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EFFECT OF ULTRASONIC IRRADIATED CHITOSAN NANOPARTICLES ON REACTIVE DYES IN AQUEOUS SOLUTIONS

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Abstract

Vibrant color clothes add flavor and confidence to our attire and imparts attraction to the fabric but the use of color for dyeing has become a big environmental hazard. Wastewater generated from the textile industries containing dye is a major source of environmental pollution. Several materials of organic and inorganic origin have been reported for degradation of such pollutants i.e dye. Chitin, a polysaccharide of animal origin, is obtained from waste material of seafood industries. It occurs in the skeletal material of crustaceans such as crabs, lobsters, shrimps, prawns and crayfish. It is extracted as a pure polysaccharide. Further deacetylation produces chitosan. The chitosan is most significant and is commonly having different applications. The present study provides a deep insight dealing with the preparation of cost effective organic chitosan nanoparticles and their effect on particular dye degradation namely Reactive red 158 and Reactive blue 5 dyes. Chitosin nanoparticles concentration of 1 g/L, removal efficiency rates at pH 4, 9, and 7 has been assessed, and optimum pH was achieved. The effect of contact time (10, 30, 60, 90, and 120 minutes) and concentration of the nanoparticles with each of the respective dyes (50 mg/L) was investigated. The percentage of color removal has been further calculated. The results obtained indicate that after increasing the nanoparticles concentration in the solution, the concentration of hydroxyl radicals that play the main role in decomposition of dye and other contaminants were increased, which provided a clear and gradual increase in degradation of Reactive red 158 and Reactive blue 5 dyes and their color removal.

Key words: *Chitosan, De-proteinization, Demineralization, nanoparticles, dye degradation, color removal, ultrasound irradiation.*

抽象的

鲜艳的颜色衣服为我们的服装增添了味道和信心，并赋予织物吸引力，但使用颜色进行染色已成为一个很大的环境危害。纺织工业产生的含有染料的废水是环境污染的主要来源。已经报道了几种有机和无机来源的材料可降解此类污染物，即染料。甲壳素是一种动物来源的多糖，是从海产品行业的废料中提取的。它存在于甲壳类动物的骨骼物质中，如螃蟹、龙虾、虾、虾和小龙虾。它被提取为纯多糖。进一步脱乙酰产生壳聚糖。壳聚糖是最重要的并且通常具有不同的应用。本研究提供了一个深入的见解，涉及具有成本效益的有机壳聚糖纳米粒子的制备及其

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对特定染料降解的影响，即活性红 158 和活性蓝 5 染料。壳聚糖纳米颗粒浓度为 1 g/L，在 pH 4、9 和 7 下的去除效率已经过评估，并达到了最佳 pH。研究了接触时间（10、30、60、90 和 120 分钟）和纳米颗粒与每种相应染料（50 毫克/升）的浓度的影响。进一步计算了颜色去除的百分比。所得结果表明，提高溶液中纳米粒子的浓度后，在染料和其他污染物分解中起主要作用的羟基自由基的浓度增加，这为活性红的降解提供了明显且逐渐的增加。158 和活性蓝 5 染料及其脱色。

关键词：壳聚糖，脱蛋白，脱矿质，纳米颗粒，染料降解，脱色，超声辐照。

Introduction

Art of applying beautiful colour to the fabric is known to mankind since 3500 BC. W.H. Perkins in 1856 discovered the use of synthetic dyes, providing a wide range of fast and bright colors. Use of synthetic dyes has introduced an adverse effect on all forms of life. The harmful chemicals present in textile effluents readily react with many disinfectants especially chlorine and leads to the formation of bi products that are often carcinogens (Saini, 2018). Colloidal matter present in the effluent along with color increases the turbidity, providing bad appearance, foul smell and prevents the penetration of sunlight into water bodies necessarily required for the photosynthesis which interfere with the oxygen transfer mechanism and hence imparts a negative effect on marine life (Rita, 2012). If textile dyes effluents are allowed to flow in drains and rivers it affects the quality of drinking water making unfit for human consumption. Hence, it is very important to remove these pollutants from the waste water before its final disposal into water bodies (Sharma & Bhattacharya, 2016).

