

# Synthesis of chitin from exoskeleton of Shrimp and factors affecting adsorption of dye on Chitin

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**ABSTRACT:** The control of water pollution is becoming increasingly important these days. Release of dyes into the environment constitutes a small portion of water pollution. The adsorption process is one of the effective methods for removal of dyes from the waste effluent. Chitin has been utilized as an adsorbent for a variety of substrates. Chitin can be used as adsorbent in the treatment of effluents from the textile industry, especially for triphenyl methane. In present research, preparation of chitin was done from fishery waste. Study of the effect of pH and agitation time on adsorption of xylenol orange dye on chitin was also carried out. The maximum adsorption of xylenol orange dye was observed at 445 nm under the U.V Spectrophotometer. It was interesting to note that the pH range for maximum adsorption at different agitation times remains between 3 to 4 only. It was observed that maximum adsorption of dye occurs at 10 minutes agitation time and in a pH range of 3 to 4.

**KEYWORDS:** *Chitin, Xylenol orange dye, adsorption, textile effluents.*

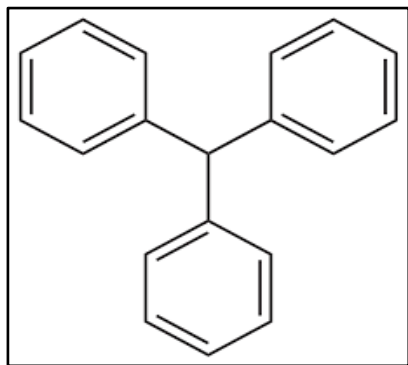
## 1. INTRODUCTION:

Environmental pollution can be defined as the unfavorable changes in our environment largely due to the impact of human society. It may bring about undesirable alteration in the physical and chemical characteristics of our environment [1]. The prevention of water contamination is becoming more and more crucial these days. Release of dyes from various industries into the environment constitutes only a small portion of water pollution. Legislation from the government is requiring the textile industry to treat its waste effluent. A large amount of water and chemicals are used in the wet processing of textiles in the textile industries. It is highly visible and undesirable when colors, even in very low concentrations, are present in wastewater. Currently, removal of dyes from effluents is by the physico-chemical means[2]. Such methods are often very costly and though the dyes are removed, accumulation of concentrated sludge creates a disposal problem. There is a need to find alternative methods of treatment that are effective in removing dyes from large volumes of effluents and are low cost such as biological or combination systems[3].

Synthetic dyes are chemically divided into azo, triphenylmethane or heterocyclic / polymeric structures. They are used extensively in the biochemical, foodstuff, plastic, and textile industries; where it is estimated that 10-14% of the dye is lost in the effluents during the dyeing process. Synthetic dyes share a common feature is that they are not readily biodegradable and when discharged into the environment they are therefore persistent and many of them are also toxic[4]. Dyes are sustained in substrates through physical adsorption, the formation of metal complexes, or the establishment of covalent chemical bonds[4], and they acquire their color from electronic transitions between various molecular orbits where intensity of the color is determined by the probability of transitions. In our present work Xylenol Orange dye is selected, which belongs to triphenylmethane class.

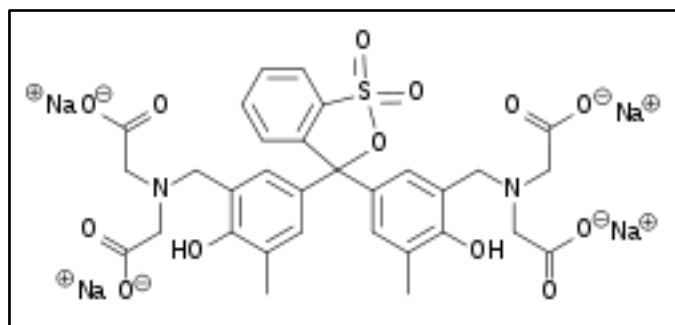
### 1.1 TRIPHENYLMETHANE DYES:

