2.0 Introduction

This chapter deals with the materials used for the study and the methods applied in preparation and characterization of the materials developed. The preparation of polymeric composite nanofibers was carried out using electrospinning technique. The technique has been described and the important parameters in obtaining uniform and continuous nanofibers have been discussed. Various techniques used for characterization of polymeric composite nanofibers like Optical microscopy, Scanning electron microscopy (SEM & EDAX), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), X-ray photon spectroscopy (XPS), Thermo gravimetric analysis (TGA) have been discussed. Other measurements techniques include Atomic Adsorption Spectroscopy (AAS) and Ultra violet spectroscopy (UV) which have been employed for carrying out batch adsorption studies for heavy metal removal were also discussed in this chapter. This chapter includes the details of instrument used and the method in general. And specific details of experiments carried have been discussed in related chapters. The adsorption isotherms for adsorption process in details were also discussed in this chapter like Langmuir and Freundlich isotherm. It is important to study the kinetics of developed materials to know the rate of adsorption of heavy metals on the developed materials. Various kinetic models like pseudo first and pseudo second order models have been discussed. The interference study for all developed polymeric composite nanofibers for various metal ions has been conducted to see their effect on adsorption of heavy metal ions. The regeneration study of all the developed materials has also been carried out to check the reusability for successive adsorptions of heavy metals.
2.0 Experimental

2.1 Materials

Chitosan

It is a linear polysaccharide composed of randomly distributed β-linked D-glucosamine and N-acetyl-D-glucosamine. It is obtained from shrimp and other crustacean shells by treating them with the alkali sodium hydroxide. Chitosan (M.W:100000-300000) was purchased from Across Organic, New Delhi.

![Chemical structure of Chitosan](image)

Polyvinyl Alcohol

It is a white and odorless, water-soluble synthetic polymer and is used in papermaking, textiles, and a variety of coatings. Polyvinyl-alcohol (PVA), (M.W: approx. 125000) was obtained from CDH.

![Chemical structure of Polyvinyl Alcohol](image)

Polyacrylonitrilie

It is semi-crystalline organic polymer resin, thermoplastic and does not melt under normal conditions. PAN co-polymer with Poly acrylonitrile (6% monomer methyl methacrylate) was used as source for nanofibers.
Di-methyl formamide

It is a common polar (hydrophlic) solvent for chemical reactions Di-methyl formamide (DMF) (99% purity, B. Pt. = 157°C) and obtained from CDH, India

![Chemical structure of Di-methyl formamide](image)

Polyvinyl pyrrolidone

It is also called as polyvidone or povidone, made from the monomer N-vinylpyrrolidone. Polyvinyl pyrrolidone (PVP) was purchased from Loba Chemia, India.

![Chemical structure of Polyvinyl pyrrolidone](image)

Cerium (III) nitrate hexahydrate

(Ce (NO$_3$)$_3$.6H$_2$O), (M.W; 434.23) was purchased from Chemica-biochemica reagents, India

Heavy metal for Adsorption studies

As (III) removal studies were conducted using (NIST-SRM-3103a) USA make standard reference solution.

Hg (II) adsorption studies were carried out using reference standard of SCP Science. USA after appropriate dilutions

Lead removal studies were conducted by Lead acetate trihydrate (MW 325.29) was obtained from Fischer Scientific.

All the standards were used after the appropriate dilutions.
Other chemicals and glassware

Zirconyl oxy-chloride octa-hydrate (ZrOCl$_2$.8H$_2$O) was obtained from CDH, India. Ethylene diamine tetra acetic acid (EDTA) (MW 294.24) and Ortho phosphoric acid (H$_3$PO$_4$) were purchased from Fisher Scientific, India. Other analytical grade reagents like formic acid (HCOOH), Sodium borohydride (NaBH$_4$), Hydrochloric acid (HCl), Sodium hydroxide (NaOH) were obtained from E. Merck India. All the synthesis work has been carried in double distilled water, while Atomic adsorption spectrophotometer- hydride generator (AAS-HG) experimental work has been done using de-ionized water of 18.2 mega Ω resistivity water of Millipore, USA make water purification system. All the glassware like pipette, volumetric flask, beakers used were of Borosil make India. All the dilutions and synthesis work has been carried out in a fume hood with exhaust system. All weighing work has been carried out using Shimadzu make, model- AX-200, max.= 200g, d=0.1mg specification balance.

2.2 Fabrication of Polymeric Nanofibers by electrospinning

All the polymeric nanofibers prepared and studied in this research work have been divided into three categorized.

(a) Polyvinyl/alcohol (PVA/CHT) composite nanofibers and PVA/CHT composite nanofibers modified with the help of Ce (III).

(b) PAN nanofibers and PAN nanofibers modified with Zirconia to produce PAN/Zr composite nanofibers

(c) Hydrothermally treated PAN/Zr with H$_3$PO$_4$ to produce (PAN/Zr-P) composite nanofibers.
The all polymeric composite (pure and modified) were fabricated by using electro spinning equipment (ESPIN-NANO, procured from Physics Instrument Company, Chennai) by optimizing processing parameters as discussed in earlier studies (Figure 2.1) [26-28].

Polymer solution or the melt that has to be spun is filled in a syringe and forced through a syringe pump to form a droplet at the tip of the capillary in form of pendent. High voltage potential is then applied to the polymer solution inside the syringe with the help of an immersed electrode to produce free charged ions into the polymer solution. In the presence of electric field, the pendant hemispherical polymer drop takes a cone like projection at the tip of the capillary. The electric force of attraction between the charged ions overcomes the surface tension of the liquid and liquid is pulled out from the tip of the cone tip in the form of fibers through Rayleigh instability [1]. After the cone initiation, the jet undergoes a chaotic motion or bending instability and is field directed towards the appositively charged rotating or stationary
collector [2]. The solvent evaporates during this process when the jet travels through the atmosphere, leaving behind a dried fiber on the collector. The jet breaks up into droplets low viscosity solutions, while for high viscosity solutions it travels to the collector as fiber jets [3]. Figure 2.2 shows the schematic of electrospinning setup.

