Synthesis and Characterization of Carboxymethyl Chitosan and its Effect on Turbidity Removal of River Water

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Abstract: The Chitosan derivative named Carboxymethyl Chitosan was synthesized by direct Alkylation method. The Chitosan was obtained by Chemical method from White Prawn/Indian Prawn (FenneropenaeusIndicus) and was used for preparation of Carboxymethyl Chitosan. The Chitosan composition was found 51.17%Carbon, 41.61% Oxygen and 5.22% Nitrogen with 81.3% degree of deacetylation. The prepared Carboxymethyl Chitosan was characterized by SEM, FT-IR and TGA. The Carboxymethyl Chitosan examined as flocculant for turbidity removal of Indusriver water samples. The experimental condition was optimized and found that the maximum separation efficiency was achievedat pH-7 and 0.2 mg/L dose. The separation efficiency range placed between 81.2% and 87.1%. The total suspended solids and turbidity relation was also evaluated.

Keywords –Carboxymethyl, Characterization, Chitosan, Prawn, River Indus

I. INTRODUCTION

The Carboxymethyl Chitosan is an amphiprotic ether derivative. It is derived from Chitosan. The Chitosan was isolated from Chitin. The Chitosan have much industrial applications. It is used in pharmaceutical industry, biomedical and water treatment etc. The Chitosan is composed of amino group and hydroxide group at primary and secondary positions which took part in reaction. The amino group is weak base, it protonated at pH less than 6.5 of suspension. In alkaline medium it was insoluble and forms precipitous. The internal structure of Chitosan, its Hydrogen bonding, cationic nature and degree of deacetylation influence its solubility of in aqueous solutions. Due to limited solubility, its application did goes in narrow spectrum especially in pharmaceutical industry where solubility is prime factor.

The chitosan properties were altered by chemical modification by means of grafting onto chitosan chain and make macro molecular chain. Also done by substitution of hydrophilic group in place of amino or hydroxyl group of Chitosan. The modification improves and enhances the physiochemical proprieties of Chitosan. The degree of acetylating, reactivity, solubility and molecular weight were altered. The Chitosan derivatives are the modified and altered form of Chitosan with improved physiochemical proprieties. The hydrophilic group which took part in modification and alteration were hydroxyl propyl, hydroxyalkylamine, hydroxyethyl, and Sulfate phosphate and carboxyalkyl group. The carboxyalkyl group included carboxybutyl, carboxyethyl and carboxymethyl [6]. Among all Chitosan derivatives the carboxymethyl group placed dominant position in consumption and usage. It happened because of ease of synthesis, solubility, reactivity, biodegradability, biocompatibility and non-toxicity [6]. The Carboxymethyl Chitosan were found O-Carboxymethyl Chitosan, N-Carboxymethyl Chitosan and N,O-Carboxymethyl Chitosan.

There are two methods followed up for the substitution.These are Reductive alkylation method and direct alkylation method. In Reductive alkylation method the Chitosan reacted with aldehyde – Glyoxylic acid. The N_aBH_4 and N_aCNBH_3 used for hydrogenation. In this method the O-Substitution were not happened because of its absence while the amino group were took part in reaction.Hence Carboxymethylationis done on N-atom. The presence of glucoamine unit in Chitosan chain responsible for monoCarboxymethylation and diCarboxymethylation. The other factors were the regent concentration and reaction conditions. By the varying the conditions number of Carboxymethyl Chitosan species were formed differentiated by molecular weight distribution, molecular sizes and degree of deacetylation.

The second method was the direct alkylation. In this method the Chitosan reacted with monochloroacetic acid. The isopropanol in water were used as solvent. In this method the O-Carboxymethyl and N-O Carboxymethyl were formed by varying the pH. At mild pH 8 to 8.5, the amino group active took part in reaction and formed N-Carboxymethyl. When Sodium Hydroxide concentration increase >20% and pH