Chitin is among one of the most abundant renewable biopolymer on earth that can be obtained from marine sources (Muzarelli, 1997). It is biocompatible, biodegradable and bio-

absorbable, with antibacterial and wound-healing abilities and low immunogenicity; several studies have been published on its biomedical applications (Jolles & Muzzarelli, 1999). Wide range of applications of Chitin in different fields such as food technology, material science, microbiology, agriculture, wastewater treatment, drug delivery systems, tissue engineering, bio-nanotechnology have been reported. Chitin, a polysaccharide of animal origin, can be obtained from waste material of seafood industries it is also present in the skeletal material of crustaceans such as crabs, lobsters, shrimps, prawns and crayfish (Elieh-Ali-Komi & Hamblin, 2017). It is an important component of exoskeleton of Arthropods and it is also found in fungus, Chitin is a structurally important component of the fungal cell wall located closest to the plasma membrane. Chitosan is the deacetylated product formed by treatment of chitin with concentrated (50%) caustic alkali. Over 60% deacetylation of chitin yields chitosan (Tsugita, 1990). Chitin and Chitosan are described as a family of linear polysaccharides consisting of varying amounts of linked residues of β (1, 4) linked N-acetyl-2 amino-2-deoxy-D-glucose and 2-amino-2-deoxy-Dglucose residues. Chitin samples have a low amount of D units and hence the polymer is insoluble in acidic aqueous media, on the other hand, the amount of

D units in Chitosan samples is high enough to allow the polymer to dissolve in acidic aqueous media. Chitin is the second most abundant natural polymer in nature after cellulose and it is found in the structure of a wide number of invertebrates (Rinaudo, 2006). In the present study, Chitosan nanoparticles have been prepared and their utility for treatment of reactive dyes in aqueous solutions under the effect of ultrasonic irradiation has been studied.

Materials and Methods

Collection and Demineralization of chitinous wastes

The chitinous wastes of fresh water crustaceans were collected from the river bank areas of Ganga river, from Doiwala region, Dehradun, Uttarakhand, India. Initially the material was washed with water properly in order to remove the sand debris present on the surfaces. Further the demineralization of chitinous wastes was performed according to the method adopted by (B & N, 1993) with some modifications. The chitinous wastes were treated with 1.75 N Acetic acid for about 12-15 hours at room temperature. The ratio of waste to solvent were maintained (1:15 w/v). The demineralized material was then recovered by filtration and rinsing with de-ionized water and then the demineralized material was air dried in forced hot air oven at 65°C. The dried demineralized chitinous material obtained was then powdered; the powdered material obtained was used as chitin for present study.

De-proteinization: Removal of Lipids and Preparation of Chitosan

The new and advanced methodology for de-proteinization of proteins from demineralized chitinous wastes has been designed and reported

by using deproteinization agents (Mathur A, 2011). This process was performed by using proteolytic enzymes such as proteinase-K/Trypsin dissolved in buffer containing 0.05 M Tris-base (pH, 6.5-9.1) in a ratio 1:20 (w/v) in flasks at various temperatures in incubator-shaker for about 72 h and adding mixture of solvents (phenol: chloroform, 1:1 ratio) again and again to the residue obtained and centrifuging the mixture until the residue gives no test for the presence of protein content. After repeating the procedure for 3-4 times, finally the residue was treated with 2N sodium hydroxide (1:25 w/v) at 70°C for 1 hour. The lipid content get dissolved in phenol:chloroform mixture and were removed from the chitinous wastes (Mótyán, Tóth, & Tózsér, 2013). Greese spot test has been performed in order to determine qualitatively the presence of lipid content if any present in the residual material. The residual material thus obtained was then dried in hot air oven at 60°C and percent yield of chitin extracted from crustacean waste has been calculated. The resulting chitin was further deacetylated in 40% sodium hydroxide in 120°C for 1-3 h. This treatment produces 70 % deacetylated chitosan (Gerhard, Jürgen, & Jon, 2010).

Preparation of Chitosan-Tripolyphosphate (CS-TPP) nanoparticles

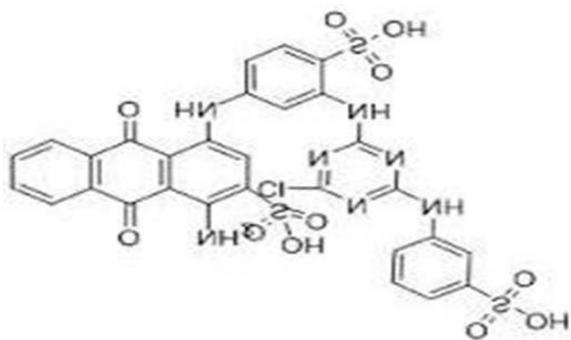
CS-TPP nanoparticles were prepared according to the procedure adopted and reported (Calvo P, 1997) incorporating few modifications. Chitosan (CS), 1 mg/ml was dissolved in 1% (w/v) acetic acid and sonicated till the solution became transparent. The addition of tripolyphosphate (TPP) solution, at a concentration of 1 mg/mL, to CS solution (pH-5), with stirring at room temperature, produced the formation of CS-TPP

