CHAPTER 3
MATERIALS AND METHODS

3.1 CHEMICALS

Chitosan (Extrapure, SRL India), Poly vinylalcohol (Sigma Aldrich), Poly acrylonitrile (Sigma Aldrich), Zinc sulphate, Lead acetate (Merck Chemicals, India), Eosin Yellow (Merck chemicals, India), Rose Bengal (Merck chemicals, India), Hydrochloric acid, Tri sodium polyphosphate, Glutaraldehyde, Acetic acid, Dimethyl sulfoxide, Sodium hydroxide, Nitric acid and Ceric Ammonium nitrate. The deionized water was used throughout the experimental studies. All the chemicals used were of analytical grade and of highest purity.

3.2 INSTRUMENTAL CHARACTERIZATION OF ADSORBENTS

The TEM analysis of prepared chitosan nanoparticles was performed using Computer aided Transmission electron microscope of Philips make. The morphological surface of the chitosan blended PVA and chitosan blended PAN before and after adsorption of Zn(II) and Pb(II) ions, chitosan blended PVA before and after adsorption of Eosin Yellow and Rose Bengal dye were analysed using a Quanta FEG SEM with an accelerating voltage of 10 kV.

X Ray Diffraction analysis was carried out for chitosan blended PVA and chitosan blended PAN before and after adsorption of zinc (II) and Pb (II) ions, chitosan blended PVA before and after adsorption of Eosin
Yellow/Rose Bengal dye. The different functional groups attached with the adsorbents have been identified through FTIR spectra in the range of 4000 – 400 cm\(^{-1}\) in a FTIR spectrometer (Perkin Elmer spectrum, 2000) under dry air at room temperature.

Atomic absorption spectroscopy (AAS) is a simple technique used to determine trace (g/mL) levels of metals in the given samples, with good accuracy and acceptable precision and the instrument is AAS, SL 176, Elico Model, India. Atomic Absorption Spectrophotometer (AAS) was operated at specific wavelength 217 nm, current 05.5 mA, EHT 600 V, air/acetylene flame type for Lead samples and at wavelength 214.4 nm, current 07.5 mA, EHT 555 V and air/acetylene type of flame for Zinc samples.

3.3 **PREPARATION OF ADSORBENTS**

3.3.1 **Preparation of Chitosan Nanoparticles**

Chitosan nanoparticles were prepared in accordance with ionotropic gelation process (Koukaras et al 2012). Nanoparticles were formed instantaneously upon the drop wise addition of 0.2% TPP solution to chitosan solution in the ratio of 5:2. An opaline suspension was formed spontaneously upon stirring at room temperature for an hour. Finally, the chitosan nanoparticles were separated by centrifugation at a maximum speed of 10,000 rpm and temperature at 4°C for 1 hour and supernatant was discarded. The origination of nanoparticles was a result of the interaction between the negative groups of Trisodium Poly Phosphate and the positively charged amino groups of chitosan. The samples were dried at room temperature and then examined using Transmission Electron Microscope (TEM).
3.3.2 Chitosan Blended Poly Vinyl Alcohol (PVA)

Chitosan compatibly forms a uniform, firm and homogenous membrane with Polyvinyl alcohol. Chitosan/Polyvinylalcohol blend was prepared by mixing solution of prepared chitosan nanoparticles with Polyvinyl alcohol solution. These two polymers were blended in the ratio of 1:3 and 20 ml of 5% of glutaraldehyde was added as a cross linking agent. The solutions were stirred using magnetic stirrer for 2-3 hours and kept for 24 h for the formation of homogenous blend at room temperature. Then the membrane was dried at 40°C for about 24 h and crushed to the required size.

3.3.3 Chitosan Blended Poly Acrylo Nitrile (PAN)

The prepared chitosan nanoparticles of 2 grams were dissolved in 100 mL of acetic acid. To this solution, 10 ml of 0.1 M ceric ammonium nitrate was added, followed by the addition of 1% Polyacrylonitrile solution (prepared in dimethyl sulfoxide) drop by drop under gentle stirring maintained at 70°C. Thus, chitosan blended polyacrylonitrile formed was precipitated under alkaline conditions and washed with double distilled water and dried at 45°C (Acharyulu 2013). Thus the copolymer formed was used as an adsorbent in the powder form for the adsorption of Zn(II) and Pb(II) ions.