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increases the hydroxyl group became active hence substitution of Hydroxyl group done on C6 and C3 while some amino group substitution done on C2. The degree of deacetylation puts no impact on degree of substitution however the sodium Hydroxide concentration affects the chemistry of Carboxymethyl Chitosan [3].The Carboxymethyl Chitosan exists in dirty white to pure white powder form. The properties strictly influenced by experimental conditions of temperature and reactants' concentrations.The presence of Carboxy group in Carboxymethyl Chitosan chain enables its solubility in neural, acidic and basic medium. It is highly soluble polymer. Another property of Carboxymethyl Chitosan was its moisture adsorption and retention. This was very prominent characteristic which make its importance high in cosmetic industry and drug formation. The large hydrodynamic volume of Carboxymethyl Chitosan feasible in film formation and hydrogels making. The Carboxymethyl Chitosan was antioxidant and antimicrobial agent specially used in wood healing drugs and cell cultures agricultural and food industry for preservation and storage of fruits and vegetables from fungus and bacterias. The Carboxymethyl Chitosan was most suitable organic polymer for metal adsorption and metal Chelation. The flexible structural chemistry and hydrophilicity make feasible for metal complexation. The amino group and hydroxyl group were the active legends for metal Chelation. The hydroxyl group easily protonated in acidic solution creates the electrostatic force which attracts the anionic part of metal complexes while amino group provided the active sites for metal Chelations [6].

II.   METHOD FOR PREPARATION OF CARBOXYMETHYL CHITOSAN

Carboxymethyl Chitosan was prepared from Chitosan by acid–base treatment method [1],[2],[4],[5],[7]. As Chitosan exists in exo-skeleton of crustaceans, hence IndianPrawn or White Shrimp of Arabian Sea was selected for extraction of Chitosan. The White Prawn or Indian Prawn are from class of Crustacean, belongs to Penaeidae family and its Phylum is Arthropoda. The biological name of White Prawn/Indian Prawnis *FenneropenaeusIndicus*. Prior to preparation of Carboxymethyl Chitosan, it was analyzed and found that degree of deacetylation of Chitosan was 81.3%. According to the EDX report the prepared Chitosan composed of 51.17% Carbon while the Oxygencontent was41.61% andNitrogen content was 5.22%.

The 3gm of extracted Chitosan was taken in conical flask than 65mL isopropanol were added. It was stirring well up to 30 min till uniform suspension was obtained. The 40 gm sodium Hydroxide was dissolved in double distilled water and makes 40% solution. This solution was added in conical flask the reaction was started at room temperature. The room temperature was noted $50^{\circ}C$ in those days.The monochloroacetic acid solution were also prepared and added in flask. The 14.4 grams monochloroacetic acid was dissolved in 100mL isopropanol. The Monochloroaceticacid solution was pouring in conical flask. The reaction followed up. The suspension was kept five hours at room temperature for complete the reaction. After five hours the suspension were filtered in glass filtered. The filtrate was recovered and washed with 100 mL methanol. Than it was filtered and methanol was discarded. The filtrate was neutralized by glacial acetic acid and finally ethanol was added to make it in organic phase. Finally it was filtered and dried at room temperature. The dirty white type Carboxymethyl Chitosan were obtained in lumps form. It was bump with glass rod to make small size and passes through the 100mμ screen.

III.   CHARACTERIZATION

The synthesized Carboxymethyl Chitosan was characterized by FT-IR and TGA (Thermal Gravimetric Analysis). The SEM (Scan Electron Microscopy) was also done. The details of all are discussedin the following sections.