nanoparticles by ionic gelation mechanism. The nanoparticles were then separated from the solution after centrifugation at 3600 rpm for 1 hour. The residual pellet was pooled out and washed thrice with de-ionized water and freeze dried to form the powder for further analysis. The freeze-dried chitosan nanoparticles was suspended in water for characterization and directly used for other experiments (Debnath, 2018; Qian-ying Deng, 2006).

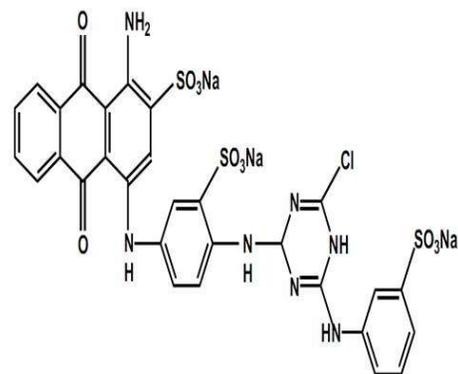
Characterization of Chitosan-TPP Nanoparticles

Characterization of Chitosan-TPP Nanoparticles by Scanning Electron Microscopy (SEM)

Chitosan-TPP nanoparticles has been utilized at first place for surface morphological studies of nanoparticles performed by Scanning Electron



Reactive blue 5



Reactive Red 158

Image 1: Different dyes selected in present study

Treatment/Degradation studies of dyes in aqueous solutions

The treatment of dyes has been achieved by different treatment sets: (a) Chitosan-TPP nanoparticles solution at different concentration (viz. 10 to 100 mM/l) irradiated with ultrasonic

Microscope (SEM). The diameter of the AgNP has been determined (T.López-Leóna 2005).

Analysis of Chitosan and Chitosan-TPP nanoparticles by High Performance Liquid Chromatography (HPLC)

The 20 μ l of each of the sample was chromatographed using linear gradients of CH₃CN–H₂O from 70% to 55% in 30 minutes at a flow rate of 1 ml/minute. The nanoparticles were monitored at 205 nm with a spectrophotometric detector.

Preparation of aqueous solution of dyes

In the present study, Reactive red 158 and Reactive blue 5 dyes have been utilized as the model pollutants (S.Senthilkumaara 2006). Aqueous solutions of each of the dyes have been prepared by dissolving calculated amount of dye in distilled water.

radiations and (b) ultrasonic degradation separately. Advanced oxidation has been performed in different experimental set-ups for different dyes under study viz. using ultrasonic degradation with a frequency of 37 kHz; power (200W-500W) and in the presence of CS-TPP

nanoparticles irradiated with ultrasonic radiations to remove the dyes from the aqueous solutions. The stock solutions of dyes viz. reactive red 158 and reactive blue 5 were prepared separately by dissolving 1 g of each dye in deionized water (1000 mg/l), stock solutions were refrigerated to avoid any variations in concentration. The stock solutions of each of the dyes were prepared at 50 mg/l concentration and the standard method was used to determine the most appropriate wavelength for measuring the concentrations of the dye under study. Finally, the wavelength which achieved maximum absorption for the desired dye was taken in consideration. The important variables pH, concentration of CS-TPP nanoparticles, initial dye concentration, and contact time were also assessed. In the first step of the process, the optimum pH was obtained under constant conditions, each of the dye concentration of 50 mg/L, contact time of 60 minutes, nanoparticles concentration of 1 g/L, removal efficiency rates at pH 4, 9, and 7 has been assessed, and optimum pH was achieved. The effect of contact time (10, 30, 60, 90, and 120 minutes) and concentration of the nanoparticles with each of the respective dyes (50 mg/L) was investigated. The percentage of color removal was further calculated.

Dye removal ratio (%) = $[(1-C_t/C_0) \times 100]$

Where C_0 is the initial dye concentration and C_t is the dye concentration at contact time "T". Blanks containing no dyes were used in each series of experiments. The solutions were collected and dried for further identification by UV-VIS spectra.

Results and Discussions

The average particle size of CS-TPP synthesized nanoparticles was found to be 30 μm (**Figure 1**).

