

Chapter-II

Experimental

EXPERIMENTAL**2.1 MATERIALS****2.1.1 Reagents (Chemicals)**

The following chemicals were employed in the present investigation. All chemicals used were of analytical reagent grade unless otherwise specified.

- | | |
|-------------------------------|---------------------------------|
| 1. Methanol | 24. Benzene |
| 2. Ethanol | 25. Ethyl acetate |
| 3. Carbondisulphide | 26. Stannous chloride |
| 4. Acetone | 27. Zinc chloride |
| 5. Hydrochloric acid | 27. Cobalt chloride |
| 6. Nitric acid | 28. Lead acetate |
| 7. Sulphuric acid | 29. Ammonium ferrous sulphate |
| 8. Acetic acid | 30. Manganese sulphate |
| 9. Ammonia solution | 31. Thorium nitrate |
| 10. Sodium hydroxide | 32. Copper sulphate |
| 11. Sodium chloride | 33. Uranyl acetate |
| 12. Potassium hydroxide | 34. Ammonium molybdate |
| 13. Potassium chloride | 35. Cadmium chloride |
| 14. Sodium acetate | 36. Dimethyl formamide |
| 15. Ammonium chloride | 37. Dimethyl sulphoxide |
| 16. Amberlite XAD-2 | 38. Phosphoric acid |
| 17. Amberlite XAD-16 | 39. Disodium hydrogen phosphate |
| 18. Carbandisulfide | 40. Sodium dihydrogen phosphate |
| 19. Ethyl benzoate | 41. Chloroform |
| 20. Thiosemicarbazide | 42. Magnesium sulphate |
| 21. pyridine | 43. Ammonium sulphate |
| 22. 2-nitrobenzoyl chloride | 44. Magnesium nitrate |
| 23. 2-hydroxybenzoyl chloride | 45. Calcium carbonate |

2.1.2 Purification of solvents

Acetone, ethanol, carbon disulphide and methanol were purified by the standard methods described in the literature [1-3].

- (a) **Deionised doubly distilled water** was prepared by double distillation of deionised water in a quartz distillation unit. This was used for the preparation of stock and standard metal solutions and also used in experimental work wherever is required.
- (b) **Acetone** (S. D. Fine Chem Ltd., India) was purified by heating under reflux with successive quantities of potassium permanganate until the violet colour persists. Then the solution was dried over anhydrous potassium carbonate, filtered and distilled using a fractionating column. The fraction was collected between 55-56 °C.
- (c) **Ethanol** (S. D. Fine Chem Ltd., India) was purified by heating in a muffle furnace with successive quantities of calcium oxide and allowed to cool in a dessicator. Then it is taken in a flask fitted to a double surface condenser carrying calcium chloride guard tube, and refluxed gently for 6 h (preferably using a heating mantle) and allowed to stand overnight. Then the condenser is resembled in the condenser for downward distillation via a splash head adapter to prevent the calcium oxide in the vapour stream. Then ethanol is gently distilled by gently discarding the first 20 ml of distillate. The fraction was collected between 77-78 °C.
- (d) **Carbon disulphide** (S. D. Fine Chem Ltd., India) was shaken for 3 hours with three portions of potassium permanganate solution (5 g per litre), twice for 6 h with mercury (II) sulphate (2.5 g per litre). Then the solution was dried over anhydrous calcium chloride and distilled using a fractionating column. The fraction was collected between 55-65 °C.
- (e) **Methanol** (S. D. Fine Chem Ltd., India) was purified by heating under reflux with successive quantities of furfural and sodium hydroxide for 6-12 h. A resin is formed which carries down all acetone present. Then the alcohol is then fractionated through a fractionating column. The fraction was collected between 64-65 °C.

2.1.3 Preparation of buffer solutions

- (a) **Phosphate buffer:** 0.2 M of phosphoric acid and sodium dihydrogen-phosphate solutions were prepared and mixed in a suitable proportion to get the pH 2 and 3.
- (b) **Acetic acid-sodium acetate buffer:** 0.2 M of acetic acid and sodium acetate solutions were prepared and mixed in a suitable proportion to get the pH from 4 and 7.
- (c) **Ammonium chloride-ammonium hydroxide buffer:** 0.2 M of ammonium chloride and ammonium hydroxide solutions were prepared and mixed in suitable proportions to get the pH from 8 and 12.