3.1 Scan Electron Microscopy of Carboxymethyl Chitosan

The Morphology of Carboxymethyl Chitosan was observed by Scan Electron Microscope (SEM) in Scanning Electron Microscope Laboratory, Centre of Pure and Applied Geology University of Sindh Jamshoro using Electron Microscope Model (JEOL 6490 LV – SEM by JAPAN). The prepared CarboxymethylChitosan from Chitosan extracted from Indian white Prawn (*FenneropenaeusIndicus*) was subjected for scan electron microscopy having 200μm particle size as shown in Figure-1. The scan prints showed that prepared CM-Chitosan having Crystalline, Angular and Nodular Crystals. The prints were obtained by zooming the sample 8000 times under 12kW power.
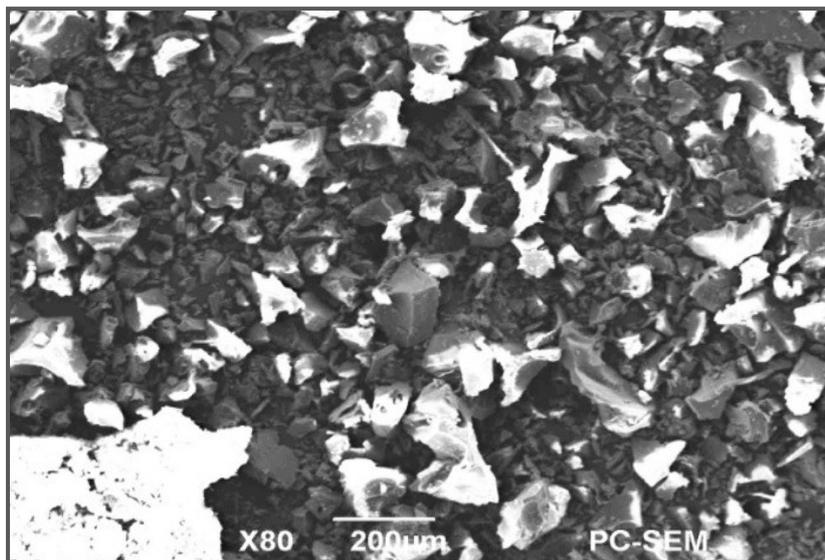
Fig.-1: The Scanning Electron Micrographs of Carboxymethyl Chitosan prepared from
Chitosan extracted from White Prawn (FenneropenaeusIndicus)

3.2 Energy Dispersive X-Ray Spectroscopy Carboxymethyl Chitosan

Energy Dispersive X-Ray Spectroscopy (EDX or EDS) of Carboxymethyl Chitosan was done in PCSIRLab Karachivide model no JSX-3400RII Energy-Dispersive X-ray Fluorescence Spectrometer as shown in Figure-2.According to spectrum results, the content of Carbon was 55.65%, Oxygen 34.36%, and Nitrogen5.30%, Sodium 1.63% and Chlorine 1.91%.The compositions were determined via emission of electron from K-shell.
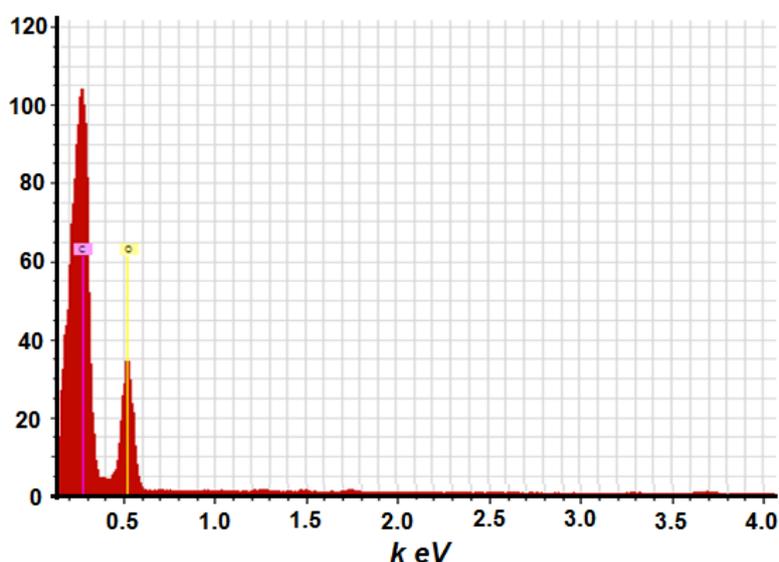
Fig.-2: EDX Spectrum of Prepared Carboxymethyl Chitosan. The profiles of Carbon, Nitrogen, Oxygen, Sodium and Chlorine
are distributed on the surface of each Carboxymethyl Chitosan Skeleton.