![Electrospinning setup](image)

**Figure 2.2: A schematic diagram to interpret electrospinning of polymer nanofibers**

The polymer solution jet is subject to opposing forces like electrostatic, visco-elastic and surface tension. In the polymer solution to be electrospun, visco-elastic force is produced by entanglement of polymer chains that resists against the stretching of the jet by electrical force. The nature and mechanical properties of the polymer solution including its viscosity and surface tension are determining factors for privilege of one of these forces to the other one [4, 5]. For the solutions containing long-chain molecules that cannot be easily broken up into discrete droplets, Rayleigh instability creates a “pearls-on-a-string” morphology (so-called “beading”). Formation of the beaded nanofibers strongly depends on the processing variables including viscosity (i.e. concentration of the polymer solution) also surface tension [4]. Other than the polymer solutions, polymer melts can also be electrospun into nanofibers. Similarly, the polymer melt is introduced
into the capillary tube, however the whole electrospinning process is done in a vacuum condition [6].

2.2.1 Factor affecting the electrospinning process for nanofibers

Electrospinning of nanofibers is affected by several different parameters including:

(a) The properties of polymer solution such as viscosity, elasticity, conductivity and surface tension.

(b) Governing parameters such as pump feed pressure, electrical voltage and distance between the tip and the collector.

(c) Ambient parameters such as temperature, humidity and air velocity in the electrospinning chamber [4].

The electrospun nanofibers having morphological characteristics can be optimized considering the (1) consistency and controllability of the diameter of nanofiber, (2) absence of any surface defects like pores and beads, and (3) continuity of single nanofibers as much as possible [6]. The optimization of consistent, beads free and continuous nanofibers can only achieved by regulating the above mentioned parameters. The diameter of fiber is mainly controlled by solution viscosity adjustment. A higher viscosity solution results in a larger fiber diameter. Since, the solution viscosity is proportional to the polymer concentration, hence increase of the polymer concentration leads to formation of thicker nanofibers. In fact, the higher polymer concentration makes more chain entanglement and restricts chain mobility; as a result less extension of the solution jet thickens the fibers. Increase of fiber diameter with polymer concentration follows a power law relationship. Other than the importance of control over the fiber diameter and its uniformity, the formation of defects such as beads and surface pores as shown in Figures 5 should be minimized as well. In contrary to the beads which lower the surface area of the
nanofibers, the pores can enhance it and for some special applications such as catalysis and tissue engineering they are also beneficial. The formation of the beads is also dependent on the polymer concentration such that higher polymer concentrations result in fewer beads. It is also believed that when surface tension of a polymer solution decreases, beadless fibers could be obtained. This does not mean that solely increase of polymer concentration decreases surface tension. Rather than the polymer concentration, the surface tension is assumed to depend more to solvent compositions. Moreover, adding filler materials such as salt into a polymer solution increases the electric charge density on the surface of the solution jet thereby make beadless fibers. Increase of the charges density of the jet equals with increase of electrostatic repulsion force and applying a higher stretching force to the jet and subsequently formation of thinner nanofibers with smaller beads. However, increase of the electrical voltage has no effect on the number of beads or surface morphology of nanofibers i.e. formation of smoother nanofibers. Inversely, it has been shown in Figure 2.3 that with increase of the electrical potential, the electrospun nanofibers become rougher.
2.3 Synthesis of Zirconia and Zirconium-phosphate nanoparticles

Zirconia (Zr) nanoparticles were prepared using zirconyl oxy chloride and PVP, whereas Zirconium Phospahte (Zr-P) nanoparticles were prepared by treating the Zr with orthophosphoric acid using different preparation routes. The details of the experiments are given in chapter 4 and 5.

2.4 Determination of Point Zero charge (pHᵢₑₓ)

The point zero charge pHₑₓ is defined as the pH value at which the charge of sorbent surface takes a zero value [7], means at this point the charge of both positive and negative surface...
becomes equal. The knowledge of pH\textsubscript{pzc} is useful to know about the ionization of functional group and their interaction with metal species in solution. When the pH of solution is greater than pH\textsubscript{pzc} value, it indicates that sorbent surface is negatively charged and could interact with metal species in the solution. While lower pH values than pH\textsubscript{pzc} indicates that sorbent site is positively charged and could interact with negative charge species. In other words The pH\textsubscript{pzc} is a point at which the acidic or basic functional groups on sorbent surface no longer contribute to the pH value of the solution [8]. The pH\textsubscript{pzc} is of fundamental importance in surface science, it determines how easily a substrate is able to adsorb harmful ions. It also has numerous applications in technology of colloids, e.g., flotation of minerals. At pH\textsubscript{pzc}, the colloidal system exhibits zero zeta potential (i.e., the particles remain stationary in an electric field), minimum stability (i.e., exhibits maximum coagulation/flocculation rate), maximum solubility of the solid phase, maximum viscosity of the dispersion, and other peculiarities. There are several techniques for the determination of pH\textsubscript{pzc} of oxides, hydroxides, soil, and other heterogeneous solid materials.

In present research work the pH\textsubscript{pzc} of CHT/PVA and PVA/CHT/Ce composite nanofibers were determined using Potentiometric titration curve between blank and all nanofibrous materials. The pH\textsubscript{pzc} of PAN/Zr composite nanofibers were obtained using Mass titration method. The pH values in all the titrations were recorded on pH meter (Orion 3 star, pH benchtop).