Triphenylmethane dyes are those dyes in which a central carbon atom is bonded to two benzene rings and one p-quinoid group (chromophore). The auxochromes are  $-NH_2$ ,  $-NR_2$  and  $-OH$ . These dyes have brilliant colors but are not very fast to light and wash. Triphenylmethane dyes are used extensively in the textile industries for dyeing of nylon, polyacrylonitrile, modified nylon, wool, silk and cotton. Some of the triphenylmethane dyes are used in medicine as biological stains, in paper and leather industry; for coloring plastics, in gasoline, paper and leather industries are the major consumers of azo and triphenylmethane dyes. Food and cosmetic industries also use different types of dyes [5]



**Figure 1. Triphenylmethane dye**

Xylenol orange [3,3'-Bis[N,N-bis(carboxymethyl) aminomethyl] (XO) is a fully miscible and completely water soluble dye. It is a common synthetic dye that is usually utilized as a complexometric indicator and potentiometric reagent for the detection of metal ions in laboratories. As a result, the XO effluents from manufacturing plants and laboratories will eventually bind heavy metal ions, resulting in a variety of disorders for the living body. It is toxic if inhaled, swallowed or absorbed through skin. Exposure to this dye may cause gastrointestinal irritation with nausea, vomiting and diarrhea. If inhaled, it affects the respiratory tract [6]. Only a few research have so far documented the removal of XO from an aqueous solution.



**Figure 2. Structure of Xylenol orange**

One of the more pressing environmental problems that the textile industry has been facing is the removal of the color from dye bath effluent prior to discharge to local sewerage treatment facilities or adjoining watercourses. Considerable efforts have been made on developing suitable treatment systems for these effluents. Only biotechnological approaches are able to completely eliminate the dyestuff while concurrently reducing both the biological oxygen demand (BOD) and chemical oxygen demand (COD)[7]. Basic and diazo direct dyes were found to have the highest toxicity rates.

Some triphenyl methane either are toxic or carcinogenic compounds. There are no universally useful methods available for the treatment of dye wastes probably because of the complex and very varied chemical structures of these compounds[8].

Presently most of the processes used for the treatment of dye wastewaters are chemical processes, physical or physico-chemical processes which are generally expensive and of limited applicability. All these methods possess significant differences in color removal, volume, capability, operating speeds and capital costs. The color removal concentration in the textile industries varies between 1100 - 1300 mg/L. During coloration more than 15 % of the dyes is wasted which enters into the effluents. These effluents are a source of environmental contamination characterized by high levels of chemical and biochemical (COD, BOD) oxygen demand, suspended matter solids, toxic and hazardous materials. The BOD of dye waters typically varies between 200- 3000/ LSS and a pH of 4 - 12.[9]

Biological processes are getting more attention as they are cost effective, environment friendly and do not produce large amounts of sludge[10]. For this purpose natural material available in large quantities and certain agricultural waste products are considered as promising such as chitin, chitosan, coir pith, activated carbon, cellulose containing materials (cotton & jute fibers), starch based materials[11], for removal of toxicity from the environment as shown in table-1.

One of the efficient processes for removing colors from waste effluent is adsorption. Adsorption has an advantage over other processes since it is sludge-free, clean, and totally removes dyes even from diluted

solutions[12]. Activated carbon (powdered or granular) is the most widely used adsorbent because it has excellent adsorption efficiency for the organic compound. Activated carbon, however, is commercially expensive[13]. This had led to further studies for cheaper substitutions[14,15]. Many inexpensive, commercially accessible adsorbents have been utilized to remove dye in the modern era.. However, as the adsorption capacities of the above adsorbents are not very large, the new adsorbents which are more economical, easily available, and highly effective are still needed[16].