2.1.4 Preparation of stock metal solutions

- (a) **Iron (1mg ml^{-1}):** 7.26 g of ferric chloride was dissolved in deionised doubly distilled water containing 25 ml of concentrated hydrochloric acid and diluted to 1000 ml with deionised doubly distilled water.
- (b) **Cobalt (1mg ml^{-1}):** 2.2806 g of cobalt chloride was dissolved in deionised doubly distilled water containing 5 ml of concentrated sulphuric acid and diluted to 1000 ml with deionised doubly distilled water.
- (c) **Lead (1mg ml^{-1}):** 1.000 g of lead metal was dissolved in 50 ml 5M HNO_3 and diluted to 1000 ml with deionised doubly distilled water.
- (d) **Manganese (1mg ml^{-1}):** 3.0765 g of manganese sulphate ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$) was dissolved in deionised doubly distilled water containing 5 ml of concentrated sulphuric acid and diluted to 1000 ml with deionised doubly distilled water.

Standard solutions of each element were prepared freshly by appropriate dilution of individual stock solutions with deionised doubly distilled water.

- (e) **Multielement standard solution ($100\ \mu\text{g ml}^{-1}$):** This was prepared by appropriate dilution of ICP standards of Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn (Merck, Germany).
- (f) **Diverse ion solutions:** Solutions of counter ions for the interference studies were prepared according to the standard procedures [4, 5].

2.2 INSTRUMENTS USED IN THE PRESENT STUDY

- (a) Shimadzu AU7 220 digital electronic balance was used for weighing the substances.
- (b) The pH measurements were made using a pH meter Elico, Model LI-129, India in conjunction with a combined glass and calomel electrode.
- (c) Thermo-Nicolet FT-IR, Nicolet IR-200, USA Fourier Transform Infra-Red Spectroscopy using potassium bromide (KBr) pellet was used for IR studies.
- (d) Gemini-200 Varian, Switzerland and Avance - 300 Bruker, Switzerland were used for NMR studies.
- (e) Vario EL, Elementar, Germany and Elementar Vario EL III, Carlo Erba 1108, Heraeus CHN-O rapid analyzer, Automatic C/H/N analyzer, were used for elemental analysis.
- (f) A micro wave digester (CEM, Model MDS-2000) was used for digestion of leafy vegetable and vegetable samples.
- (g) Atomic emission spectrometry (ICP-AES, Varian, Liberty Series II, Australia) with an wipro acer computer was employed for the determination of metals in samples. The instrumental operating parameters are given in Table 2.5.

Table 2.1: ICP-AES operating parameters

Photomultiplier tube voltage/V	700
Incident power/Kw	1.1
Plasma gas flow/l min ⁻¹	15.0
Auxillary gas flow/l min ⁻¹	1.5
Observation height/mm	14.0
Pump rate/rpm	15.0
Sample uptake times	25.0

Wavelength of elements

Element	Wavelength (nm)
Cu	324.754
Cd	226.502
Co	228.610
Mn	257.610
Ni	221.647
Pb	220.382
Zn	213.856

2.3 INSTRUMENTAL TECHNIQUE

2.3.1 Inductively coupled plasma-atomic spectrometry (ICP-AES)

Inductively coupled plasma-atomic emission spectrometry (ICP-AES) is one of the modern, sensitive and promising emission techniques for multi elemental analysis. It was developed independently by Greenfield et al. [6] in UK and Fassel et al. [7] in USA in the 1970s. It is a versatile method, which finds use in many fields including inorganic chemistry, bio-inorganic chemistry, pharmaceutical industries, biological sciences, geology, oceanography, food industries, polymer industries, pesticide industries, environmental studies and pollution monitoring of water and air and catalyst industries. The method is based on measurement of electromagnetic radiation consequent to the relaxation from excited state of an atom by plasma. Plasma is a conducting gaseous mixture containing a significant concentration of cations and electrons.

(a) Principle

An ICP spectrometry is based on the principle that atoms are promoted to higher electronic energy levels when heated to high temperature (10, 000 K) in plasma. As the excited species leave the high temperature region, the absorbed energy is released as uv or visible photons when the excited atoms decay to the ground electronic state. The plasma temperature is sufficient to ionize most atoms. So most sensitive lines arises from an ion rather than an atom. Measurement of the wavelength and intensity of emitted lines provide both qualitative and quantitative determination of elements.

(b) Plasma torch

The inductively coupled argon plasma torch [8] derives its sustaining power by induction from a high frequency magnetic field. The plasma torch generally consists of three concentric quartz tubes. The solution is aspirated by a nebulizer system and a fine liquid aerosol is transported through the central tube and through the toroidal core of plasma by a flow of argon gas at a sample uptake rate of above 1 ml min^{-1} . The high temperature produced by plasma generally require a second stream of gas usually argon, to provide a tangential flow of argon (15 l min^{-1}) to cool the inside quartz walls of the torch. This flow also serves to center and stabilize the plasma. The intermediate tube provides argon gas flow for sustaining the plasma. In the direct current plasma sources [9, 10], a high velocity inert gas produces a high temperature plasma and separates the excitation region from the analytical observation zone.