2.5 Determination of Zeta potential ($\zeta$)

Zeta potential is also known as electro kinetic potential\textsuperscript{11} in colloidal dispersions and denoted using the Greek letter '$\zeta$'. It is caused by the net electrical charge contained within the region bounded by the slipping plane, and also depends on the location of that plane. The zeta potential is the important factor to describe possible behavior of the dispersion. Colloids with high zeta
potential value (negative or positive) are considered as electrically stabilized, whereas colloids with low zeta potentials tend to coagulate [9, 10]. The zeta potential for particles between minus 30 to plus 30 mV show tendency to the coagulation. The zeta potential becomes zero mV at the isoelectric point. The measurement of zeta potential also helps to explain the principles of the particle surface and its surroundings interactions. For examples; heavy metals adsorption or surfactant adsorption on carbonaceous materials. The Zeta potential is also help in many other areas e.g. oxidative catalysts, pigments, waste slurries, etc.

In the present study the zeta potential measurement for ZrO$_2$, Zr-P, PAN, PAN/Zr and Pan/Zr-P were conducted by Zetasizer Ver.7.11 (Malvern).

2.6 Thermal degradation study

2.6.1 Thermo-gravimetric analysis (TGA)

Thermo-gravimetric analysis (TGA) is a method of thermal analysis which measures the changes in physical and chemical properties of materials as a function of increasing temperature (with constant heating rate), or as a function of time (with constant temperature and/or constant mass loss) [11]. The measurement is normally carried out in air or in an inert atmosphere, such as Helium or Argon, and the weight is recorded as a function of increasing temperature. Sometimes, the measurement is performed in a lean oxygen atmosphere (1 to 5% O$_2$ in N$_2$ or He) to slow down oxidation. TGA provides the information about physical and chemical phenomena’s, such as vaporization, sublimation, absorption, adsorption, desorption, chemisorptions, dehydration, decomposition, and redox reactions [12]. In TGA change in mass of a substance is recorded as a function of temperature or time. In other words, TGA is a technique in which, upon heating a material, its weight increases or decreases. The plot of change in weight verses temperature known is as thermogram (Figure 2.4). The instrumentation of TGA mainly consist of a thermo-
balance, whose basic components are: balance, Furnace, Programmer unit for temperature measurement and control, recording unit for mass and temperature change (Figure 2.5). Balances are of two type Null type balance and Deflection balance. The linear heating is provided with the help of furnace over a wide range of operating temperature (-150°C-about 2000°C) depending on the requirement. Thermocouples regulate the temperature variations and recording unit makes use of a microprocessor which allows for digital data acquisition and processing using a personal computer.

Figure 2.4 Figure showing thermogarm for TGA

Figure 2.5: Instrumentation of TGA
TGA analysis is mainly used for characterizing thermal stability of polymers and fibers. This technique not only provides qualitative and quantitative data for kinetics of thermal degradation thereby weight losses of the samples, but also it can characterize the effect of additives and chemical modification on fibers and textile materials and the behavior of copolymer or polymer blends.

TGA of PVA/CHT nanofibers neat and loaded with Ce, PAN nanofibers neat and loaded with Zr and ZrP were carried out by instrument METTLER TOLEDO TGA/SDTA851e. TGA runs were carried out in a temperature range of 0-800°C, under air and with a constant heating rate at 10°C/minute. The sample size of 4-10 mg was weighed and the mass of the sample pan was continuously recorded as a function of temperature.

2.6.2 Differential scanning colorimeter (DSC)

Differential scanning calorimeter (DSC) monitors heat effects related to phase transitions or chemical reactions as a function of temperature. DSC records the difference in heat flow to the sample and a reference at the same temperature as a function of temperature. The reference is an inert material such as alumina, or just an empty aluminum pan. The temperature of both the sample and reference are increased at a constant rate. Or it is a technique in which the energy necessary to establish a zero temp, difference between the sample and reference material is measured as a function of temperature. The samples and the reference material are heated in such a way that their temperature are maintained the same while these temperature are increased or decreased linearly. The DSC consists of a sample holder and a reference holder, which are made up of platinum to allow high temperature operation (Figure 2.6).

Each holder is attached to resistance heater and a temperature sensor. Current is applied to both the heaters to increase the temperature at the selected rate and the difference in the power
necessary to maintain the holders at the same temperature, is used to calculate $\frac{dH}{dt}$. A flow of nitrogen gas is maintained over the samples to create a reproducible and dry atmosphere and also used to eliminate air oxidation of the samples at high temperatures. The sample (up to 10mg) sealed into a small aluminum pan is placed in sample holder while the reference is usually an empty pan and cover.

![Diagram of DSC setup](image)

**Figure 2.5: Instrumentation of DSC**

A DSC scan is showing in the figure 19. The heat capacity of the sample is calculated from the shift in the baseline at the starting transient consisting of exothermic process and endothermic process. The exothermic process includes: glass transition temperature, melting and boiling points, crystallization time and temperature, percent crystallinity, heats of fusion and reactions, specific heat capacity, oxidative/thermal stability and Purity of the samples.