3.4 PREPARATION OF SYNTHETIC SOLUTIONS

**Stock solution:** 4.45g of zinc sulphate was dissolved in 1000ml of double distilled water, which gives 1000mg/L concentration of zinc ions. Zinc standards of 10, 20, 30, 40 and 50 ppm were prepared from stock solution using double distilled water.

**Stock solution:** 1.83g of lead acetate was dissolved in 1000ml of double distilled water which gives 1000mg/L concentration of lead ions. Lead
standards were prepared from stock solution by diluting to different concentration using double distilled water. Lead ion solution was prepared for 10, 20, 30, 40 and 50 mg/L. The pH of the working solution was adjusted to 5.0 by using 0.1 M HCl or 0.1M NaOH.

**Stock solution:** 1000mg/L of Eosin Yellow dye solution was prepared as stock solution. Eosin Yellow standards were prepared from stock solution by diluting to different concentrations using double distilled water and prepared for 10, 20, 30, 40 and 50 mg/L.

**Stock solution:** 1000mg/L of Rose Bengal dye solution was prepared as stock solution. Rose Bengal standards were prepared from stock solution by diluting to different concentration using double distilled water and were prepared from 10, 20, 30, 40 and 50 mg/L.

### 3.5 BATCH ADSORPTION EXPERIMENTS

The adsorption capacity of the chitosan blended PVA and chitosan blended PAN was optimized by varying physical parameters and the readings were obtained using Atomic Absorption Spectrophotometer (AAS).

Batch adsorption experiments were carried out by varying the operating physical parameters such as solution pH, adsorbent dose, contact time, temperature and initial metal ion concentration and the results were based on the removal of metal ions and dye from the aqueous solution. In each batch experimental study accurately weighed quantity of adsorbent was added to 100 mL of aqueous solution taken in a 250 mL conical flasks and the system was agitated at 160 rpm in a rotary shaker. The analyses of samples were done after filtering it using Whatman 42 filter paper. The concentration of Zn(II)/Pb(II) ions/dye in the filtrates were analyzed utilizing Atomic absorption spectrometer. Each experiment was repeated thrice and the results
obtained were their average values. The obtained data from these studies were used to calculate the percentage removal of metal ions by using the following mass balance relationship:

\[
\% R = \frac{C_o - C_e}{C_o} \times 100
\]  

(3.1)

where \(C_o\) and \(C_e\) are the initial and equilibrium concentration (mg/L) of Zn(II)/Pb (II) ion concentrations, respectively (Kumar et al 2014).

### 3.5.1 Effect of Adsorbent Dose

Lead (II)/Zinc (II) ions of 10 mg/L concentration in five different conical flasks were taken, to which adsorbent dose (chitosan blended PVA) ranging from 1 to 10 g/1000 mL was added to each of the aqueous ion solution. The pH of the solution was adjusted to 5 using 0.1 M HCl or 0.1 M NaOH. After which, the solutions were kept in the rotary shaker for 60 minutes about 160 rpm. The temperature of the solution was maintained at about 30° C. Then the supernatant was collected by filtering the solution and the samples were analyzed using Atomic Absorption Spectrophotometer. A graph was plotted against percentage removal of adsorbate versus various amount of adsorbent dose.

Zn (II)/Pb (II) solution was taken of 10mg/L concentration in five different conical flasks. The chitosan blended PAN (adsorbent) of 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4g/1000 mL was added to each of Zn(II)/Pb(II) working solutions. The pH of the solution was adjusted to 5 using 0.1M HCl or 0.1 M NaOH. The solutions were kept in the rotary shaker for 60 minutes about 160 rpm, 30° C. Then the supernatant was collected by filtering the solutions. Then the samples were analyzed using Atomic Absorption Spectrophotometer. Then the graph was plotted against percentage removal of adsorbate versus various amount of adsorbent dose.
Eosin Yellow/Rose Bengal dye of working concentration was taken at 10mg/L in five different conical flasks. The chitosan blended PVA of 1, 2, 3, 4, and 5g/1000 mL was added to each EY/RB working solutions. The pH of the solution was adjusted to 6 for EY dye and pH at 7 for RB dye. The solutions were kept in the rotary shaker for 60 minutes about 160 rpm, 30°C. Then the supernatant was collected by filtering the solutions and the samples were analyzed at 520 nm (EY dye) and 540 nm (RB dye) wavelength. A graph was plotted against percentage removal of adsorbate versus various amount of adsorbent dose.