**Table-1: Advantages and disadvantages of physical and chemical process of absorption of dye.**

| Physical and Chemical methods   | Advantages  | Disadvantages  |
|---------------------------------|---|--|
| Fenton's reagent[17]            | Effective decolorization of both soluble and insoluble dyes | Sludge generation                                      |
| Ozonation [17]                  | Applied in gaseous state; no alteration of volume           | Short half-life (20 min.)                              |
| Photochemical[18]               | No sludge production  | Formation of by- products                              |
| NaOCl[19]                       | Initiates and accelerates azo-bond cleavage                 | Release of aromatic amines                             |
| Activated carbon[20]            | Good removal of wide variety of dyes                        | Very expensive   |
| Peat[20]                        | Good adsorbent due to cellular structure                    | Specific surface areas are lower than activated carbon |
| Wood chips[21]                  | Good sorption capacity for acid dyes                        | Requires long retention times                          |
| Membrane filtration[22]         | Removes all dye types                                       | Concentrated sludge formation and very expensive       |
| Ion exchange[23]                | Regeneration, no adsorbent loss                             | Not effective for all dye types                        |
| Electrokinetic Coagulation [23] | Economically feasible                                       | High sludge production                                 |
| Irradiation[24]                 | Effective oxidation at lab scale                            | Requires a lot of dissolved oxygen                     |
| Silica gel[25]                  | Effective for basic dye                                     | Side reactions prevent                                 |

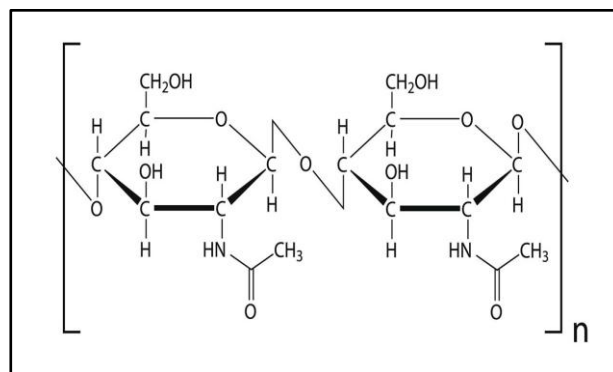
|                                 | removal                                 | commercial application   |
|---------------------------------|---|--------------------------|
| Electrochemical destruction[26] | Breakdown compounds are non – hazardous | High cost of electricity |

All above mentioned and available methods have few limitations, hence in the present work a new and novel material, chitin, is used for adsorption.

## 2. CHITIN:

**2.1 STRUCTURE OF CHITIN:** Chitin is one of the most abundant organic materials, after cellulose which is produced annually by biosynthesis. It occurs in animals,

particularly in crustacea, mollusks and insects, where it is the major constituent of exoskeleton, and in certain fungi, where it is the principal fibrillar polymer in the cell wall. Chitin consists of 2-acetamido-2-deoxy- $\beta$ -D-glucose through a  $\beta(1\rightarrow4)$  linkage. It is a highly insoluble material like cellulose in its solubility and low chemical reactivity. Chitin is a white, hard, inelastic, nitrogenous polysaccharides and the major source of surface pollution in a coastal region[27]



**Figure 3. Structure of Chitin**

As most of the present day polymers are synthetic materials, their compatibility and biodegradability are much more limited than those of natural polymers such as cellulose, chitin, chitosan and their derivatives. In this respect, chitin and chitosan are recommended as suitable functional materials, because these natural polymers have excellent properties such as biodegradability, non-toxicity, adsorption properties etc[28].

## 2.2 PROPERTIES OF CHITIN

Chitin and chitosan are highly basic polysaccharides, their unique properties include polyhydroxy salt formation, ability to form films and chelate metal ions. Chitin is highly hydrophobic and insoluble in water and most organic solvent..

The nitrogen content of chitin varies from 5 to 8% depending on the extent of deacetylation. Nitrogen in chitin is in the form of amido group[29]. When chitin undergoes acetylation, the amido group is converted into aliphatic amino group.