3.3 Fourier Transform Spectroscopy of Carboxymethyl Chitosan

Fourier Transform Spectroscopy of Carboxymethyl Chitosan was conducted in M. A.KaziInstitute of Chemistry, University of Sindh, Jamshorovide equipment Nicolet AVATAR 330 FT-IR with an Attenuated Total Reflectance (ATR) accessory, smart performer (Thermo Nicolet, Thermo Electron Corporation USA) with Zn-Se probe. According to FT-IR Spectraas shown in Figure-3, the bend stretched at 3436 cm^{-1} attributed for O-H and N-H group. The 2900cm^{-1} stretching bend express the C-H group. The asymmetrical stretched attributed at 1634cm^{-1} for C=O. At 1420cm^{-1} bend stretched for the-CH$_2$- and -CH$_3$- group. The C-O bend stretched at 1053cm^{-1} and 1327 cm^{-1}.
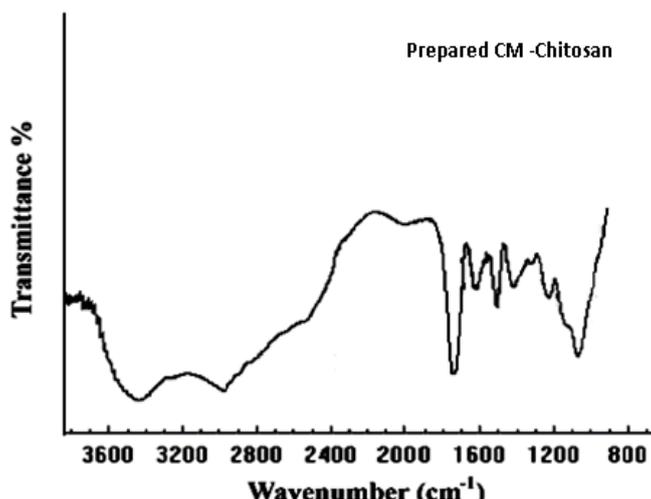
Fig.-3:FT-IR Spectra of Prepared Carboxymethyl Chitosan

3.4 Thermal Gravimetric Analysis (TGA) of Carboxymethyl Chitosan

The Thermal Gravimetric Analyses describes the thermal degradation of samples. The TGA of Carboxymethyl Chitosan was conducted in PCSIR Lab Karachi vide Equipment NetzschTC 209ÎL Libra Thermal Analyzer. According to TGA Curve the thermal degradation of Carboxymethyl Chitosan was took place in three places as shown in Figure-4. The curve showed that in between 35^oC - 240^oC, the degradation started and weight losses occurred. The reason of weight lost were moisture content and decomposition of the cyclized product containing COC. The curve showed the removal of COO from polysaccharide and it was possible that at this stage carbon dioxide released from samples. As CM-Chitosan content -COOH group in its chain structure hence it was decarboxylated near 300^oC. The third degradation was reported and that was possible that NH_2 group detached in the form of ammonia (NH_3) released. The degradation of Carboxymethyl Chitosan were done in nitrogen atmosphere.
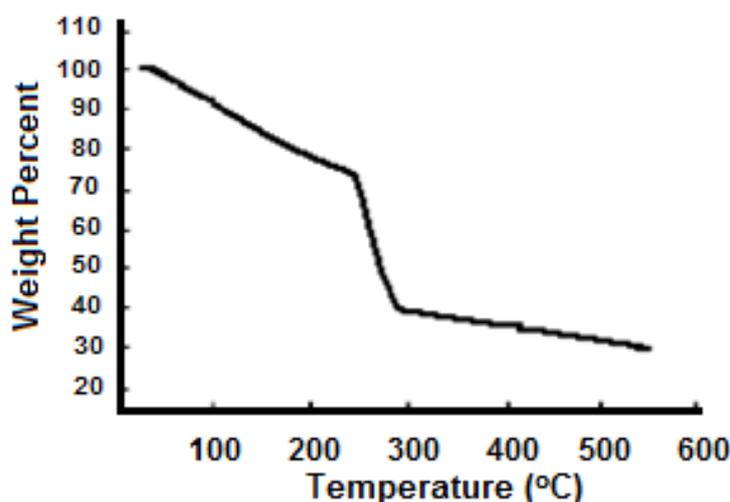
Fig.-4:Thermogravimetric Analysis (TGA) of Carboxymethyl Chitosan

IV.   EXPERIMENT

The water sample was collected from River Indus. The samples were collected according to standard procedure and analyzed without delay. Before collection of the sample all the samples bottles were soda washed rinsed with double distilled water and sterilized. All the samples bottle were make free from all the contaminations. The samples were analyzed by the calibrated equipments. The samples were collected in each month of 2011. Each and every sample was collected repeatedly in order to validate. The samples were collected in air tight sampling bottles and capped properly. The analyses were carried out without delay. The standard procedure was followed in sampling.