### 3.5.2 Effect of Initial Concentration

Different concentrations of zinc (II)/lead (II) ion solution were taken from 10, 20, 30, 40, and 50 mg/L. To each zinc (II) ion solution of 100 mL, 0.5g of chitosan/PVA was added and 0.6 g of chitosan/PVA was added to each lead (II) ion solution. The pH of the solution was adjusted to 5 using 0.1M HCl or 0.1M NaOH. These solutions were kept in a rotary shaker for 60 minutes at about 160 rpm and the temperature of the solution at 30°C. After one hour agitation, the supernatant was collected by filtering the solution and the residual solution samples were analyzed using Atomic Absorption Spectrophotometer (AAS). A graph was plotted against percentage removal of adsorbate versus various concentrations of adsorbate dose. Similarly the same procedure was repeated with 0.2 g of chitosan blended PAN in all the different working concentrations of Zn(II) and Pb(II) ion concentrations.

Eosin Yellow/Rose Bengal dye of varying working concentrations were taken as 10, 20, 30, 40, and 50mg/L. To each Eosin Yellow/Rose Bengal solution, 0.2g /100mL of chitosan blended PVA was added. The pH of EY dye solution was adjusted to 6 using 0.1M HCl or 0.1M NaOH and pH of RB dye maintained at 7. These solutions were kept for agitation for 60 minutes,
160 rpm and 30° C. After which, the supernatant of the EY dye samples were analyzed at 520 nm wavelength and RB samples at 540 nm. A graph was plotted against percentage removal of adsorbate versus various concentration of adsorbate dose.

### 3.5.3 Effect of pH

20 mg/L working concentration of Lead (II)/Zinc (II) ion solution was adjusted to vary its pH from 2, 3, 4, 5, 6, 7 and 8. The optimum adsorbent (chitosan/PVA) dose of 0.5g/100ml in zinc ion solution and 0.6 g/100 mL in lead ion solution was added. Similarly, adsorbent (chitosan blended PAN) of 0.2 g/100 mL was added to 20 mg/L working concentration of Zn(II) and Pb(II) ion solution and pH was varied from 2 to 8. All these flasks were kept in the rotary shaker for 60 minutes, 160 rpm, 30° C. After one hour, the supernatant was collected by filtration and the samples were analyzed using AAS. A graph was plotted against percentage removal of adsorbate versus different pH values.

The working solutions of EY and RB dye (20 mg/L concentration) was adjusted to vary in its pH as 2, 3, 4, 5, 6, 7, 8 and 9 using 1N HCl or 1N NaOH. Adsorbent (chitosan blended PVA) of 0.2g/100ml was added in each working solution. Then they were kept in the rotary shaker for 60 minutes about 160 rpm, 30°C. After one hour, the supernatant was filtered and the samples were analyzed at 520 nm (EY dye) and 540 nm (RB dye) wavelength. Then the graph was plotted against percentage removal of adsorbate versus various pH values.

### 3.5.4 Effect of Temperature

The Lead (II)/Zinc (II) solutions were prepared in four conical flasks with lead and zinc solution at 20mg/L concentration. Then adsorbent
(chitosan/PVA) was added 0.6g/100ml to each of the Pb (II) working solutions and 0.5g/100 mL in each of zinc (II) ion solution (pH 5). Then they were kept in an incubator shaker for agitation at different temperatures. Thus temperature varied for each solution as 30°C, 40°C, 50°C and 60°C. The samples were kept for agitation at various temperatures for 60 minutes about 160 rpm. After one hour the supernatant was filtered out and analyzed using AAS. A graph was plotted against percentage removal of adsorbate versus different temperatures. Similarly, the same procedure was repeated at different temperatures from 30°C to 60°C with 0.2g/100 mL of chitosan blended PAN to each of 20 mg/L Zn(II) and Pb(II) ion working solutions (pH 5).

Eosin Yellow/Rose Bengal dye of working concentration at 20mg/L, added with 0.2g/100 mL chitosan blended PVA as adsorbent in each flask. At different temperatures ranging from 30°C, 40°C, 50°C and 60°C, the samples were analyzed at the respective wavelengths as stated in the above procedure. A graph was plotted against percentage removal of adsorbate versus different temperatures.