Thermal behavior of all composite nanofibers were carried out by (DSC) instrument (DSC1 METLER TOLEDO from star system) up to 400°C in an air or nitrogen atmosphere at a rate of 10°C/min
2.7 Adsorption study

Adsorption is defined as the adhesion of atoms, ions, or molecules from a gas, liquid, or dissolved solid to a surface. In this process a film of the adsorbate on the surface of the adsorbent is created. This process is different from absorption, in which a fluid permeates/dissolve by a liquid or solid. Adsorption is a surface phenomena, while absorption involves the whole volume of the material. The term sorption encompasses both processes, while desorption is the reverse of it. Adsorption is a mass transfer operation in that a constituent in the liquid phase is transferred to the solid phase. In the process of adsorption, the substance to be removed from the liquid phase at the interface is considered as adsorbate. And, the solid, liquid or gas phase onto which the adsorbate accumulates is called as adsorbent. The term adsorption is also used to describe two kinds of forces of interaction between the adsorbate and the adsorbent, like physiosorption (physical adsorption) and chemisorptions (chemical adsorption) [13]. Physical adsorption (physiosorption) occurs due to the operation of weak forces between molecules which are not affixed to a particular site on the solid surface; it is free to move over the surface [14]. The physical interactions among molecules, based on electrostatic forces, include dipole-dipole interactions, dispersion interactions and hydrogen bonding. Chemical adsorption (chemisorptions) is also based on electrostatic forces, but much stronger forces act a major role on this process. In chemisorptions attraction between adsorbent and adsorbate is a covalent or electrostatic chemical bond between atoms, with shorter bond length and higher bond energy [15]. The enthalpy of chemisorptions is (200 kJ/mol) higher than that for physiosorption (20 kJ/mol). Chemisorptions is exothermic in nature in special cases.
In present study batch adsorption studies of heavy metals using PVA/CHT/Ce, PAN/Zr and PAN/Zr-P were carried out by suspending known weight of adsorbents with different concentration of heavy metals solution in 100 ml beaker. The content was stir for definite time to achieve equilibrium on magnetic stirrer at lowest speed (50-100 rpm). The stock solutions were diluted to desirable concentrations for studies and pH was adjusted with the help of dilute Hydrochloric acid or Ammonium hydroxide solutions. After removing the adsorbent from the solution, the analyte concentration in the remnant was determined by using Hydride Generator Atomic Absorption Spectrophotometer (AAS-HG model- Vario-6-Analytik Jena, Germany)[16, 17]. The adsorption capacity ($q_e$) and efficiency were calculated by using following equations:

$$
\text{Adsorption capacity (} q_e \text{)} = \frac{(C_i - C_f) \times V}{m} \quad \text{------- (i)}
$$

$$
\% \text{ Removal} = \frac{(C_i - C_f) \times 100}{C_i} \quad \text{------- (ii)}
$$

**Figure 2.6: Figure showing sorption phenomena**

[Diagram showing gas-liquid and liquid-solid interfaces with labels for gas film, liquid film, gas bulk, liquid bulk, and solid bulk.]
Where, ‘$C_i$’ and ‘$C_f$’ (mg/L) are the initial and final concentration of metal solution, respectively; $V$ is the volume (L) of the solution and ‘$m$’ is the weight (g) of composite nanofibers.

A series of adsorption experiment were conducted by varying conditions like pH, adsorbent dose, Ce content in PVA/CHT/Ce composite nanofibers, Zr and Zr-P content in PAN matrix for adsorption of heavy metals. Kinetics and equilibrium studies were conducted for Langmuir and Freundlich isotherms at optimized conditions with varying concentrations of metal ion solutions. Recyclability and reuse of PVA/CHT/Ce, PAN/Zr and PAN/Zr-P composite nanofibers used in adsorption process was ascertained [18]. Various cycles of regeneration were carried out by treating metal loaded adsorbents with 0.01 M HCl.

2.7.1 Adsorption Isotherms

Adsorption isotherms are useful tool to understand the adsorption process like the equilibrium relationship between the solid and liquid interface. When adsorption equilibrium data are plotted with the mass adsorbed on the y-axis and the mass in the fluid on the x-axis at constant temperature results in adsorption isotherm. Adsorption isotherms are considered as mathematical models, which are used to describe the distribution of the adsorbate specie among liquid and solid phases. The isotherm models are based on a set of assumptions that are related to the heterogeneity/homogeneity of the solid surface, the type of coverage, and the possibility of interaction between the adsorbate species. Adsorption isotherms can be expressed in the form of Langmuir, Freundlich, Temkin and Dubinin-Radushkevich [19]. Langmuir and Freundlich isotherms are most commonly used adsorption isotherm models and are used in this research study. Langmuir isotherm which describes the adsorption of adsorbate onto the surface of the adsorbent is based on following assumptions: The adsorbent’s surface is in contact with a solution having adsorbate attracted to the surface. Adsorbent surface has a specific number of
active sites to bind solute’s molecules. Only monolayer adsorption occurs on the surface of adsorbent. Whereas Freundlich isotherm commonly describes the adsorption characteristics for the heterogeneous surface or shows multilayer’s adsorption of adsorbate onto the surface of adsorbent [20].

2.7.1.1 Langmuir Isotherm

Langmuir isotherm explains about the formation of a monolayer adsorbate on the outer surface of the adsorbent, and after that no further adsorption takes place. Thereby, the Langmuir represents the equilibrium distribution of metal ions between the solid and liquid phase. The Langmuir isotherm is valid for monolayer adsorption onto a surface containing a finite number of identical sites. The Langmuir model assumes that the uptake of metal ions occurs on a homogenous surface by monolayer adsorption without any interaction between adsorbed ions (Figure 2.7).

![Langmuir isotherm](image)

**Figure 2.7: Langmuir isotherm**

The Langmuir adsorption isotherm was developed by assuming that:

(a) A fixed number of active sites are available on the adsorbent surface having the same energy.

(b) Adsorption process is reversible.

(c) Monolayer adsorption occurs or on surface only.
(d) There are no lateral interactions between molecules of adsorbate. The Langmuir adsorption isotherm is expressed as:

\[
\frac{C_f}{q_e} = \frac{1}{K_L q_m} + \frac{C_f}{q_m} \quad \text{----- (iii)}
\]

Where, ‘q_m’ and ‘q_e’ in mg/g is the maximum adsorption capacity and adsorption capacity at equilibrium, respectively; ‘C_f’ in mg/L is the final concentration of solution (adsorbate) after adsorption; and K_L in L/mg is the energy of reaction. The linear plot of ‘C_f/q_e’ vs. ‘C_f’ obeys Langmuir adsorption model. The value of ‘q_m’ and ‘K_L’ can be determined by slope and intercept of a linear plot of ‘C_f/q_e’ against ‘C_f’.