The dominant peak of CS-TPP nanoparticles, when purified, through HPLC showed retention time of 5.089 minutes (**Figure 2**). The removal of reactive red 158 and reactive blue 5 has been studied by preparing a concentration of 50 mg/L of dye, exposing it to CS-TPP nanoparticles with ultrasonic irradiation (50 mmol/l) and ultrasonic degradation as such and sampling it at test times (10, 30, 60, 90, and 120 minutes). Results showed that, during these times, the initial dye concentration was unchanged, and ultrasonic degradation alone did not affect in dye degradation or removal. In order to obtain the best concentration of CS-TPP nanoparticles (optimum concentration), solution concentrations of 10, 15, 20, 50, and 100 mMol/l were prepared irradiated with ultrasonic radiation (200 W) and placed in contact with 50 mg/L concentrations of each of the dyes for 60 minutes respectively. For the prepared nanoparticle concentrations, removal efficiency rates were variable and it was found that maximum percentage of color reduction of each of the dyes solution, treated occurs at, 100 mMol/l concentration (**Table 1; Figure 3**). Further, the treatment sets were compared with ultrasonic radiation as such at power 200-500 W. The results showed that at high power, maximum color reduction occurs but not much in comparison to the chitosan-TPP nanoparticles treated with ultrasonic irradiation (**Table 2; Figure 4**). The optimal pH and wavelength maxima of the dyes were determined (**Table 3; Figures 5 & 6**). Further, after 120 minutes, the treatment of dyes at different concentration of Chitosan-TPP nanoparticles and ultrasonic radiation treated as such on dyes were studied (**Table 4; Figure 7 & Table 5; Figure 8**). The results of experimental sets of treatments are shown (**Images 1-4**). By increasing the

nanoparticles concentration in the solution, the hydroxyl radicals that play the main role in decomposition of dye and other contaminants were increased; the collision probability of these very active radicals with the target contaminants

were also increased, and decomposition and removal were conducted. The results are in correlation with the previous findings reported (Li JT, 2013; Ghaderpoori M, 2016; Zaroni, 2005).

