Present section of the thesis describes the synthesis and characterization of Starch-Fe\(^0\)Nps-Silica hybrid biocomposites (SFS). A series of starch-Fe\(^0\)Nps-Silica composites (SFS\(_1\) to SFS\(_8\)) with different starch/Fe\(^0\)Nps/silica ratios were prepared using an inexpensive and simplistic sol gel technique and mango leaf reduction strategy. Various process parameters were varied for optimizing the synthesis and to obtain the optimum sample (SFS\(_1\)) in terms of homogeneity, gelling time, and dye degradation. The optimum hybrid composite has been characterized using FTIR, HR-TEM, EDX, and FE-SEM techniques. The results of HR-TEM study showed that SFS\(_1\) was decorated with spherical Fe\(^0\) nanoparticles of approx 4 nm average size. FE-SEM images showed that the surface of nanocomposite was dispersed (somewhat agglomerated) with Fe\(^0\) nanoparticles which were spherical in shape. The mapping shape of iron, silica, carbon, and oxygen elements showed that they are evenly distributed throughout the composite. The activity and stability of the Fe\(^0\)Nps was significantly improved on gelation with starch and TEOS.

**INTRODUCTION**

Starch is low cost, abundantly available carbohydrate reserve of plants and is found in plant tubers and seed endosperm. Starch has been considered as a
renewable raw material for many different industrial applications because it is an excellent biocompatible, environmental friendly, non-toxic, and biodegradable material with many hydroxyl groups that can be chemically modified to design materials with new or improved properties [70]. Starch granules consist of several million amylopectin molecules and smaller amylase molecules. Both polymers consist of D-glucose units in the $^4\text{C}_1$ conformation. In amylose, D-glucose units are linked through $\alpha$-1,4 glycosidic bonds with limited branching points at the $\alpha$-1,6 positions, whereas amylopectin is essentially a linear chain of D-glucose units linked by $\alpha$-1,4 glycosidic bonds and is highly branched at the $\alpha$-1,6 positions [71]. Soluble starch is a modified starch which dissolves in hot water, forming a limpid solution. Soluble starch is derived by treating starch with mild acids or by oxidation of starch or by heating starch with glycerol. Soluble starch has a linear structure that consists of $\alpha$-1,4 linked D-glucose units (Fig.20). Starch based nanocomposites have potential applications in many areas such as food industry, biomedicine, environmental remediation, and agriculture. Incorporation of metal nanoparticle in starch-silica matrix has been the subject of great research interest.

In the present study, synthesis and characterization of starch-Fe$^0\text{Nps}$-Silica nanocomposite have been undertaken. The nanocomposites have been
prepared by impregnating Fe$^0$Nps (derived from mango leaf extract) within starch templated silica matrix. The starch and silica were chosen as the constituents materials because of their low cost and environmental friendliness.

RESULTS AND DISCUSSION

Synthesis of Fe$^0$Nps

Fe$^{3+}$ ions were reduced into Fe$^0$ nanoparticles using mango leaf extract. The formation of the zerovalent iron nanoparticle was visualized by change in color of the ferric nitrate solution from yellow to black. Mango leaf extract was used as a reducing agent. Fe$^0$Nps were prepared by mixing Mango leaf extract to a freshly prepared Fe(NO$_3$)$_3$.9H$_2$O solution in 1:1 ratio. The formation of iron nanoparticles was evidenced by the appearance of SPR peaks of Fe$^0$Nps (at 225 nm and 265 nm) (Fig. 21 (a)) [17] and by a visible change in the color of the solution (from yellow to black).