### 2.3 SOURCES OF CHITIN

The main sources of materials for laboratory preparation of chitin are the exoskeletons of various crustacea, principally crab and shrimps[30,31]. Composition is shown in figure.4

Shrimps are the producers of a hard shield to protect their body. This structural material is made out of chitin. This natural material is typical for crustaceans such as lobsters, crabs. From the shell waste, producers can harvest chitin and process it into chitosan to make valuable products for the medical, food, fiber and textile industries. Chitin is the most abundant organic resource on earth[32].

| Chitin Sources                      |        |        |         |
|-------------------------------------|--------|--------|---------|
|                                     | Crabs  | Shrimp | Fungi   |
| Composition (Based upon Dry Weight) |        |        |         |
| Chitin                              | 25-30% | 30-40% | 15-40%  |
| Protein                             | 15%    | 35%    | 5-10%   |
| CaCO <sub>3</sub>                   | 55%    | 30%    | Glycans |
| Lipids                              | 2-5%   | 5-10%  | 5-10%   |

**Table-2 : Sources of Chitin [33]**

| Source       | Percentage of Chitin |
|--------------|----------------------|
| Fungi        | 05 - 20 %            |
| Scorpions    | 25 - 30 %            |
| Worms        | 20 - 38 %            |
| Spiders      | 25 - 38 %            |
| Cockroaches  | 30 - 35 %            |
| Water Beetle | 30 - 37 %            |
| Shrimps      | 35 - 40 %            |
| Silkworm     | 02 - 04 %            |

**Figure 4. Sources of Chitin**

### 3. EXPERIMENTAL WORK:

#### 3.1 MATERIAL:

Laboratory prepared Chitin, Xylenol orange dye, 1% Sodium hypochlorite, 1M NaOH laboratory grade, 2 M HCl laboratory grade.

#### 3.2 PREPARATION OF CHITIN:

Chitin is closely associated with proteins, inorganic material that is mainly  $\text{CaCO}_3$  pigments and lipids. Exoskeletons of shrimps were treated with 1 M NaOH for 24 hrs. The process was repeated three times with fresh portions of alkaline solution. This process was known as deproteinization. The material was washed with water until the pH became neutral. Then it was treated with 2 M HCl at room temperature for 2 hours, again material was washed with water till the pH became neutral. The exoskeletons of crustacea contain coloring matter, principally carotenoids, which was be removed by NaClO 1 % W/V. Then the material was washed with water until the pH becomes neutral. The material was then washed and dried at  $100^\circ\text{C}$  for 2 hrs & pulverized at 100 mesh. Chitin is obtained[33]. This process can be presented in the flowchart as below-

#### 3.3 ABSORBANCE SPECTRA OF XYLENOL ORANGE:

The 500  $\mu\text{g}$  XO dye is dissolved in a suitable solvent system (water 25 mL) i.e 20 ppm and scanned through UV-Visible spectrophotometer. Identification of the dye by this method involves an empirical comparison of the details of the spectrum, i.e. maxima and minima point of the unknown with those of pure compounds. Particularly if the spectrum has several distinct, short peaks, a close match is thought to be strong evidence of the chemical identity[34]. Instrument used is the UV -Visible recording spectrophotometer of Shimadzu Corporation. The instrument has facility of scan from 190 nm to 900 nm and can express results in the form of absorbance, transmittance & unknown concentration of compounds etc. Absorption range is 4 to 5 and transmittance range is 0 to 999.9 %. Light source used is a 50 W halogen lamp and a photomultiplier detector of type R928. Absorbance of XO is as recorded in table 3.