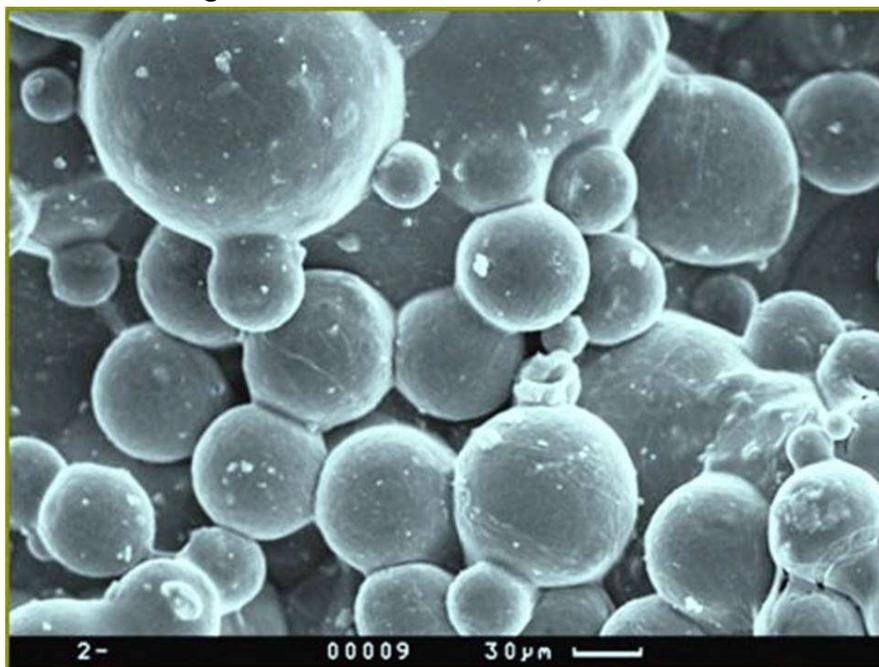


Figure 1: Chitosan-TPP (CS-TPP) nanoparticles as determined by SEM

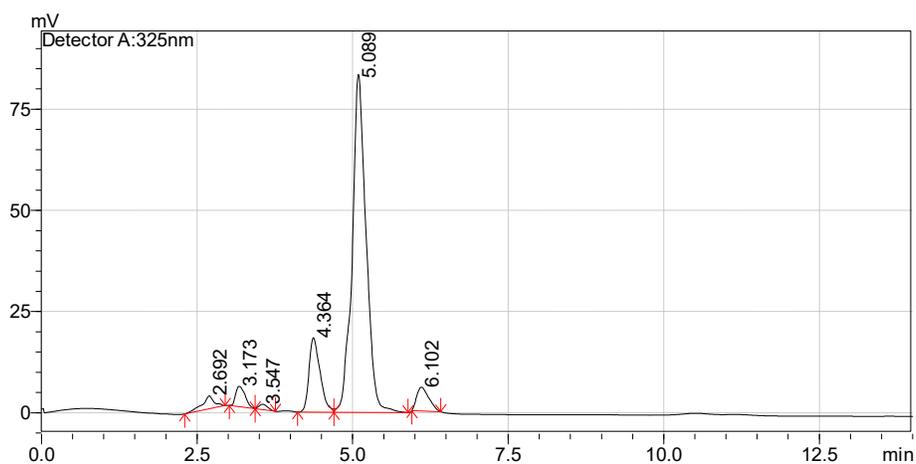


Figure 2: HPLC chromatogram of Chitosan-TPP (CS-TPP) nanoparticles

Table 1: Chitosan-TPP irradiated nanoparticles treatment on different dyes after 60 minutes

S.No.	Chitosan-TPP (mMol/l) irradiated with ultrasound irradiation	Percent removal of dyes (50 mg/l) in first 60 minutes	
		Reactive red 158	Reactive blue 5
1	10	23.0	28.45