3.5.5 Effect of Contact Time

Lead(II) solution / Zinc(II) ion solution was prepared for 20mg/L concentration in six different flasks. The optimum chitosan/PVA (adsorbent) dosage was added in each flask (for Zn(II) adsorbent dose 0.5g/100 mL; pH 5 and Pb(II) 0.6g/100 mL; pH 5). Then they were kept in a rotary shaker for different contact times for each flask. Thus they were kept at 30°C for 10, 20, 30, 40, 50 and 60 minutes for Zn(II) ions and 10 min to 80 min in increments of 10 for Pb(II) ions in a rotary shaker. The supernatant was separated by filtration and the samples were analyzed using AAS. A graph was plotted against percentage removal of adsorbate versus different contact time with the adsorbent.
The same above procedure was repeated for Zn(II) and Pb(II) metal ions with 0.2g of chitosan blended PAN from 10 to 60 minutes time intervals. Also, Eosin Yellow and Rose Bengal dye of working concentration at 20mg/L each, added with 0.2g/100 mL chitosan blended PVA as adsorbent in each flask. After regular intervals of time ranging from 10 to 60 min, the supernatant was filtered and the samples were analyzed at respective wavelengths. A graph was plotted against percentage removal of adsorbate versus different contact time.

3.6 ADSORPTION ISOTHERMS

The experimental data on the effect of an initial adsorbate concentration of chitosan blended PVA and chitosan blended PAN of the test medium were fitted to the different adsorption isotherm models such as Langmuir (1918), Freundlich (1906) and Temkin (Temkin & Pyzhev 1940). Perfect describing adsorption isotherm helps to design an adsorption system.

3.6.1 Langmuir Isotherm

The Langmuir model is based on the presumption that maximum adsorption takes place when a saturated monolayer of solute molecules is present on the adsorbent surface, the energy of adsorption is uniform and there is no migration of adsorbate molecules in the surface plane.

The effect of initial concentration data were fitted to the Langmuir adsorption isotherm model. The adsorbate concentration in fluid phase (C_e) was obtained from the effect of initial concentration plot and the amount of adsorbate ions adsorbed onto the adsorbent at equilibrium, q_e (mg/g), was calculated by using the following equation:

\[ q_e = \frac{(C_l-C_e)V}{m} \]  

(3.2)
where $C_i$ and $C_e$ is the initial and equilibrium concentration of adsorbate [Zn (II)/ Pb (II)/EY/RB dye] in the solution at equilibrium (mg/L). $V$ is the volume of the solution taken (L) and $m$ is the mass of the adsorbent (g). The Langmuir adsorption isotherm equation is given as follows:

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e}$$  \hspace{1cm} (3.3)

where, $q_e$ = equilibrium adsorption capacity (mg/g),

$q_m$ is the maximum monolayer adsorption capacity (mg/g),

$K_L$ is the Langmuir constant related to the adsorption of Zn(II)/Pb(II) ions onto adsorbent (chitosan/PVA or chitosan/PAN) (L/mg)

$C_e$ is the equilibrium concentration of adsorbate ions solution (mg/L).

The $C_e$ and $q_e$ values were taken from the plot of effect of initial concentration. Then the inverse of the values were also found for the plot of Langmuir isotherm and the slope predicts the value of $K_L$ and the intercept gives $q_m$.

The separation factor $R_L$ is:

$$R_L = \frac{1}{1 + K_L C_o}$$  \hspace{1cm} (3.4)

$C_o$ is the initial adsorbate ions concentration in the solution (mg/L),

$K_L$ is the Langmuir constant (L/mg).

The value of separation factor $R_L$ plays an important role to provide data about the nature of adsorption. If $R_L = 0$; the adsorption is irreversible; $0 < R_L < 1$, then the adsorption is favourable; $R_L = 1$, the adsorption is linear; $R_L > 1$; the adsorption is unfavourable.
3.6.2 Freundlich Isotherm

The Freundlich isotherm model is an empirical relationship describing the adsorption of solutes from a liquid onto a solid surface. The Freundlich adsorption isotherm relates the amounts of lead adsorbed per unit mass of adsorbent \( q_e \) and the concentration of the adsorbate at equilibrium \( C_e \).