**Figure 5. UV-Visible Spectrophotometer**





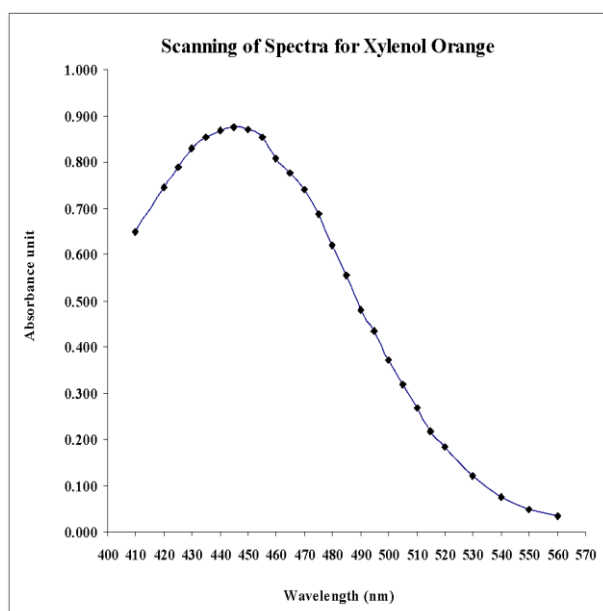
**Table-3 : Absorbance spectra of XO**

| Sr. No. | Wave length in(nm) | Absorbance |
|---------|--------------------|------------|
| 1.      | 410                | 0.648      |
| 2.      | 420                | 0.744      |
| 3.      | 425                | 0.787      |
| 4.      | 430                | 0.828      |
| 5.      | 435                | 0.853      |
| 6.      | 440                | 0.867      |
| 7.      | 445                | 0.874      |
| 8.      | 450                | 0.870      |
| 9.      | 455                | 0.854      |
| 10.     | 460                | 0.807      |
| 11.     | 465                | 0.777      |
| 12.     | 470                | 0.739      |
| 13.     | 475                | 0.686      |
| 14.     | 480                | 0.620      |
| 15.     | 485                | 0.555      |
| 16.     | 490                | 0.480      |
| 17.     | 495                | 0.433      |
| 18.     | 500                | 0.370      |



|     |     |       |
|-----|-----|-------|
| 19. | 505 | 0.317 |
| 20. | 510 | 0.268 |
| 21. | 515 | 0.217 |
| 22. | 520 | 0.182 |
| 23. | 530 | 0.121 |
| 24. | 540 | 0.074 |
| 25. | 550 | 0.047 |
| 26. | 560 | 0.033 |

From the scanned spectra of XO in figure-6 maximum absorbance was observed at 445nm. It is observed that as the wavelength increases, absorbance of Xylenol Orange dye increases and reaches to maximum absorbance 0.874 at wavelength 445 nm, after which absorbance decreases with decrease in wavelength. This shows maximum absorbance at wavelength 445nm.



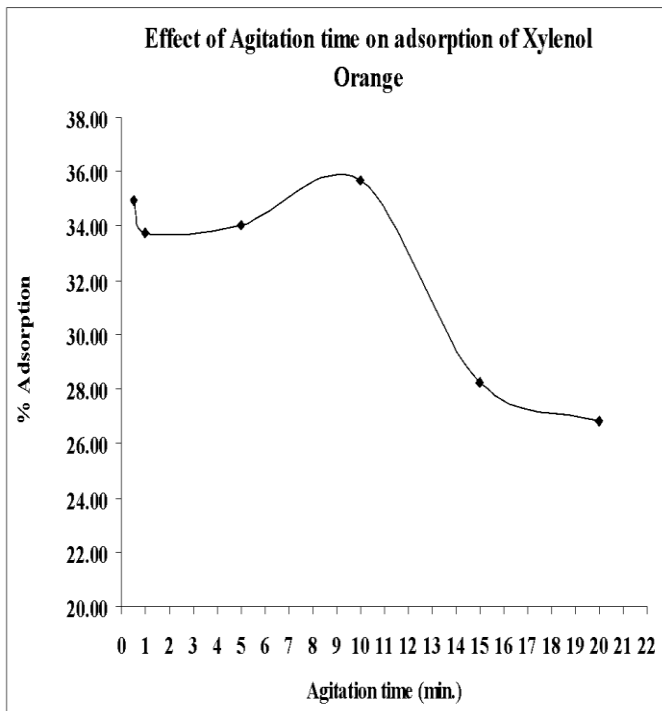
**Figure 6.** Scanning of spectra for XO (20 ppm of Xylenol orange dye)