Langmuir isotherm can also be expressed by a dimensionless constant which is known as the equilibrium parameter or separation factor ‘R_L’. Separation factor is very useful in predicting feasibility and shape of the isotherm of the adsorption process by equation:

\[
R_L = \frac{1}{1 + (K_L C_i)} \quad \text{----- (iv)}
\]

Where: ‘K_L’ is related to energy of adsorption called as Langmuir Constant. On the basis of ‘R_L’, nature of adsorption can be predicted as unfavorable (>1), linear (=1), favorable (>0 or <1) or irreversible (=0). The Langmuir isotherm is limited in its application to adsorption in monolayer. It applies well to chemical adsorption and to physical adsorption when saturation is approached.

2.7.1.2 Freundlich Isotherm

This is commonly used to describe the adsorption characteristics for the heterogeneous surface. It is based on the assumption that adsorption occurs at the heterogeneous surfaces and any foreign material or pollutant can be allowed to adsorb in multilayer of an adsorbent [21]. A brief
empirical equation often used to represent adsorption data is called the Freundlich equation. The empirically derived Freundlich isotherm is defined as follows:

\[
\log q_e = \log K_f + \frac{1}{n} \log C_f
\] -----(v)

The plot of ‘log q_e’ Vs ‘log C_e’ represents the applicability of Freundlich isotherm (Figure 2.8).

**Figure 2.8: Freundlich isotherm**

where; \( q_e \) : Amount adsorbed per unit weight of adsorbent at equilibrium (mg/g), (mol/g) 
\( C_f \): Equilibrium concentration of adsorbate in solution after adsorption (mg/g), (mol/L) 
\( K_f \): Empirical Freundlich constant or capacity factor (mg/g), (mol/g), \( 1/n \): Freundlich exponent. The exponent \( n \) is an index of the diversity of free energies associated with the adsorption of the solute by multiple components of a heterogeneous adsorbent. When \( n=1 \), the isotherm is considered as linear and system has a constant free energy at all adsorbate concentrations. When \( n < 1 \), the isotherm is considered as concave and adsorbate are bound with weaker and weaker free energies, finally, when \( n > 1 \), the isotherm is convex and presence of adsorbate in the adsorbent increase the free energies for adsorption [22]. The good fit of Freundlich isotherm to an adsorption system means there is almost no limit to the amount adsorbed and there is a multilayer adsorption.
2.7.2 Kinetic study

The rate at which adsorption takes place is called as adsorption kinetics, and rate is considered as the change of a given quantity over specific period of time. Since after a particular time period the sorption is reversible when enough adsorbate had been adsorbed and desorption had become more active. At this point, the concentration of the adsorbate depends on the rate differences between the sorption and desorption reactions. But by neglecting the desorption reaction, reaction rate becomes dependant only on the concentrations of the adsorbate. Change in concentration of a reactant or product per unit time is called as reaction rate. The concentrations of the products do not appear in the rate law because the reaction rate is being studied under the conditions where the reverse reactions do not contribute to the overall rate. There are various kinetic models, like pseudo-first-order (equation vi), pseudo-second order (equation vii) to understand the adsorption kinetics [23-26].

\[
\ln(q_e - q_t) = \ln q_e - k_1 t \quad \text{------ (vi)}
\]

\[
\frac{t}{q_t} = \frac{1}{k_2 q_c^2} + \frac{t}{q_c} \quad \text{------ (vii)}
\]

If time ‘t’ is zero then ‘h’ becomes:

\[
h = k_2 q_c^2 \quad \text{------(viii)}
\]

Where ‘q_e’ and ‘q_t’ (mg/g) represents the adsorption capacities at equilibrium and at time ‘t’, respectively; ‘h’ (mg/g*min) is the initial adsorption rate and ‘k’ (g/mg *min) is the rate constant.
According to previous studies it is found that pseudo second order model in liberalized (type 1) form gives better understanding than in its other form [27, 28].

### 2.7.3 Atomic adsorption spectrophotometer

Atomic absorption spectroscopy (AAS) is one of the popular instrumental methods for analyzing metals and some metalloids. But because of interferences, poor reproducibility, and poor detection limits hydride generation HG-AAS is an alternative method for some elements--mostly metalloids. In HGAAS, metalloids like antimony, arsenic, selenium, and tellurium are now routinely analyzed by [29]. AAS is a spectro analytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state. In analytical chemistry the technique is used for determining the concentration of a particular element (the analyte) in a sample to be analyzed. HGAAS consists: a hollow cathode lamp, air/acetylene flame, and optical system but include (in most systems) an optical cell and the relatively complex hydride generation system with continuous flow system.
Many oxyanions of metalloids react with sodium borohydride and HCl to produce a volatile hydride: $\text{H}_2\text{Te}$, $\text{H}_2\text{Se}$, $\text{H}_3\text{As}$, $\text{H}_3\text{Sb}$, etc. In case of AAS, the oxidation state of the metalloid is crucial and care must be taken to produce the specific metalloid oxidation state before the sample is introduced into the hydride generation system. The time from reagent mixing and when the volatile hydride is separated from the liquid and sent to the optical cell is also important. The timing of that process is controlled by flowing reagents together using a peristaltic pump and tubing of specific lengths. After being mixed together the liquid mixture flows through a tube of a specific length (read this as a controlled reaction time) and is ultimately flowed into a gas/liquid separator where the hydride and some gaseous hydrogen (produced by the $\text{NaBH}_4 + \text{H}_2$ reaction) bubble out and are purged (via a high purity inert gas) into the optical cell via a gas transfer line. Most of the reagents introduced into the system flow to a waste container, and since the acid content is very high, often approaching 50%, as with AAS, the waste container is glass and must be handled carefully and labeled well. Atomic absorption spectrometry has many applications like:

1. Clinical analysis: Analyzing metals in biological fluids and tissues such as whole blood, plasma, urine, saliva, brain tissue, liver, muscle tissue, semen.
2. Pharmaceuticals: In some pharmaceutical manufacturing processes, minute quantities of a catalyst that remain in the final drug product.
3. Water analysis: Analyzing water for its metal content. Figure 2.9 represents the picture of AAS.