2	15	58.0	52.45
3	20	64.6	64.65
4	50	82.6	88.0
5	100	93.12	95.43

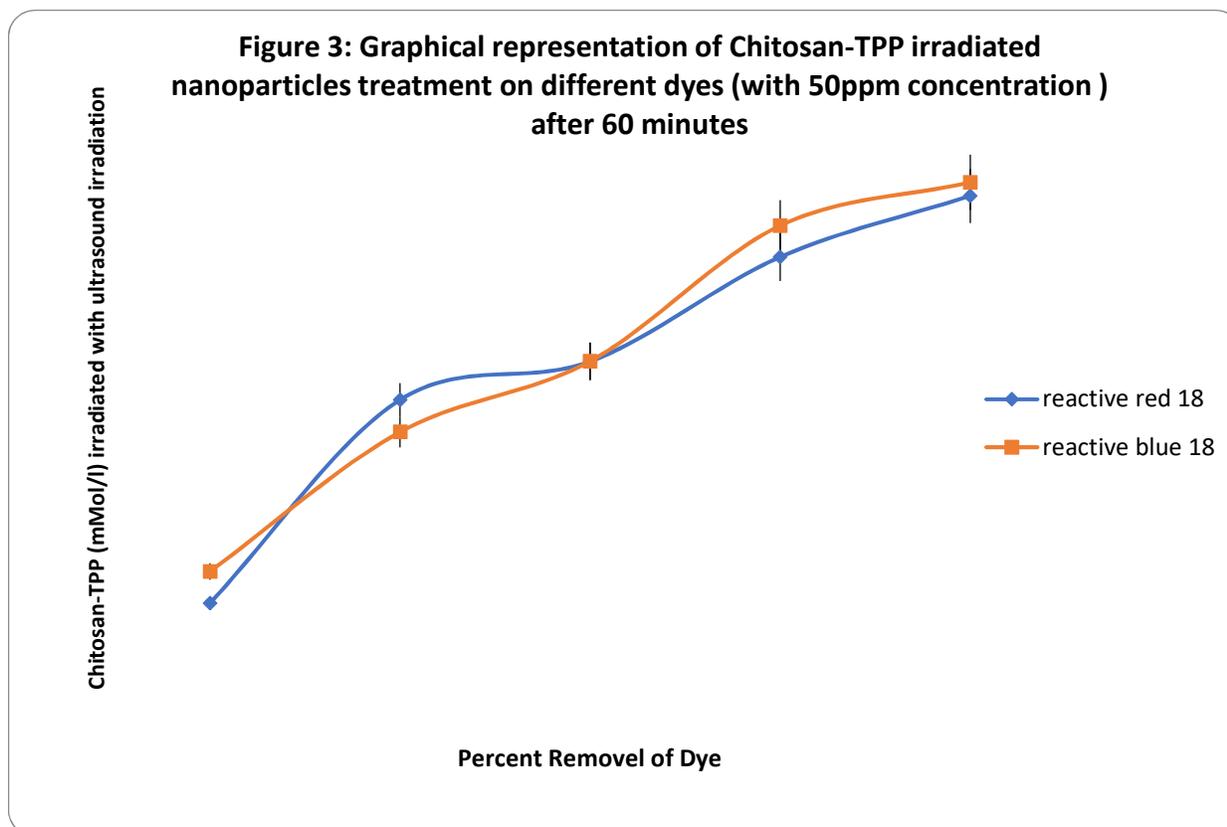


Table 2: Ultrasonic radiation treatment on different dyes after 60 minutes

S.No.	Power (W)	Percent removal of dyes (50 mg/l) in first 60 minutes	
		Reactive red 158	Reactive blue 5
1	200	11.23	12.56
2	250	20.56	21.56
3	300	32.23	32.65
4	350	42.0	54.45
5	500	67.45	78.45

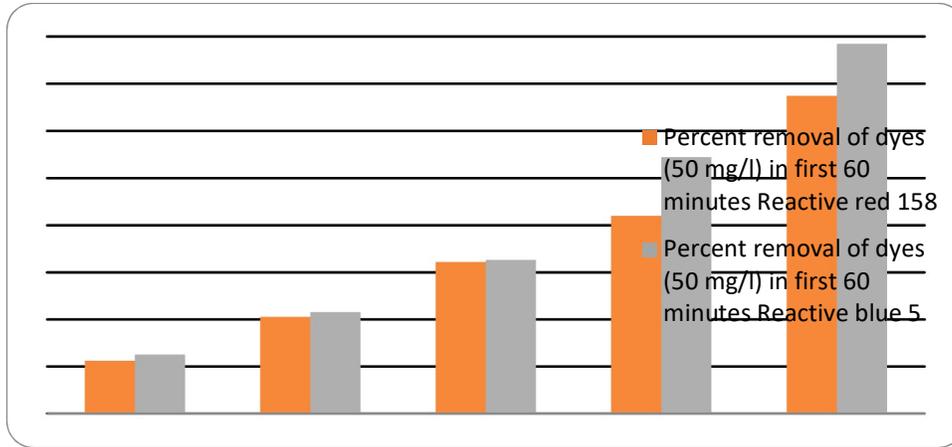


Figure 4: Graphical representation of Ultrasonic radiation treatment on different dyes after 60 minutes

Table 3: Absorption maxima and optimum pH of dyes during degradation studies

S.No.	Dyes	Maximum absorption of the light at wavelength (nm) during degradation studies	Optimum pH of dyes during degradation studies
1.	Reactive red 158	513	6.3
2.	Reactive blue 5	615	5.3