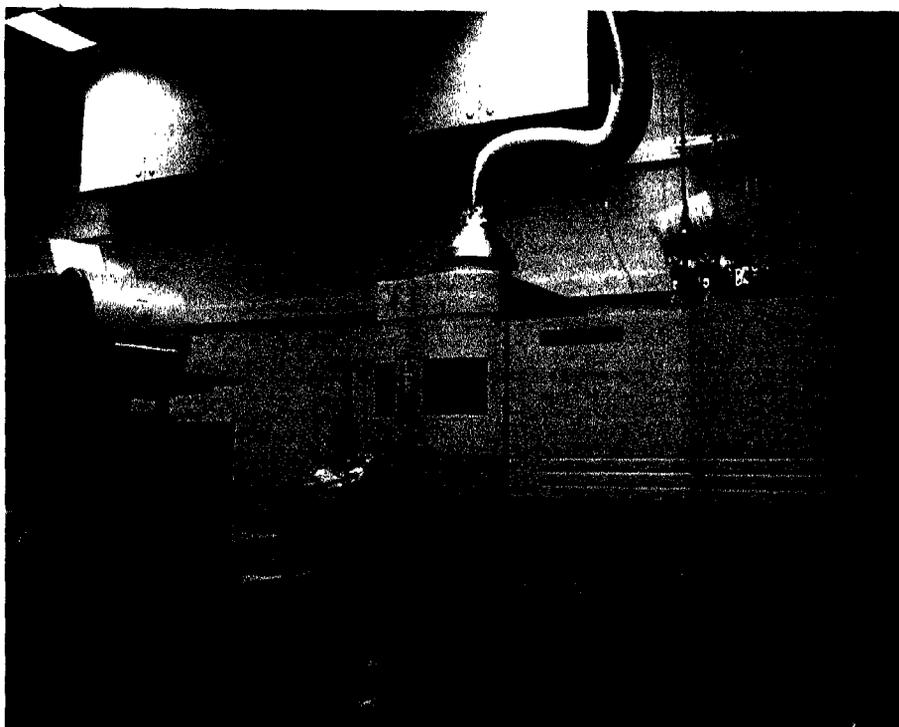


Fig. 2.1: Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) image

(c) Nebulization system

The introduction of liquid sample into the plasma is mostly effected through pneumatic nebulization systems. In these nebulizers gas is used to generate the aerosol and also carry to the plasma torch. Clogging of the nebulizer due to introduction of concentrated solution frequently occurs in pneumatic nebulizers. Among the pneumatic nebulization systems, cross-flow and meinhart nebulizers are commonly used. In the ultrasonic waves and the gas is used only to carry aerosols to the plasma. Further the aerosol is finer in size compared to that obtained with pneumatic nebulization. The above factors dictate that these type of nebulizer help in improving the detection power significantly.

Another kind of nebulizer, the Babington type, is used for viscous samples and samples containing particulates or high dissolved solid content ($> 5000 \text{ mg l}^{-1}$). The design of this nebulizer provide free from blockage in analysis.

(d) Excitation process

The excitation is provided by two or three metal induction tube which is coiled on the top of the silica quartz tubes. A radio-frequency current flows through this tube where the standard frequency of operation is 27.12 M Hz while the forward power is 1 to 2 KW. This type of high frequency, low power operation ensures satisfactory introduction of sample aerosols into plasma. The temperature profile of the plasma varies from 6,000-10,000 K with temperature decreasing as a function of the height above the r.f. coil. Typical observation height is 15-20 mm. The excitation of atom/ion is achieved by one or more of the three mechanisms.

- i. Collisional transfer of excitation energy
- ii. Transfer of excitation energy to the analyte through metastable states of argon atoms/ions.
- iii. Charge transfer between argon ions and the analyte atoms.

(e) Measurement of analytical signal

The plasma emission source is coupled to a high resolution spectrometer with a printer or video terminal. The spectrometer may be of the sequential (monochromator) or simultaneous (polychromator) type with airpath, inert gas purged, or vacuum optics. In

the sequential mode of operation normally a single photomultiplier tube is used as the detector or over a wide spectral region and the grating movement is effected in an ingenious way. The most common simultaneous systems are multi channel with one or more detector for each analyte element. The direct reading spectrometers are invariably accompanied by computerized data acquisition and processing facility. The analytical software available with the computer is often able to correct for background spectral contribution, inter-elemental interference, matrix effects etc.