In present work the HGAAS has been used to determine the heavy metal concentrations without any interference of other metal using different composite nanofibers like PVA/CHT/Ce, PAN/Zr and PAN/Zr-P in batch study with (3% sodium borohydride, 1.5% sodium hydroxide and 2% hydrochloric acid in presence of argon gas having 36 ml/min flow rate).
2.7.4 UV spectrophotometer

Generally UV/Vis spectrophotometer measures the intensity of light passing through a sample (I), and compares it to the intensity of light before it passes through the sample (I₀). The ratio (I/I₀) is called as transmittance and can be (%T). The absorbance, (A), related to the transmittance by following equation:

\[ A = - \log (\%T/100\%) \]

A spectrophotometer may be of single beam or double beam. It mainly consists of a light source, sample holder, a monochromator or a prism to separate the different wavelengths of light, and a detector (Figure 2.10). The radiation source in UV may be of Tungsten filament (300-2500nm), a deuterium arc lamp, which is continuous over the ultraviolet region (190-400nm), Xenon arc lamp, which is continuous from 160-2,000nm or light emitting diodes (LED) for the visible wavelengths. The different types of detector used UV spectrophotometer are photomultiplier tube, photodiode, a photodiode array or a charge-coupled device (CCD). In scanning monochromator, single photodiode detectors and photomultiplier tubes are used, which filter the light so that only light of a single wavelength reaches the detector at one time. The scanning
monochromator moves the diffraction grating to "step-through" each wavelength to measure the intensity as a function of wavelength. CCDs and photodiode arrays are used with fixed monochromator. It is very useful in analytical chemistry for the quantitative determination of transition metal ions, highly conjugated organic compounds, and biological macromolecules. The sample are analysed in solution form but solids and gases may also be studied.

![Instrumentation of spectrophotometer](image)

**Figure 2.10: Instrumentation of spectrophotometer**

For Lead removal studies for PAN/Zr-P composite nanofibers all the samples were analyzed using UV-Vis Double beam Spectrophotometer (Shimadzu UV-1800).

### 2.8 Characterization techniques

Optical microscope and Scanning electron microscopy (SEM) were used to see the morphology of composite nanofibers fabricated from electrospinning technique. FTIR was used to study the functional groups present in composite nanofibers and their interactions. TGA and DSC were used to see the thermal behavior of composite nanofibers. XRD was used to see the XRD patterns of composite nanofibers. XPS technique was used to characterize the available
functional group present on composite nanofibers and their interaction after adsorption of metal ions.

2.8.1 Morphology of nanofibers

2.8.1.1 Optical microscope

The optical microscopic examination of the nanofibers was done on a Leitz Mettaloplane Optical Microscope to initially examine the microstructure of nanofibers. The optical microscope (light microscope) is a type of microscope which uses visible light and a system of lenses to magnify images of small samples. Optical microscopes are the oldest design of microscope and were possibly invented in their present compound form in the 17th century. Basic optical microscopes can be very simple, although there are many complex designs which aim to improve resolution and sample contrast. Optical microscope captures the image by normal light-sensitive cameras to generate a micrograph. In past time images were captured by photographic film but now digital images are taken by using modern Complementary metal-oxide semiconductor (CMOS) and charge-coupled device (CCD) cameras. Digital microscope uses a CCD camera to examine a sample showing the image directly on a computer screen. Conventional optical microscopes are having two basic configurations: the simple and the compound microscope. The majority of modern research is focused on using compound microscopes while some cheaper commercial digital microscopes are simple single lens microscopes. Generally, microscope optics are static in nature, distance to sample and lens can be adjusted and objective lens are used to get a wider or narrower field of view a different magnification or a separate set of optics can be used for illuminating the sample. Microscope objectives are characterized by two parameters, namely, magnification and numerical aperture. The former typically ranges from 5× to 100× while the latter ranges from 0.14 to 0.7, corresponding to focal lengths of about 40 to 2 mm, respectively.
Objective lenses with higher magnifications normally have a higher numerical aperture and a shorter depth of field in the resulting image. Some high performance objective lenses may require matched eyepieces to deliver the best optical performance.

![Optical microscope](image)

**Figure 2.11: Optical microscope**

In the present research investigation a small piece of nanofibers was cut into square shape and mounted on the specimen for microscopic examination. The specimen was then observed for its microstructure under reflected polarized light and a camera attached to the microscope produce photograph of the microstructure in the form of micrograph.