Synthesis of Starch-Fe$^0$Nps-Silica composites (SFS)

The nanocomposites were crafted by adding known volumes of tetraethoxysilane (TEOS) and ethanol (EtOH) to the starch solution of known concentrations. To these mixtures, known volumes of 12N NH$_4$OH and Fe$^0$Nps were added. The mixtures were stirred for a known time period
till the hybrid hydrogels were formed. The dried and powdered samples were evaluated for their catalytic activity for the dye degradation. The optimum sample (SFS₁) in terms of homogeneity, gelling time, and dye degradation was obtained when the ratio of Fe⁰Nps : TEOS : EtOH : H₂O : NH₄OH was 2 : 1.66 : 1.66 : 4.66 : 1 (Table. 5). The presence of Fe⁰Nps had an expediting influence on the sol-gel polymerization of TEOS and this significantly minimized the gelling time.
<table>
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<tr>
<th>Hybrid</th>
<th>Starch (g)</th>
<th>H₂O (mL)</th>
<th>TEOS (mL)</th>
<th>EtOH (mL)</th>
<th>Fe⁰Nps (M)</th>
<th>NH₄OH (mL)</th>
<th>Gel/ppt time (min)</th>
<th>Yield (mg)</th>
<th>Dye degradation (%)</th>
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<td>2.5</td>
<td>-</td>
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<td>2.5</td>
<td>-</td>
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<td>2.5</td>
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Table 5: Optimization of nanocomposite synthesis by varying various process parameters while keeping the total reaction volume fixed (16.5 mL); dye degradation was done using 0.05 g nanocomposite, 20 mL of 100 mg L⁻¹ dye solution, 1 mL of H₂O₂ (3M), temperature 35°C, contact time 55 min, rpm 150, and pH 2.
Soluble starch molecule bears hydroxyl (-OH) functional group. It can be proposed that the hydrogen bonding between hydroxyl moieties of starch (molecule backbone) and shell of zerovalent iron reduced the Vander Waals and magnetic attraction forces to result well dispersed Fe$^0$Nps [72]. Fe$^0$Nps were also stabilized through hydrogen bonding with silica silanol and siloxane groups [73] (Scheme 3). Silica has been used as an efficient dispersant for Fe$^0$Nps. It prevented agglomeration of Fe$^0$Nps by electrostatic repulsion and/or steric hindrance.

Scheme 3: Mechanism of nanocomposite (SFS) synthesis
Characterization of \( SFS_1 \)

\textit{Fourier Transform Infrared Spectroscopy}

The FTIR spectrum of pure starch (Fig. 22 (a)) has sharp absorption peaks at 3427 cm\(^{-1}\) and 2928 cm\(^{-1}\) due to O-H and C-H stretchings respectively. The stretching peaks due to C-O bond are seen at 1158 cm\(^{-1}\), 1081 cm\(^{-1}\) and 1016 cm\(^{-1}\), while the peaks at 928 cm\(^{-1}\), 859 cm\(^{-1}\), 759 cm\(^{-1}\), 709 cm\(^{-1}\), 574 cm\(^{-1}\) and 525 cm\(^{-1}\) corresponded to the anhydroglucose ring stretching vibrations [74]. The absorption peak at 1643 cm\(^{-1}\) was attributed to the water adsorbed in the amorphous region of starch granules. The C-O-H bending and CH\(_2\) bending modes of starch are visible at 1372 cm\(^{-1}\) and 1417 cm\(^{-1}\) respectively [75].

Silica content in \( SFS_1 \) was confirmed by the appearance of SiO-H, Si-OH, and O-Si-O absorption peaks at 3467 cm\(^{-1}\), 974 cm\(^{-1}\), 1092 cm\(^{-1}\) and 801 cm\(^{-1}\) respectively (Fig. 22 (b)) [22]. Silanol O-H stretching peak was invisible separately as it is merged with starch O-H stretching peak at 3467 cm\(^{-1}\). The peak observed at 468 cm\(^{-1}\) (bending vibrations) signified the formation of siloxane domains (SiO\(_4\)) [23]. The peaks at 2077 cm\(^{-1}\), 1632 cm\(^{-1}\), 1401 cm\(^{-1}\), 3467 cm\(^{-1}\) are seen shifted due to mutual interaction between starch and silica which has changed the electron density around the chemical bonds [76].
In FTIR spectrum of nanocomposite (SFS<sub>1</sub>), the SiO–H, Si–OH and O–Si–O stretching are seen at 3442 cm<sup>-1</sup>, 952 cm<sup>-1</sup>, 1145 cm<sup>-1</sup>, 475 cm<sup>-1</sup> and 809 cm<sup>-1</sup> respectively (Fig. 22(c)). Another peak shift (from 3467 cm<sup>-1</sup> to 3442 cm<sup>-1</sup>) was observed in the case of SFS<sub>1</sub>. The shift suggested that -OH (combined SiO–H and starch-O–H) groups played an important role in the formation of the Starch-Fe<sup>0</sup>Nps-Silica nanocomposite. Absorption peaks due to C–O–H bending, CH<sub>2</sub> bending, Si–OH and O–Si–O (stretching) also showed significant shift after entrapment of Fe<sup>0</sup>Nps.