### 3.4 EFFECT OF AGITATION TIME ON DYE EXTRACTION:

Graph of percentage adsorption versus agitation time as shown in figure-7, shows increase in percentage adsorption from 1 minute agitation time till 10 minute agitation time. After 10 minutes of agitation time it shows a decreasing trend. This may be due to increase in agitation inturn increases temperature. The temperature when increased reduces adsorption capacity by chitin, due to the enhancement of the desorption[33]. This shows that maximum adsorption of dye 35.69 % on chitin takes place at 10 minutes.

**Table-4 Effect of agitation time on dye extraction:-**

| Sr. No. | Agitation time (minutes) | Absorbance (before addition of chitin) | Absorbance (after addition of chitin) | Percent adsorption (%) |
|---------|--------------------------|--|---------------------------------------|------------------------|
| 1.      | 0.5                      | 0.776                                  | 0.505                                 | 34.92                  |
| 2.      | 1                        | 0.776                                  | 0.514                                 | 33.76                  |
| 3.      | 5                        | 0.776                                  | 0.512                                 | 34.02                  |
| 4.      | 10                       | 0.776                                  | 0.499                                 | 35.69                  |
| 5.      | 15                       | 0.776                                  | 0.557                                 | 28.22                  |
| 6.      | 20                       | 0.776                                  | 0.568                                 | 26.80                  |



**Figure 7 Effect of Agitation time on adsorption of XO.** ( Reaction condition -100 mg Chitin - 100mg, Xylenol orange = 20 ppm solution, pH = 4)

### 3.5 EFFECT OF pH AND AGITATION TIME ON DYE EXTRACTION:

As an important factor, pH can significantly affect the adsorption efficiency of anionic dyes. The effects of pH on XO adsorption were studied within the pH range between 3 and 6 for 20 ppm XO solution as shown in (Figure 8). The result shows greater adsorption in the pH range of 3-4 . At a pH above 4, the anions in the solution compete with the anionic XO dye, thereby decreasing adsorption since the amino groups of the polymer are deprotonated to maintain neutrality. [35]. Simultaneously varying agitation time shows that the maximum adsorption remains between 3 to 4. Percentage adsorption is shown in table-5.

**Table-5 Effect of pH and agitation time on dye extraction:**

| Contact time |    | 1 min.                | 5 min. | 10 min | 20 min. | 40 min |
|--------------|----|-----------------------|--------|--------|---------|--------|
| Sr.No.       | pH | Percentage adsorption |        |        |         |        |

|    |   |       |       |       |       |       |
|----|---|-------|-------|-------|-------|-------|
| 1. | 3 | 40.80 | 37.88 | 43.35 | 36.78 | 16.81 |
| 2. | 4 | 42.94 | 33.74 | 40.68 | 42.96 | 03.42 |
| 3. | 5 | 31.66 | 19.09 | 18.18 | 16.83 | 13.90 |
| 4. | 6 | 35.76 | 15.49 | 29.80 | 21.32 | 03.10 |

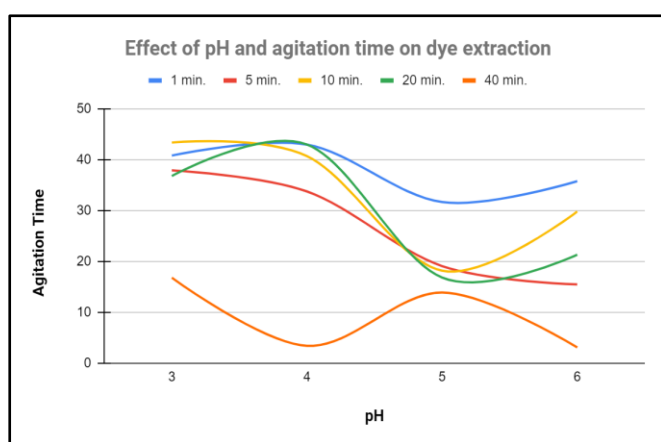


Figure 8. Effect of pH and agitation time on dye extraction.