2.4 LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

The limit of detection (LOD) for any analytical procedure, the point at which analysis is just feasible, may be determined by a statistical approach based on measuring replicate blank (negative) samples or by an empirical approach, consisting of measuring progressively more dilute concentrations of analyte. The LOD may be expressed based on blank signals ($n=10$) plus three times the standard deviation of the blank signals.

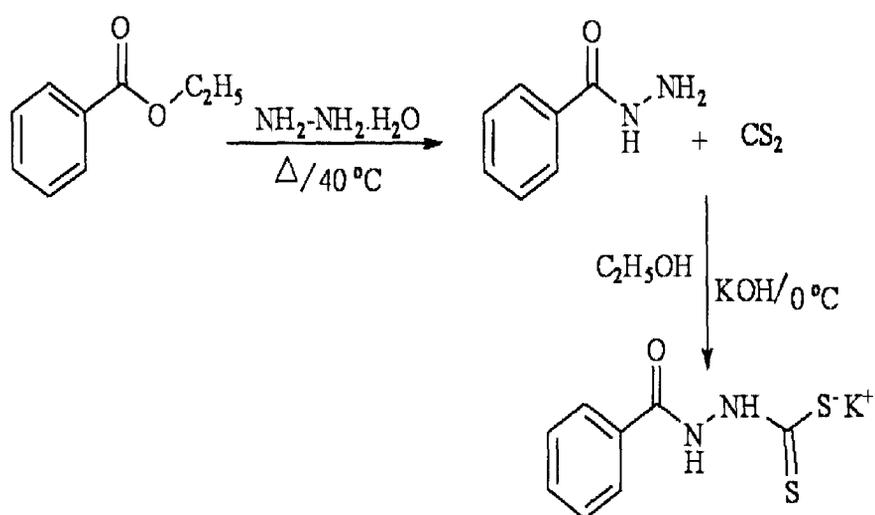
The limit of quantification (LOQ), or concentration at which quantitative results can be reported with a high degree of confidence, may likewise be determined by either approach. It is also defined as 3.33LOD [11].

2.5 SYNTHESIS OF LIGANDS

2.5.1 Synthesis of potassium 2-benzoylhydrazinecarbodithioate (PBHCD)

About 0.14 mol of ethyl benzoate (Dissolved in 10 mL of $\text{C}_2\text{H}_5\text{OH}$) and 0.28 mol of hydrazine hydrate (dissolved in 10 mL of $\text{C}_2\text{H}_5\text{OH}$) were mixed in round bottom flask in the presence of base (pyridine) and refluxed for 3 hr. After completion of the reaction the solvent was removed under reduced pressure to obtained crude product which was recrystallized from chloroform to obtain colourless crystals benzoyl hydrazide.

Benzoyl hydrazide (0.1 mol dissolved in 25 mL of $\text{C}_2\text{H}_5\text{OH}$) was placed in a round bottom flask and alcoholic KOH (0.2 mol) was added drop wise to obtain colorless liquid and then carbon disulfide (0.3 mol of solution) was added quickly to obtain yellow color solid of potassium 2-benzoylhydrazinecarbodithioate (PBHCD). Yield: 86 %, m.p: 294-290 °C. Schematic representation of synthesis of PBHCD is shown in Fig. 2.2.



Potassium 2-benzoylhydrazinecarbodithioate

Fig. 2.2: Scheme for the synthesis of PBHCD

2.5.2 Synthesis of 3-(2-nitrophenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (NPTT)

To the solution of thiosemicarbazide in absolute pyridine, 2-nitrobenzoyl chloride was added drop wise with stirring at -5 to 0 °C. The reaction mixture was stirred for 3 h at room temperature and poured into H_2O to get precipitate. It was refluxed for 3 h in NaOH (2N) solution, cooled to room temperature. Obtained precipitate was filtered off and recrystallized. Yield: 82 %. Schematic representation of synthesis of NPTT is shown in Fig. 2.3.

2.5.3 Synthesis of 3-(2-hydroxyphenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (HPTT)

To a solution of thiosemicarbazide in absolute pyridine, 2-hydroxybenzoyl chloride was added drop wise with stirring at -5 to 0 °C. Reaction mixture was stirred for 3 h at room temperature and poured into H_2O to get precipitate, it was refluxed for 3 h in NaOH (2N) solution, cooled to room temperature. Obtained precipitate was filtered off and recrystallized. Yield: 82%. Schematic representation of synthesis of HPTT is shown in Fig. 2.4.