**2.8.1.2 Scanning electron microscope**

Scanning electron microscope is a powerful and useful instrument for imaging topographies of specimens at very high magnifications. It uses electrons instead of light to form images. In comparison to traditional optical microscopes, the scanning electron microscope possesses has many advantages like larger depth of field (up to 100 times), permitting large area of a specimen to be in focus at one time. Additionally, SEM allows the specimens which are very close to each other to be detected and magnified at much higher levels (>100,000x) due to its high resolution.
power. SEM uses electromagnets rather than lenses so that it facilitates a much more control in the degree of magnification. It provides clear images of specimen or sample, giving rise to a high efficiency for various research areas. SEM produces images of sample by scanning it with a focused beam of electrons which interact with the atoms present in sample, producing various signals that can be detected and that contain information about the surface’s topography and composition of sample [30]. It provides a scan image at high spatial resolution, in a low acceleration voltage area. It is digitalized with computer controlled programmed and capable of showing images with a resolution of 3 nm at 40 kV using a LaB₆ filament. So the images are automatically analyzed. Line averaging and frame averaging can also be applied to the image in addition to increasing the dwell time in order to reduce the noise without damaging the specimen. 

During inspection, a very small amount of the specimen is bombarded by an electron beam produced at the top of the microscope by an electron gun which is a thermal emission source, such as a heated tungsten filament or a field emission cathode. The incident electron’s energy varies from as low as 100 eV to as high as 30 keV depending on the purpose of inspection. As shown in Figure 2.12, the electron beam travels vertically (top to bottom) through the microscope, under vacuum. The electromagnetic fields and lenses present on the SEM column focus the beam down toward the sample. Finally, the scanning coils located near to the end of the column are responsible for positioning and directing the focused electron beam onto the sample surface. The bombarding electrons (primary electron) force out the electrons of the specimen due to elastic and inelastic scattering events occurring at the surface and near-surface of the sample. The high-energy electrons dislodged by an elastic collision of an incident electron with a sample atom’s nucleus are called as backscattered electrons. Backscattered electrons are as energetic as
the incident electrons and their number are based on the specimen’s atomic numbers. So backscattered electron imaging is useful indistinguishing one material from another with atomic number difference of at least. The secondary electrons are low energy (50 eV or less) electrons emitted by the inelastic scattering of incident electrons. A detector/positively biased grid collects secondary electrons and translates them into a signal. Sweeping the electron beam across the area to be analyzed results in such signals, are amplified, analyzed, and translated into images of the evaluated topography.

![Figure 2.12: Working of SEM](image)

In the present research work the morphology of the PVA/CHT and PAN electrospun nanofibers as such and as modified with inorganic materials, were examined using SEM (Model EVO M-1; Ziess) after preparing the samples with a gold coating. The diameter of the electrospun nanofibers and approximate particle size of the inorganic nanoparticles were determined from the
SEM images using the Adobe Acrobat v.07 software. Additionally the elemental composition of nanofibers before and after adsorption process was determined using EDAX attached with SEM.

2.8.2 Fourier transforms infrared spectroscopy (FTIR)

FTIR is a technique in which infrared spectrum of solid, liquid or gas is taken which involves absorption, emission, photoconductivity or Raman scattering [31]. In FT-IR, IR radiation is passed through a sample, which is absorbed or transmitted by the sample. The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint, no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis. It is used to recognize bond types, structures and functional groups in organic and inorganic compounds for both qualitative and quantitative analysis, to identify molecules and compounds, and to determine the presence or absence of certain types of bonds and functional groups. IR sensitive vibrations are associated with those molecule having changes in their dipole moments. IR spectroscopy measures vibrational energy levels in molecules, for example, double and single bonds associated with carbon-hydrogen and carbon-oxygen bonding (–C-H, –C-H, C-O and C=O) can be distinguished in IR absorption. When functional groups can be bonded at different locations on molecules, IR spectroscopy can frequently identify the positions at which the functional groups are attached. An FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range of molecules. The term Fourier transform infrared spectroscopy originates from the fact that a Fourier transform (a mathematical process) is required to convert the raw data into the actual spectrum. The first low-cost spectrophotometer (Perkin-Elmer Infracord) was produced in 1957 for recording an infrared spectrum [32]. This
instrument covered the wavelength range from 2.5 μm to 15 μm (wavenumber ranges from 4000 cm⁻¹ to 660 cm⁻¹).

Attenuated Total Reflectance (ATR)-FTIR is now a day’s became fast and most widely used sampling tool that allows qualitative or quantitative analysis of samples with little or no sample preparation. The main advantage of ATR sampling comes from the very thin sampling path length and depth of penetration of the IR beam into the sample. This is in contrast to traditional FTIR sampling by transmission where the sample must be diluted with IR transparent salt, pressed into a pellet or pressed to a thin film, prior to analysis to prevent totally absorbing bands in the infrared spectrum. Characterization of either thick or highly absorbing materials which are not easily analyzed by transmission spectroscopy is also possible by this technique. So simply placing the bulk sample or thick film on the ATR crystal (Diamond MIRacle) and applying pressure generates a nearly perfect result (upper red spectrum) and identified taking the time less than 1 minute.

In ATR sampling IR beam is directed into a crystal of relatively higher refractive index. The IR beam reflects from the internal surface of the crystal and creates an evanescent wave, which projects orthogonally into the sample in intimate contact with the ATR crystal. Some of the energy of the evanescent wave is absorbed by the sample and the reflected radiation (some now absorbed by the sample) is returned to the detector (Figure 2.13).
Chemical surface analysis of all the electrospun composite nanofibers prepared (pure as well as modified with inorganic materials) was performed by (FTIR). Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectra were recorded using a Model Nexus-47; Nicolet.

2.8.3 X-Ray diffraction (XRD)

The X-ray diffraction is phenomenon in which the atomic planes of a crystal cause an incident beam of X-rays to interfere with one another as they leave the crystal. X-Ray diffraction analysis (XRD) is analytical technique which is used for the phase characterization of crystalline materials [33]. X-Ray diffraction is technique useful in measuring the average spacing between layers or rows of atoms, to determine the orientation of a single crystal or grain, to find the crystal structure of an unknown material, to measure the size, shape and internal stress of small crystalline regions [34].