( Reaction condition -100 mg Chitin - 100mg, Xylenol orange = 20 ppm solution)

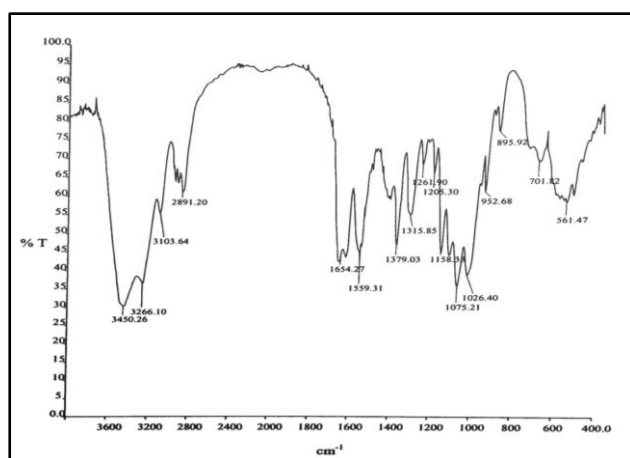
4.CHARACTERISATION AND INTERPRETATION OF IR-SPECTRA OF CHITIN

The degree of acetylation in chitin was determined by measuring the adsorption at 1655 cm<sup>-1</sup> (amide band) and 3450 cm<sup>-1</sup> (hydroxyl band) in conjunction with the standard curve. The infrared spectra were obtained with FT-IR and recorded in the region between 4000 cm<sup>-1</sup> and 500 cm<sup>-1</sup> in KBr [33].The two important areas for a preliminary examination of a spectrum are 4000 - 1300 cm<sup>-1</sup> (2.5 - 7.7 μm) and 909 - 650 cm<sup>-1</sup> (11.0 - 15.4 μm). The short wavelength portion of the spectrum is called the *functional group region*. The characteristics stretching frequencies for functional groups such as OH, NH & C=O occur in this portion of the spectrum[36].

Table- 6 : FTIR peak values of functional group in Chitin

| Sr.no | Mode of Vibration | Range | Peaks and its nature |
|-------|-------------------|-------|----------------------|
|-------|-------------------|-------|----------------------|

|   |   |                              |   |
|---|---|------------------------------|---|
| 1 | O-H stretching in primary alcohol (intramolecular)                  | 3500 - 3300 cm <sup>-1</sup> | 3450.26 cm <sup>-1</sup> (Broad)        |
| 2 | C-H stretching in CH <sub>3</sub> Group [-COCH <sub>3</sub> ]       | 3000 - 2900 cm <sup>-1</sup> | 2962.15 cm <sup>-1</sup> (Sharp)        |
| 3 | C=O stretching (Secondary amine associated)                         | 1680-1630 cm <sup>-1</sup>   | 1654.27 cm <sup>-1</sup> (Moderate)     |
| 4 | N-H in plane bending vibration in amide                             | 1570-1575 cm <sup>-1</sup>   | 1559.31 cm <sup>-1</sup> (Sharp)        |
| 5 | Associated O-H in plane bending & C-O stretching coupling vibration | 1500-1300 cm <sup>-1</sup>   | 1315.85 cm <sup>-1</sup> (sharp)        |
| 6 | C-N stretching in amide   | 1205 - 1360 cm <sup>-1</sup> | 1261.90 cm <sup>-1</sup> (Fairly sharp) |



## Figure.9 FT-IR Spectra of Chitin

**Conclusion :** This research provides a green eco friendly method of removal of dye from textile wastewater, for this purpose as a substrate chitin was used. This chitin is prepared in the laboratory by simple method. Thus the process is sustainable and as per green chemistry principle of using renewable feedstock.

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