4.1 Optimization of Dose of Carboxymethyl Chitosan for Turbidity Removal

In order to observe the optimum dose of Carboxymethyl Chitosan, the six samples were taken and their turbidity level were maintained at 10 FTU, 20 FTU, 40 FTU, 60 FTU, 80 FTU and 100 FTUas shown in Figure-5. During study it was observed that at low dose of Carboxymethyl Chitosan, the low turbidity removal percentage was obtained. The study was started by adding 0.1mg of Carboxymethyl Chitosan in one liter of water samples. The samples turbidity was already set at 10 FTU, 20 FTU, 40 FTU, 60 FTU, 80 FTU and 100 FTU. After adding the Carboxymethyl Chitosan in each tube it was stirring well. The flocculant was water soluble. The samples were kept for settling at desk for 30 minute. The turbidity was noted by turbidity meter. The experiments reflected that low dose of Carboxymethyl Chitosan exhibit the low percentage of turbidity removal. The 0.1mg/L Carboxymethyl Chitosan removed the turbidity averagely 82.67% ± 1.81%, or 82.67% ± 2.19%. As the dose of Carboxymethyl Chitosan increased at 0.2mg/L, the turbidity removal percentage were enhanced and stands at 84.9% ± 2.53%or 84.9% ± 2.98%. By followed up experimental conditions, the doses of Carboxymethyl Chitosan were slowly increased as 0.3mg, 0.4mg, 0.5mg and 0.8mg per liter of water sample. The experimental conditions revealed that doses increment beyond optimum level causes destabilization of bottom layer of settled particles. It was concluded that optimum dose were 0.2mg per one liter of sample. There was small difference observed between the doses from 0.5mg-0.7mg.However the mean turbidity removal percentages were as 83.52% ± 3.01% Or 83.52% ± 3.6% against0.3mg, 81.67% ± 3.24%or 81.67% ± 3.97% for 0.4mg, 81.33% ± 3.32% Or 81.33% ± 4.08% for 0.5mg and 80.78% ± 3.63%or 80.78% ± 4.49% for 0.8mg per liter.
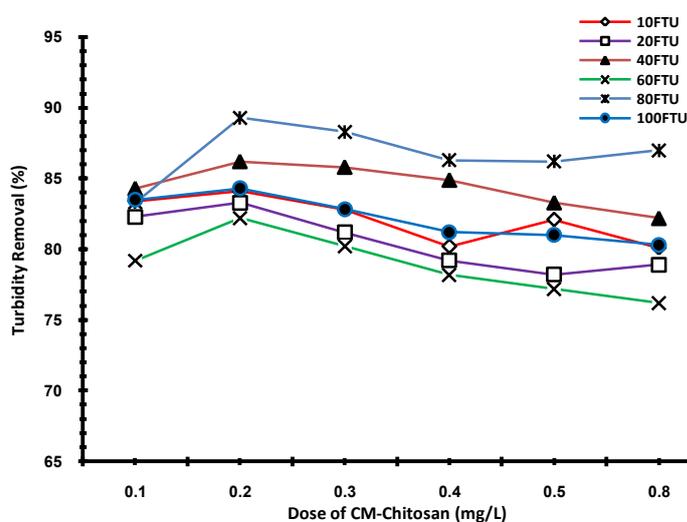
Fig.-5: Plot shows the Carboxymethyl Chitosan dosage (mg/L) for the Turbidity Removal (FTU)