Instrumentation of XRD consists of interference of monochromatic X-rays and a crystalline sample. Monochromatic X-rays further includes following sequence: X-Ray production by a cathode ray tube, filtering to get monochromatic radiation, collimation to concentrate and finally direction toward the specimen. Satisfying the condition of Bragg’s Law, X-ray waves interact with the sample giving rise to a constructive interference and a diffracted ray (Figure 2.14) [35].
\[ n\lambda = 2d \sin \theta \] ------ (x)

where \( n \) is the order of reflection \((n = 1, 2, 3, \ldots)\), \( \lambda \) the wavelength, \( d \) the distance between parallel lattice planes, and \( \theta \) the angle of incident beam and a lattice plane (Bragg angle).

\[ \text{Figure 2.14: Bragg law} \]

Constructive interference occurs and diffracted intensity is obtained as the path length in the crystal \((2d\sin \theta)\) is a multiple of the wavelength. Generally \( d \)-spacing is a function of the lattice parameters \((a, b, c)\) and angles defining the unit cell, and the Miller indices \((h, k, l)\) denoting a particular reflection. As such, it is the geometry of the crystal lattice that determines the position of the peaks in an X-ray diffraction pattern. In general, the more symmetrical the material, the fewer peaks in its diffraction pattern occurs. The radiation used in a typical diffraction measurement contains several wavelengths, denoted \( K_1, K_2, K \), which are characteristic of the material producing the X rays Figure 2.15 [36].

In present research work the X-ray diffraction analysis of all composite nanofibers prepared, bare as well modified with inorganic material was done using XRD (Expert D6 model, Japan (1-) 1.5404°A).
XPS analysis was initially conducted for adsorbents prior to adsorption in order to characterize the available functional groups. X-ray photoelectron spectroscopy (XPS) also known as ESCA (Electron Spectroscopy for Chemical Analysis) is a surface-sensitive quantitative technique which is employed to measure the elemental composition at the parts per thousand range, empirical formula, chemical state and electronic state of the elements (exist within a material) \[37\].

XPS spectra are obtained by irradiation of a material with a beam of X-rays under high vacuum \((P \sim 10^{-8}\text{ millibar})\) or ultra-high vacuum \((UHV; P < 10^{-9}\text{ millibar})\) conditions and analysing the energy of the detected electrons. Usually, MgKα \((hv=1253.6\text{ eV})\), AlKα\((hv=1486.6\text{ eV})\), or
monochromatic AlKα (hv=1486.6 eV) x-rays are used. These photons have limited penetrating power in a solid on the order of 1-10 micrometers. They interact with atoms in the surface region, causing electrons to be emitted by the photoelectric effect. The energy of the emitted electrons is given by:

\[ KE = h\nu - BE - Fs \]

where \( h\nu \) is the energy of the photon, \( BE \) the binding energy of the atomic orbital from which the electron originates and \( Fs \) the spectrometer work function. In addition to the emission of photoelectrons, Auger electrons may be emitted because of relaxation of the excited ions after photoemission. Simultaneously kinetic energy and number of electrons that escape from the top 0 to 10nm of the material to analyze is determined. While current area of development is ambient-pressure XPS, in which samples are analyzed at pressures of a few tens of millibar. XPS can be directly used to analyze the surface chemistry of a material in its as-received state, or after some treatment, for example: fracturing, cutting or scraping in air. It is also known as), an abbreviation introduced by Kai Siegbahn’s research group to emphasize the chemical (rather than merely elemental) information that the technique provides. It can detect all the elements with anatomic number \( (Z=3) \) lithium and above except hydrogen \((Z=1)\) or helium \((Z=2)\) using typical laboratory-scale X-ray sources. Sample of inorganic compounds, metalalloys, semiconductors, polymers, elements, catalysts, glasses, ceramics, paints, papers, inks, woods, plant parts, make-up, teeth, bones, medical implants, bio-materials, viscous oils, glues, ion-modified materials and many others, are usually analyzed by XPS.
In XPS spectrum number of electrons detected plotted against the binding energy of the electrons detected. Since each element show a characteristic set of XPS peaks correspond to the electron configuration of the electrons (within the atoms, e.g., 1s, 2s, 2p, 3s, etc) at characteristic binding energy values that directly identify each element (Figure 2.16 and 2.17).

So the number of detected electrons representing the characteristic peaks is directly related to the amount of element within the XPS sampling volume. To generate atomic percentage values, each raw XPS signal must be corrected by dividing its signal intensity (number of electrons detected) by a "relative sensitivity factor" (RSF), and normalized over all of the elements detected. Since hydrogen is not detected, these atomic percentages exclude hydrogen.
Figure 2.17: Schematic diagram for the XPS emission process

(XPS) spectra of all the nanofibers samples were recorded on MULTILAB 2000 (Thermo Scientific, USA) using an X-ray (Mg Kα radiation source) with a binding energy scan range of 0-1200 eV and the work function of the spectrometer was 4.1 ± 0.1 eV. The collected high resolution XPS spectra were analyzed using an XPS peak fitting software program.

2.9 Antimicrobial study

Antimicrobial study was carried out with gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *E. coli*. Microbial solutions were prepared from the stock culture in 300 mL nutrient broth having composition peptone (5g/L), NaCl (5g/L), beef extract (1.5g/L), yeast extract (1.5g/L) in separate test tubes. The test tubes were plugged with cotton, wrapped with aluminum foil and incubated for 24 h at 37°C. Each microbial solution was spread over the solidified agar plates along with fixed amount of nanofibers. Plates were then sealed with parafilm and incubated again at 37°C for 24 hours. After that zone created surrounding the nanofibers were analyzed to see the microbial activity against PVA/CHT/Ce composite nanofibers.
2.10 Reference


