead of alum, and (2) by reducing the quantity of lime required for correcting the pH of water after treatment to acceptable levels.

However, alum was found to be most effective in removing the colour of water used for retting coconut husks. The colour was almost completely removed as indicated by spectrophotometric analysis.

## Chapter 9

# Summary and conclusions

Chitin is a biopolymer similar to cellulose and present abundantly in the wastes of the sea food industry, such as the shells of crabs and prawns. Chitosan is easily produced from chitin by alkali hydrolysis. It is a biodegradeable cationic polyelectrolyte. Although the structure of chitosan was well established in the 1950s as a polyelectrolyte that should be capable of flocculating suspended particles in water, reports of actual application appeared only recently. Even then, the papers that appeared mostly dealt with chitosan as a flocculation aid along with other principal flocculants like alum or iron salts for specialised applications in the food industry [Bough (1975), Bough (1975a), Bough *et al.* (1975), No and Meyers (1989), Kawamura (1991), Pinotti *et al.* (1997), Chen *et al.* (1998), Lalov *et al.* (2000)]. Studies using chitosan alone as flocculant without any structural modification and in the absence of other flocculants were practically nonexistent.

In 1996, in a paper reporting the flocculation studies of chitosan alone on suspensions of clay minerals like montmorillonite, bentonite and kaolinite, Huang and Chen mentioned that *chitosan fails to flocculate kaolinite suspensions* and that *the effect of pH on its coagulation efficiency is insignificant.* But *they noticed appreciable flocculation of montmorillonite suspensions in water by chitosan* and concluded that *the properties of suspended particles have a significant effect on coagulation using chitosan.*

However, *contrary to the above report, flocculation of fine kaolinite clay dispersed in tap water using chitosan in our laboratory proved to be very successful.* Therefore a detailed investigation was initiated. The chitosan used in our studies was a commercial product manufactured from prawn shells, and was characterized by comparing its IR spectrum with that reported in the literature, and determining its ash content by gravimetry, degree of deacetylation by conductometric titration and the molecular mass by viscosity measurements.

We observed that flocculation of kaolinite particles in tap water using chitosan was *very effective, but strongly dependent on the pH of the medium*, as is expected from the chemical nature of chitosan. It was most effective at a pH of 7.5, with the effectiveness rapidly falling below pH 6.5 and above pH 8.0. Using suspensions having initial turbidities in the range 10 to 160 NTU, almost 95% of the turbidity could be removed at the optimum pH of 7.5. Starting from an initial turbidity of 10 NTU, turbidity could be brought down to below 1 NTU by flocculation and settling alone without resorting to filtration. Flocs were well formed and grew to maximum size in less than 30 minutes. Settling required another 15 to 30 minutes. However, contrary to the general expectation, *we could not establish any relationship between the concentration of suspended particles and the optimum dosage of chitosan flocculant.* All initial turbidities examined could be effectively removed using a chitosan dosage of 0.75 to 1.0 ppm. But a *higher initial turbidity helped more rapid formation of flocs that were larger and settled faster.* These observations were the subject matter of a paper published in Water Research

[Divakaran R and Pillai V N S (2001) Flocculation of kaolinite suspensions in water by chitosan. *Water Research*, 35:16, 3904-3908].

After this initial success, we decided to check the flocculation efficiency of chitosan using suspended particles having different properties and of interest in potable water treatment. This was to bring out any dependence of chitosan flocculation on the nature of the suspended particles.

The ability of chitosan to remove natural turbidity due to silt in Periyar river water was checked. All observations obtained using kaolinite suspensions were confirmed for river silt also. Flocs formed faster and were heavier. The optimum pH was 7.0. It was also noted that *larger than optimum doses of chitosan tend to stabilize the silt suspensions*. The results of our studies in this connection were published in Water Research [Divakaran R and Pillai V N S (2002) Flocculation of river silt using chitosan. *Water Research*, 36:9, 2414-2418].

The ability of chitosan to flocculate algae in water was studied. The presence of algae impart offensive colour, odour and taste to potable water. In addition, they reduce dissolved oxygen and release algal toxins into the water. Recently, algae like *Spirulina* have been cultivated commercially for their nutrition content and used in cosmetics and health foods. These are harvested from the water by centrifugation during which the cells are very often ruptured. In this study, the effectiveness of chitosan as a flocculant, when used alone, on the removal of algal turbidity due to three different freshwater species of algae, namely *Spirulina*, *Oscillatoria* and *Chlorella* and one brackish water species, namely *Synechocystis*, were examined. The removal of suspended algae were ascertained by measuring both turbidity and chlorophyll content of the water. The results indicated that *chitosan can be very efficient in the removal of freshwater algae by flocculation and settling*. The *optimum pH for good flocculation was again found to be 7.0*, but the optimum *dosage of chitosan required depended on the algal concentration*. In the case of the brackish water species, the high density of the salt in the medium prevented settling of the flocculated alga. The medium had to be diluted with fresh water to effect settling. The algal cells after flocculation were intact, alive and could be re-cultured. This is of great advantage in (1) preventing the poisonous contents of the cells from passing into the water in potable water treatment, (2) proper harvesting of algae like *Spirulina* for use in health foods and cosmetics since the nutritious cell contents are preserved; chitosan is edible by itself and therefore is not hazardous for such use and (3) for harvesting of genetically altered algae to mass-produce antibiotics etc. since the precious cell contents are not lost. The results of our studies in this connection have been accepted for publication in the *Journal of Applied Phycology* (reference No. JAP817) and is now in the press.