The effect of initial concentration data were further applied to the Freundlich adsorption isotherm model (Freundlich 1906). The Freundlich adsorption isotherm is the relationship between the amounts of adsorbate per unit mass of adsorbent \( q_e \) and the concentration of the Zn(II)/Pb(II)/EY/RB ions at equilibrium \( C_e \).

\[
q_e = k_F C_e^{1/n}
\]

(3.5)

where, \( q_e \) is the equilibrium adsorption capacity (mg/g), \( K_F \) is the Freundlich constant \( ((\text{mg/g})(\text{L/mg})^{(1/n)}) \) correlated to the bonding energy and \( n \) is a measure of the deviation from linearity of adsorption (g/L).

The \( C_e \) and \( q_e \) values were calculated from the plot of effect of initial concentration and this was applied to Freundlich adsorption isotherm model. For this case, the plot of log \( C_e \) Vs log \( q_e \) was employed to obtain the intercept the values of \( K_F \) and from the slope that of ‘\( n \)’.

The significance of \( n \) is as given as:

\( n = 1 \), adsorption takes place is linear; \( n < 1 \), adsorption occurs is chemical process; \( n > 1 \), adsorption is said to be physical process.
3.6.3 **Temkin Isotherm**

The Temkin adsorption isotherm assumes that the heat of adsorption decreases linearly with the sorption coverage due to adsorbent-adsorbate interactions.

The Temkin adsorption isotherm equation can be:

\[ q_e = B \ln(AC_e) \]  

(3.6)

where \( A \) is the equilibrium binding constant corresponding to the maximum binding energy, (L/mg) (Temkin isotherm constant)

\[ B = \frac{RT}{b} \]  

(3.7)

\( B \) is Temkin isotherm constant, \( R \) is the universal gas constant (8.314 J/mol K); \( b \) is a constant related to the heat of adsorption (J/mol); \( T \) is absolute temperature (K).

### 3.7 ADSORPTION KINETICS

#### 3.7.1 Lagergren’s Pseudo First Order Kinetic Model

To investigate the adsorption mechanism and rate controlling steps, the adsorption kinetic data were modelled using pseudo first order equations and pseudo second order equations.

The linear form of the pseudo first order rate equation by Lagergren (Lagergren 1898) is given as:

\[ \log(q_e - q_t) = \log q_e - \frac{k_1}{2.303}t \]  

(3.8)
where, \( q_e \) and \( q_t \) are the amount of the lead II ions adsorbed at equilibrium and at time \( t \) (min), respectively and \( k_1 \) (min\(^{-1}\)) is the rate constant for the pseudo order rate equation. A graph of \( \log (q_e - q_t) \) versus \( t \) (min) was plotted which gives \( k_1 \) and \( q_{e,\text{cal}} \) values from the slope and intercept.

### 3.7.2 Ho and Mckay Pseudo Second Order Kinetic Model

To determine the adsorption mechanism and rate controlling steps by pseudo second order kinetic model, the linear form of the pseudo second order rate equation proposed by Ho & McKay (1999) is given as:

\[
\frac{t}{q_t} = \frac{1}{k_2q_e^2} + \frac{1}{q_e}(t)
\]

(3.9)

where \( h = k_2q_e^2 \), \( h \) is the initial adsorption rate (mg/g . min) and \( k_2 \) refers to the rate constant of pseudo-second order equation (g/mg.min). A graph of \( t/q_t \) versus \( t \) (min) was plotted and the obtained values of \( q_e \) from the slope were compared with experimental values of \( q_e \).

### 3.8 ADSORPTION MECHANISM

It is most important to predict the rate-limiting step in an adsorption system to understand the adsorption mechanism associated with the phenomena. Weber and Morris intraparticle diffusion model (Weber 1963) and Boyd kinetic model (Boyd 1947) can be used to explain the adsorption mechanism. The following three steps takes place during the adsorption of adsorbate onto the adsorbent blend:

a. The movement of Zn(II)/Pb(II)/EY/RB ions from the bulk solution to the external surface of adsorbent blend (Film diffusion).
b. Movement of Zn(II)/Pb(II)/EY/RB ions into the internals of adsorbent blend (Intraparticle diffusion).