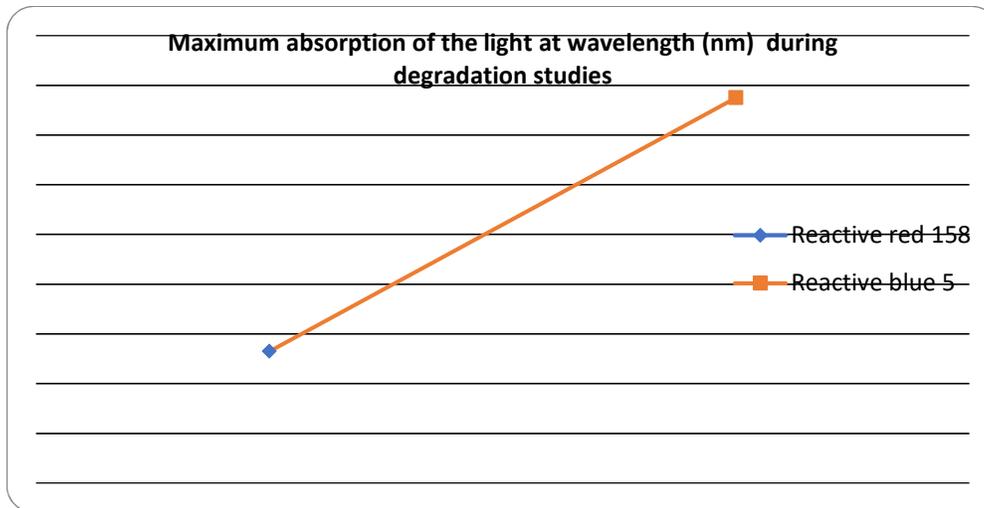


Figure 5: Maximum absorption wavelength of the dyes during treatment studies

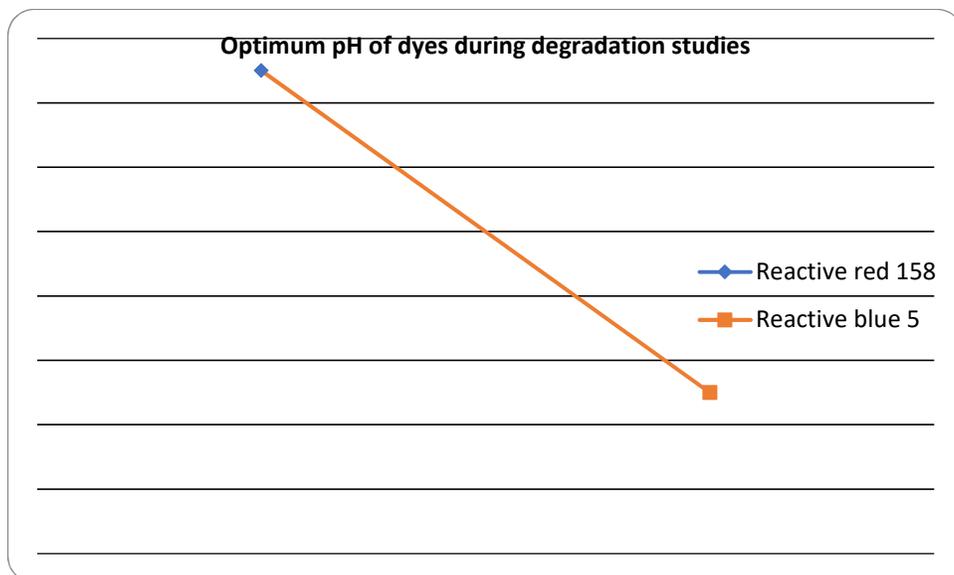


Figure 6: Optimal pH of the dyes during treatment studies

Table 4: Chitosan-TPP irradiated nanoparticles treatment on different dyes after 120 minutes

S.No.	Chitosan-TPP (mMol/l) irradiated with ultrasound irradiation	Percent removal of dyes (50 mg/l) after 120 minutes	
		Reactive red 158	Reactive blue 5
1	10	45.34	46.64
2	15	65.34	67.55
3	20	72.34	74.56
4	50	85.65	91.34
5	100	100	100

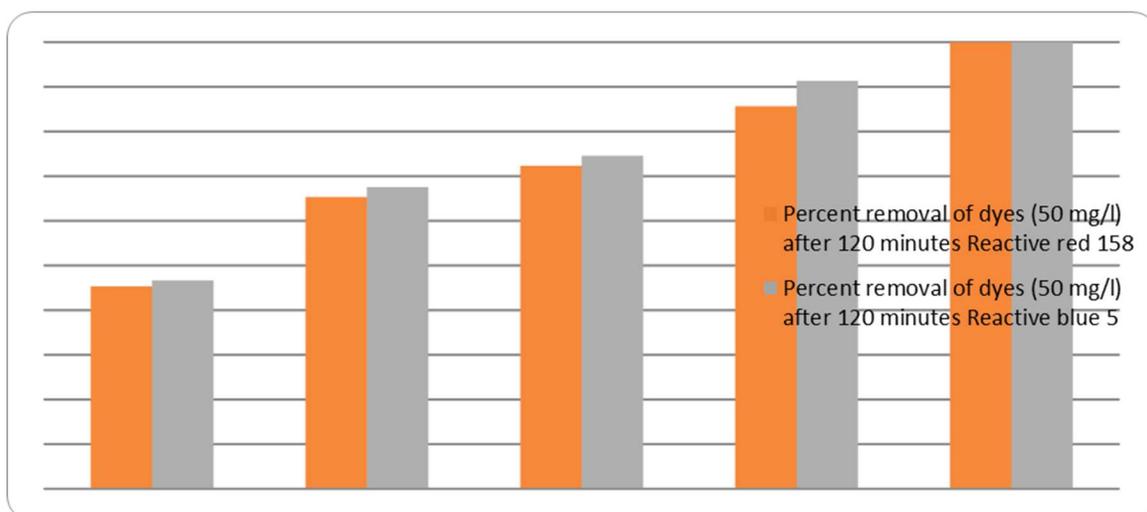


Figure 7: Graphical representation of Chitosan-TPP irradiated nanoparticles treatment on different dyes after 60 minutes