**High Resolution Transmission electron microscopy (HR-TEM) and Energy Dispersive X-ray Analysis (EDX)**

Fig. 23(a) and (b) show the HR-TEM images of St–Si and SFS<sub>1</sub>. St–Si nanocomposite is seen as a cluster of large globular particles (silica nanoparticles) (Fig. 23(a)) while in SFS<sub>1</sub>, Fe<sup>0</sup>Nps are dispersed within starch-silica matrix and have a relatively dense distribution (Fig. 23(b)). HR-TEM images revealed that the Fe<sup>0</sup>Nps are nearly spherical in shape. HR-TEM images of nanocomposite at different magnification are shown in Fig. 24. Composite exhibited crystalline nature which was confirmed through SAED pattern (Fig. 24(e). HR-TEM histogram (Fig. 24(f)) showed that the Fe<sup>0</sup> nanoparticles have an average particle size of about 4 nm. The high
resolution TEM (HRTEM) image of optimum sample showed lattice fringes having d-spacing of 0.202 nm which was in good agreement with the body-centered cubic iron (110) plane of Fe⁰ nanoparticles (Fig. 25) [77]. Fig. 26-29 presents the EDX mapping and element analysis of St-Si and SFS₁. EDX spectrum the St-Si nanocomposite revealed that it consists of carbon, oxygen, and silicon (Fig. 27). EDX spectrum of SFS₁ (Fig. 29) indicated that it has iron, carbon, oxygen, and silicon. The percentages of C, O and Si are higher than Fe (which is shown by tiny peak of Fe). The presence of carbon, silicon, and oxygen represented the presence of starch and silica. The element distribution in St-Si and SFS₁ has been done by element mapping analysis (Fig. 26 (a-d)) and (Fig. 28 (a-e)). The mapping shape of iron, silica, carbon, and oxygen elements showed that they are evenly distributed throughout the nanocomposites (SFS₁ and St-Si).

**Field Emission Scanning Electron Microscopy**

FE-SEM images of starch, St-Si, SFS₁ are depicted in Fig. 30. FE-SEM micrographs of starch revealed a smooth surface morphology with oval or ellipsoidal, and spherical shaped microparticles as observed by Odeku and Picker [78] (Fig. 30 (a)). St-Si showed rough surface that suggested that the small silica particles are deposited at the surface of starch (Fig. 30(b)). It can
be seen that (Fig. 30(c)) the surface of composite (SFS₁) is dispersed with Fe⁰ nanoparticles (somewhat agglomerated) which are spherical in shape. Fig. 31 showed the FE-SEM micrographs of the nanocomposite at different magnification.

Thus, the synthesized nanocomposite based on low-cost, eco-friendly, and natural biopolymer, starch has been designed and used for dye degradation. In future, the nanocomposite may be evaluated for the degradation of other similar dyes.

**EXPERIMENTAL**

**Materials and equipments**

Analytical reagents of tetraethylorthosilicate (98% TEOS; Merck, Germany), soluble starch, ferric nitrate (Merck), hydrogen peroxide (H₂O₂, 30 wt%), NH₄OH (30% NH₃; Merck) were purchased from Merck, India. Absolute ethanol 99.9% (AR) was purchased from Changshu Yangyuan Chemical, China. Double distilled water was used in all the procedures. The pH values were adjusted with the help of 5M HCl (Merck); or 1M NaOH (Merck). EUTECH Instruments pH meter (model 510) was used for the pH measurements. Orbital shaker Incubator, Metrex Scientific Instruments (P) Ltd., New Delhi was used.
Instrumentation

Field Emission Scanning Electron Microscopy (FE-SEM) of the composites was performed using FESEM/EDX (SUPRA 40 VP) instrument. The samples were coated with gold to avoid charging. High Resolution Transmission Electron Microscopy, EDX, and elemental mapping were conducted using a FEI Titan G2 60-300 TEM (HR-TEM) instrument. The sample was dispersed in ethanol and the dispersed sample was sonicated before placing on the grids for the HR-TEM analysis. UV-visible spectrum was recorded on a Cyber Lab double beam UV-visible spectrophotometer. FTIR was done on Perkin Elmer FTIR spectrophotometer.