4.2 Optimization of pH of Carboxymethyl Chitosan

The pH of suspension influences the flocculation process. The turbidity removal efficiency varies with the variation in pH of suspensions. To study optimum pH value the research were carried out by taken water samples maintained at the range of pH4 – pH10. The pH was maintained by 1M Sodium Hydroxide solution and 1M Hydrochloric acid. The pH was monitored by using pH Meter (Thermo Orion Model 420 A$^+$, USA) with combined glass electrode. The optimum dose of Carboxymethyl Chitosan was added to the samples.The samples were agitated well. After agitation it was settled for 30 minute. According to experimental results the maximum solids settling were done at neutral suspension. It was concluded that the maximum turbidity removed from the sample at pH 7 and at that value the residual turbidity level became high and clear interface appeared as shown in Figure-6. It was also found that when pH increased above the optimum level than destabilization of suspension was occur and clear interface were not appeared. At the pH 4 the mean turbidity removal percentage were 81.16%, at pH 5 it was stand at 82.43%, at pH6 it was 85%, and at pH7 it was 87.8%. When the pH increases from its optimum level than the turbidity removal percentage were decreases and it was noted that at pH 8 it was 85.46% and at pH 9 it stands 84.36%, and at pH10 it was 83.4%. The given figure expressed the minimum, maximum and mean value of turbidity removal in FTU. The pH4, pH5, and pH6 the Minimum and Maximum turbidity removal percentage were as 79.4%-82.4%, 80.4%-83.56% and 82.4%-86.5% respectively. The below optimum pH it was 83.4%-88.3%, 82.2%- 87.4% and 81.1%-86.4% against the pH8, pH9, and pH10 respectively.
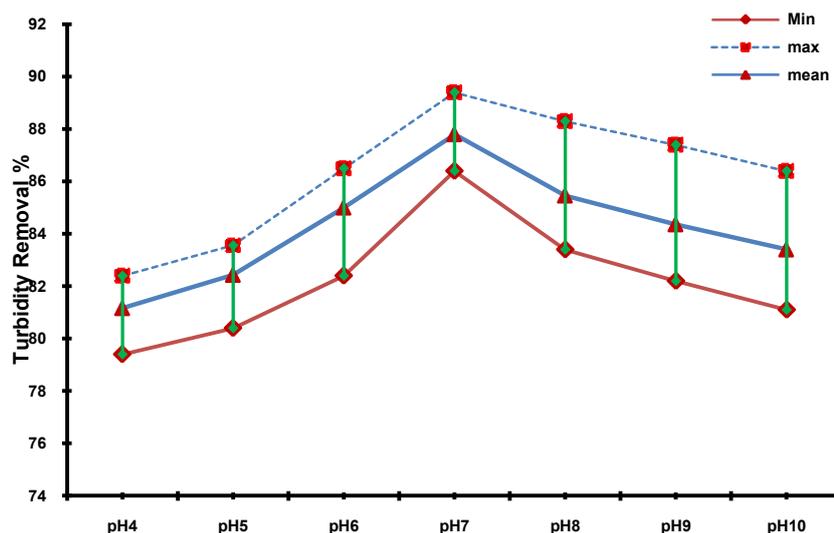
Fig.-6: The optimum pH at which maximum turbidity removed using Carboxymethyl Chitosan.
The vertical bars shows the standard deviation after six repetition

V. RESULTS AND DISCUSSION

Initial turbidity of sample refers the turbidity of sample before adding Carboxymethyl Chitosan. After adding the Carboxymethyl Chitosan at optimum condition and specific settling time the particle settling down in bottom and clear interface was obtained in top. The clear interface on top known as Supernatant, while at bottom side sediment or thick   layer observed that contain high concentration of solid particles. That bottom layer known as Residual turbidity of sample. The residual turbidity is the difference between initial turbidity and supernatant clarity of sample. The residual turbidity and supernatant clarity were interrelated term. At higher supernatant clarity the maximum residual turbidity was observed. The separation efficiency shows the effectiveness of flocculant or in other words tells us how much upper layer is free from solid particles and how many solid particles settle down. Separation efficiency computed here in terms of residual turbidity of sample.