Table 5: Ultrasonic radiation treatment on different dyes after 120 minutes

S.No.	Power (W)	Percent removal of dyes (50 mg/l) after 120 minutes	
		Reactive red 158	Reactive blue 5
1	200	34.56	37.56
2	250	63.45	65.78
3	300	74.45	73.56
4	350	85.67	84.45
5	500	100	100

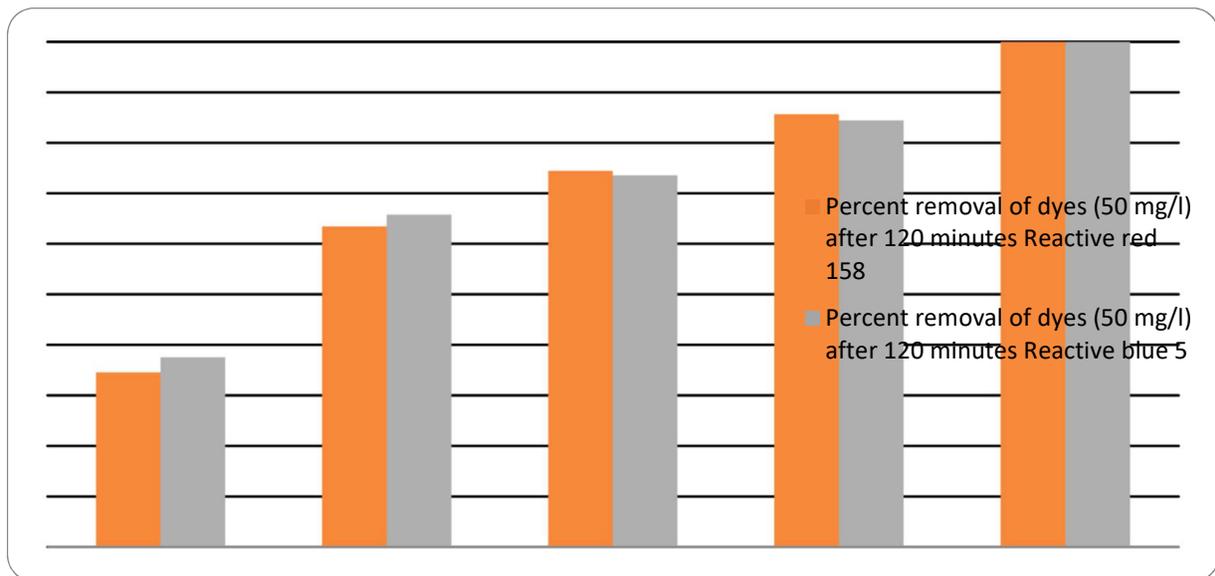
**Figure 8:** Graphical representation of Ultrasonic radiation treatment on different dyes after 120 minutes

Image 1: Reactive red 158 treated with chitosan nanoparticles irradiated with ultrasonic irradiations



Image 2: Reactive red 158 treated with ultrasonic irradiations as such



Image 3: Reactive blue 5 treated with chitosan nanoparticles irradiated with ultrasonic irradiations

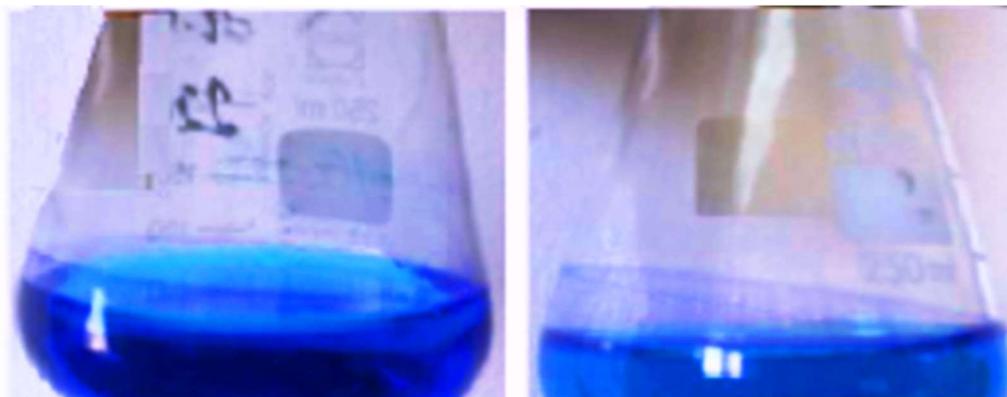


Image 4: Reactive blue 5 treated with ultrasonic irradiations

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