c. Adsorption of Zn(II)/Pb(II)/EY/RB ions onto the interior pores of the adsorbent blend (Adsorption)

3.8.1 Weber and Morris Intraparticle Diffusion Model

The intraparticle diffusion model was plotted to the extent of verifying the influence of mass transfer resistance on the binding of Zn (II)/Pb(II)/EY/RB dye to the chitosan blend. The kinetic results were analyzed by the Weber and Morris (Weber & Morris 1963) intraparticle diffusion model to demonstrate the diffusion mechanism. The model is expressed as follows:

\[ q_t = k_p t^{1/2} + C \]  \hspace{1cm} (3.10)

where \( C \) is the intercept which projects an idea about the thickness of the boundary layer and \( k_p \) is the intraparticle diffusion rate constant, (mg/g.min\(^{1/2}\)) which can be evaluated from the slope of the plot of \( q_t \) versus \( t^{1/2} \). The intraparticle diffusion model was plotted to verify the influence of mass transfer resistance on the binding of Zn (II)/Pb(II)/EY/RB to the chitosan blend.

3.8.2 Boyd Kinetic Model

The actual slowest step in the adsorption process was explained by fitting the adsorption kinetic data to the Boyd (Boyd et al 1947) kinetic model and the model equation is given as follows:

\[ F = \frac{q_t}{q_e} = 1 - \frac{6}{\pi^2} \exp(-Bt) \]  \hspace{1cm} (3.11)
The above equation can be rewritten as follows:

\[ Bt = -0.4977 - \ln(1 - F) \]  

(3.12)

where \( q_e \) is the adsorption capacity at equilibrium (mg/g), \( F \) is the fraction of adsorbate ions adsorbed at any time \( t \) in min, \( q_t \) is the adsorption capacity at any time \( t \), and \( Bt \) is a function of \( t \). The effective diffusion coefficient \( (D_i) \) values were calculated from \( B \) values which were obtained from the plot of \( Bt \) versus \( t \):

\[ B = \frac{\pi^2 D_i}{r^2} \]  

(3.13)

where \( D_i \) is the effective diffusion coefficient of Zn(II)/Pb(II) ions/EY dye/RB dye (m^2/s) and \( r \) is the radius of the chitosan blend particle in m.

3.9 THERMO DYNAMIC STUDIES

Thermodynamic parameters such as the free energy \( (\Delta G^0) \), enthalpy \( (\Delta H^0) \) and entropy \( (\Delta S^0) \) changes during adsorption can be evaluated from the following Equilibrium constant:

\[ K_c = \frac{C_{Ae}}{C_e} \]  

(3.14)

Change in Gibb’s energy: \( \Delta G^0 = -RT\ln K_c \)  

(3.15)

Linear equation to find \( \Delta H^0 \) and \( \Delta S^0 \):

\[ \log K_c = \frac{\Delta S^0}{2.303 R} - \frac{\Delta H^0}{2.303 RT} \]  

(3.16)

where \( K_c \) is the equilibrium constant, \( C_e \) is the equilibrium concentration in solution (mg/L) and \( C_{Ae} \) is the solid-phase concentration at equilibrium (mg/L). \( \Delta G^0 \), \( \Delta H^0 \) and \( \Delta S^0 \) are changes in Gibbs free energy (kJ/mol),
enthalpy (kJ/mol) and entropy (J/mol K), respectively; R is the universal gas constant (8.314 J/mol.K) and T is the absolute temperature (K). The numerical values of ΔH° and ΔS° were calculated from the slope and the intercept of the plots of plots of log Kc versus 1/T.

3.10 DESORPTION STUDIES

The desorption of metal ions and dyes from the spent chitosan blended PVA and chitosan blended PAN were studied with varying concentrations ranging from 0.05 M, 0.1 M, 0.15 M and 0.2 M of hydrochloric acid (HCl) solution. A measured quantity of spent chitosan blend was transferred to stoppered conical flasks which consist of 100 mL of HCl solution of known concentration. The flasks were shaken in a rotary shaker (160 rpm) at 30°C. After the system approached the equilibrium time of 1 h, the chitosan/PVA or chitosan/PAN was then removed by filtration. The concentrations of metal ions and dyes in the solution were determined by using Atomic Absorption Spectrophotometer.