According to data obtained it was clear that initial turbidity were stands between 10.5 FTU to 19.1FTU as depicted in Figure-7.After adding Carboxymethyl Chitosan at optimum dose and optimum pHthe particle was settle down and after 30 minute the separation efficiency was stands between 81.2%-87.1%. Without flocculant the separation efficiency was stands between 42.2%-48.32%. The mean value of separation efficiency with CM-Chitosan was 85.15% and 45.49% without CM-Chitosan.The maximum and minimum turbidity was observed in the month of March and May.
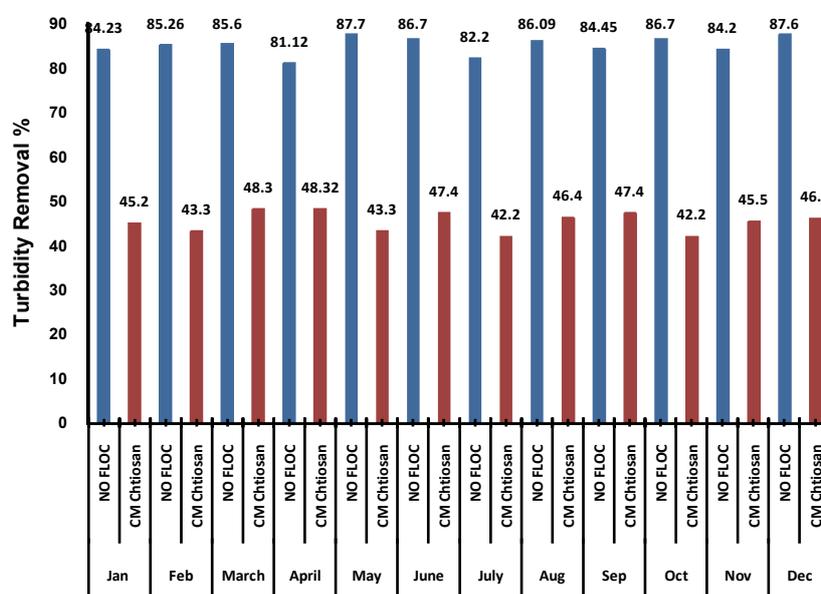
Fig.-7: The mean separation efficiencies of water samples collected from River Indus in 2011 using CM-Chitosan as flocculant

VI. CONCLUSION

6.1 Total Suspended Solids and Turbidity Relationship

It was observed after conducting the test that there is linear relationship between the total suspended solids and turbidity. The results are expressed graphically in Figure-8.
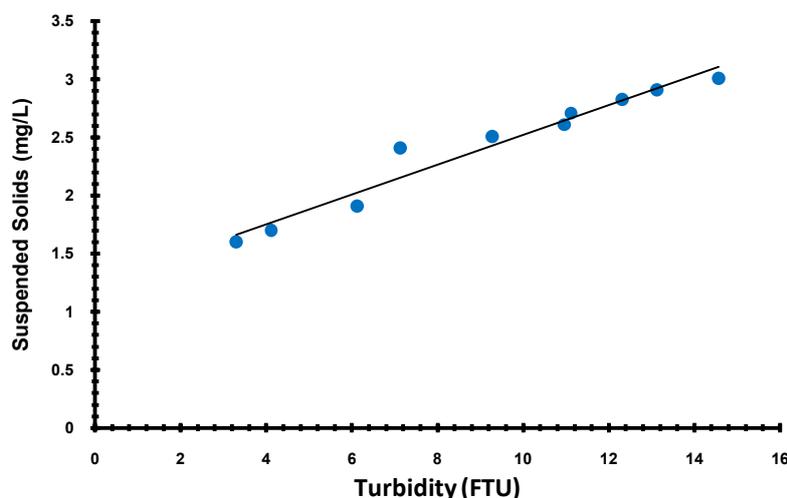
Fig.-8: Graph Plot shows the linear relationship between Total Suspended Solids (mg/L) and Turbidity (FTU)

6.2 Relationship between initial Turbidity and Dosage

Research shows that there is relationship between the flocculation dosage and turbidity removal however it is independent of initial turbidity of sample. The optimum dosage was found 3mg/100mL. At higher dosage the particle destabilize and disperse in suspension resulting suspended particle concentration increase. The maximum sediment bed obtained at dose 3mg/100mL. The turbidity removal efficiency affected by the container/vessel designing feature. The free settling promotes high settling rate. The high initial concentration of solid particles and low container/vessels volume causes hindered settling. The hindered settling takes comparatively long time to settle because the particles collides each other and walls of container.

6.3 Settling Rate and Time Relationship

When optimum condition of pH and flocculant dose was maintained in river water samples than tests were conducted to observe the settling rate verses time. It was observed that highest settling occur in staring 5 minute. The suspended particle quickly settles down in bottom and agglomerates and make floccus. The bridging mechanism would be high in starting 5 minute, here 64% settling occurs. In between 5 minute to 10 minute, the transition time starts here settling rate become slower and reaches to 19%. The particles disperse in medium and slowly move down. After 15 minute rest of the particle in suspension settle down. A porous particle bed formed bottom in 30 minute. After this time porosity of bed decrease and bed consolidate its height decrease few centimeters after 60 minutes.

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