Next, the ability of chitosan in removing fine suspended titanium dioxide from aqueous medium was examined. Titanium dioxide (rutile) has innumerable applications in ceramics, electronics, pharmaceutical, paint, paper and mineral processing industries, to name only a few. The fine particles in the suspended form pose removal problems in the effluent from all these industries. Results of our studies in this connection indicate that *removal of fine titanium dioxide from* *aqueous suspensions using chitosan is not very effective* since the residual turbidities attained are still very high. Again as in the earlier cases, optimum flocculation was obtained at a pH of 7.0 and chitosan dosage in the range 0.75 to 1.0 ppm. The results of these studies have been consolidated into a paper which is currently being reviewed for publication in *Water Research.*

However, all studies that gave good results using kaolinite and titanium dioxide as suspended particles were carried out using the local tap water as the medium. Quite surprisingly, these results could not be obtained when distilled water was used and appeared to support the observations made by earlier workers. The tap water used was very soft and contained no organic matter that was detectable by UV measurement. Still, something in the tap water was greatly assisting flocculation using chitosan. Most inorganic species that may be present in the water were examined for such assistance but were found to be ineffective. The only species which appeared to have any effect was soil extract. This led suspicion to rest on humic matter. In this connection, it is of interest to note that Gidas *et al.* (1999) reported that flocculation ability of chitosan depends on the physico-chemical characteristics of raw water, but what those characteristics were was not very clear.

In further studies using soil extract added to distilled water as medium, the flocculation characteristics of chitosan obtained using tap water could more or less be duplicated, indicating that NOM may indeed be the hidden agent. But exact duplication of results were not possible primarily because the organic matter, if any, present in the tap water was not detectable using methods available to us. Therefore an exact concentration of the NOM that was required to duplicate the results could not be arrived at. Further, the exact nature and concentration of NOM in the soil extract was also not known.

However, based on the knowledge obtained, we propose a mechanism for the assistance of flocculation by humic materials. Clay minerals such as montmorillonite and bentonite that have rough surfaces and are always associated with NOM are reportedly [Huang and Chen, 1996] flocculated and removed from suspensions in distilled water by chitosan. On the other hand, flocculation of mineral particles having smooth and clean surfaces like (commercial) kaolinite and titanium dioxide are not flocculated from distilled water. Our studies have shown that such particles can also be flocculated well in presence of minute amounts of humic materials. Humic materials reportedly contain [Gjessing (1976) and Orlov (1985)] very large molecular mass compounds incorporating large numbers of phenolic –OH, acidic –COOH and basic $-NH_2$ functional groups. Part of the acidic and basic functions combine among themselves and exist as $-O^-$ and $-COO^-$ anions and $-NH_3^+$ cations at neutral pH.

Suspended colloidal particles generally carry negative surface charges. Flocculation generally requires at least a partial neutralization of these surface charges. Chitosan somehow fails to directly adhere to the surface of smooth particles and flocculate them by the bridging mechanism. But *when humic substances are present, the* $-NH_3^+$ *cationic groups are attracted towards the* *negative surface charges of the suspended particles and neutralize them; the electrostatic attraction between the charges also keep the humic substances adhereing to their surface with all their anionic groups pointing outwards. Thus the exposed surfaces of the particles are still negatively charged; therefore the effect of the humic materials generally is to stabilize the suspension. This has indeed been observed to be the case during our experiments.*

At the same time, *the adhering humic materials tend to increase the individual particle size and make the surface rougher.* In work unconnected with ours, Malekani *et al.* (1997) have also made similar observations. *The* $-NH_3^+$ *cationic groups in polymeric chitosan chains are able to neutralize the surface charges on the roughened particles and collect a large number of such particles in the chain, leading to growth of flocs by the bridging mechanism.* Higher concentration of suspended particles will help in the rapid formation of such bridges between particles and result in the formation of heavier flocs since more particles per bridge are obtained. This has been observed in our experiments. *Since the charges present on humic materials as well as on chitosan and the solubility of chitosan are controlled by pH, flocculation properties of chitosan should be very much dependant on the pH of the medium. That this is actually so has indeed been confirmed in our studies.* The results of our studies in this connection have been communicated to *Water Research* and are currently being reviewed. We have observed such assistance by humic materials in the case of alum flocculation also. However, much more work using more sophisticated equipment will be required to confirm the conclusions reached from our exploratory work and throw more light on the mechanisms involved.

As a minor offshoot of this work, the ability of chitosan to reduce the colour of tea extract was examined. Chitosan being an edible substance, tea of a lighter colour and less bitter taste may become a successful consumer product. In our studies, the ability of chitosan to remove much of the dark coloured substances from tea decoction and produce a lighter coloured extract without appreciable reduction in the quantity of caffeine, which is the active principle, was demonstrated. The results may also prove valuable in the purification of caffeine extracted from tea leaves.

Poly Aluminium Silicate Sulphate (PASS) is a patented material proven to be very effective in flocculation of suspended solids in North American and European countries where the temperature of water bodies is below 16°C. Part of our studies has also demonstrated the efficacy of this partially-hydrolysed polymeric aluminium flocculant at much higher ambient temperature of around 28°C. A byproduct of the local rutile processing industry, containing ferric chloride, was also examined and found to be superior to both alum and PASS in flocculation characteristics under identical experimental conditions. However, alum proved to be much superior to the other two in removing the brown colour due to the presence of polyphenolic compounds present in water used for coir retting.

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