Synthesis of Fe$^0$Nps

Fe$^0$Nps have been crafted using a mango leaf extract ($Mangifera indica$) reduction method [79]. In brief, 1 g of dried mango leaves were boiled in 25 mL distilled water until volume was reduced to 10 mL. The extract was filtered and collected in a clean beaker (leaf extract). The leaf extract was then added drop wise to 0.1M ferric nitrate solution in 1:1 volume ratio. Immediately after addition of the leaf extract, the color of the ferric nitrate solution turned black which indicated the formation of zerovalent iron nanoparticles (Fe$^0$Nps). Their formation was further evidenced by the
appearance of strong SPR absorption peaks (at 225 nm and 265 nm) in UV-VIS spectrum of the solution.

**Synthesis of nanocomposite (SFS)**

A known weight starch (0.05 - 0.2 g) was dissolved in a known volume (6.5 -10 mL) of distilled water in 50 mL conical flask under magnetic stirring to obtain a homogenous solution of starch. Separately, a known volume of TEOS was mixed with a known volume of EtOH and this mixture was subsequently added to the starch solution of known concentration (w/v). The mixture was stirred on a magnetic stirrer at room temperature for 10 min. The known volume of Fe$^0$Nps and 12N ammonium hydroxide solutions were rapidly added to this stirred mixture and the stirring was continued till a hybrid gel was obtained. The whole process was carried out at room temperature. The gel was dried in an electric oven at 40$^\circ$C and powdered well. The powdered nanocomposite was used for dye degradation.

**Synthesis of the controls**

The starch-silica composite (St–Si) and control silica (CS) were synthesized by the same procedure as mentioned above. St–Si have been synthesized by using of 0.05 g starch, 10 mL of H$_2$O, 2.5 mL TEOS, 2.5 mL EtOH, and 1.5
mL of 12N NH₄OH followed by stirring at room temperature till the hydrogel was formed. Similarly control silica (CS) was fabricated by sol gel method using 2.5 mL TEOS, 2.5 mL EtOH, 10 mL of H₂O, and 1.5 mL of 12N NH₄OH solution as catalyst. The resulting mixture was stirred till the hydrogel was obtained which was dried.

**Dye degradation using SFS**

Stock solution of 1000 mg L⁻¹ was prepared by dissolving 1 g RBV dye in 1000 mL distilled water, which was later diluted with requisite volume of distilled water to achieve a desired initial concentrations (25 to 200 mg L⁻¹). A series of batch experiments were conducted on a temperature controlled incubator shaker set at 150 rpm and maintained at 35±2ºC for 55 min to measure the degrading efficiency of the samples. The degradation of RBV dye was studied as a function of pH (2-8), nanocomposite dose (0.02-0.08 g), initial dye concentration (25 to 200 mg L⁻¹), and temperature (20-50ºC). The nanocomposite was added to the dye solution (100 mg L⁻¹) and the reaction medium was maintained to a known pH using an appropriate volume of 1M NaOH or 5M HCl. Subsequently, 1 mL of H₂O₂ (3M) was added to the above suspension and the degradation reaction was initiated. The flasks were kept in a temperature controlled orbital shaker with constant
stirring during the reaction. Each flask was shaken for a desired time and the suspensions were filtered using Whatman 0.45 mm filter paper and the supernatants were analyzed for the remaining dye concentrations spectrophotometrically at 560 nm wavelength using UV-visible spectrophotometer. The degradation of RBV dye was calculated by the equation-1:

$$\text{% dye degradation} = \frac{(C_0 - C_e)}{C_0} \times 100 \quad \text{-------- (1)}$$

Averages and standard deviations were obtained from duplicate experiments which were less than 3%.