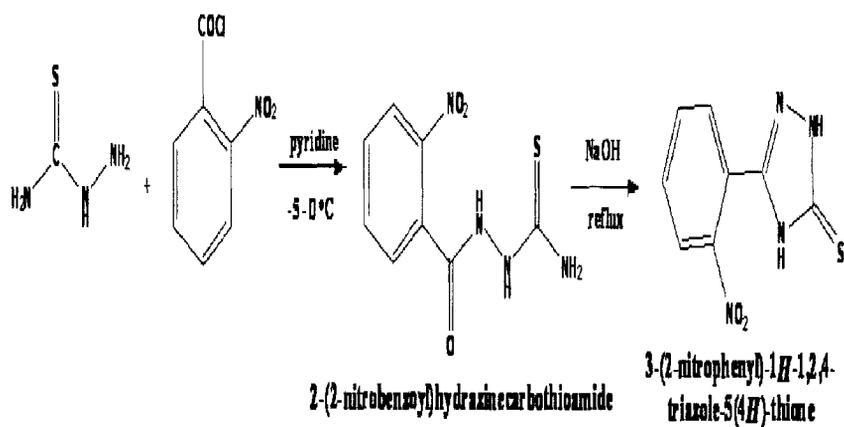
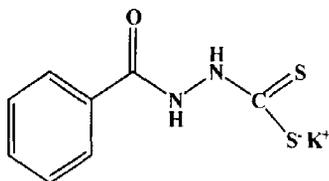
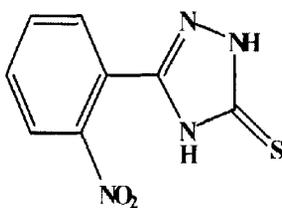


Fig. 2.3: Scheme for the synthesis of 3-(2-nitrophenyl)-1H-1,2,4-triazole-5(4H)-thione (NPTT)

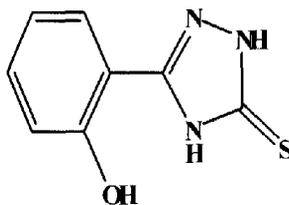
2.6 PROPOSED STRUCTURES OF SYNTHESIZED LIGANDS



Structure of potassium 2-benzoylhydrazinecarbodithioate (PBHCD)



Structure of 3-(2-nitrophenyl)-1H-1,2,4-triazole-5(4H)-thione (NPTT)



Structure of 3-(2-hydroxyphenyl)-1H-1,2,4-triazole-5(4H)-thione (HPTT)

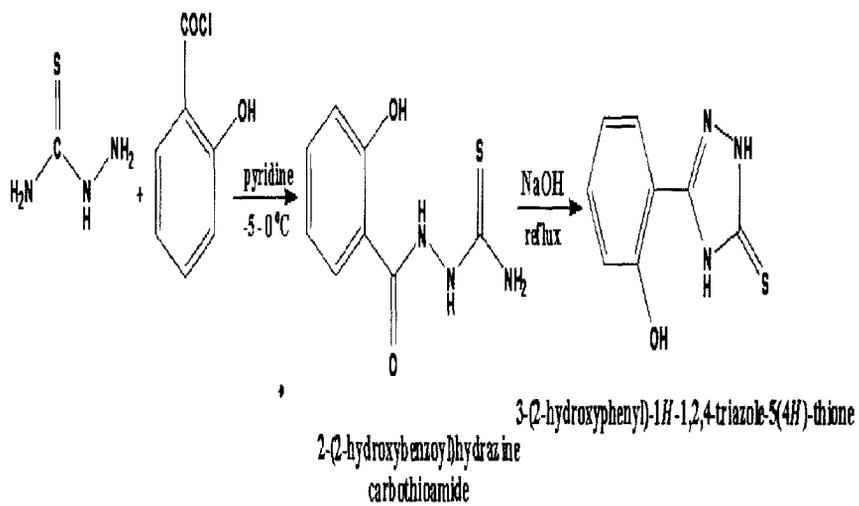


Fig. 2.4: Scheme for the synthesis of 3-(2-hydroxyphenyl)-1H-1,2,4-triazole-5(4H)-thione (HPTT)

2.7 ADSORBENTS

2.7.1 Amberlite XAD-16 resin

The Amberlite XAD-16 resin (styrene-divinyl-benzene copolymer) having a bead size of 20-50 mesh and surface area of $825 \text{ m}^2 \text{ g}^{-1}$ was used. Before use, the resin beads were purified by treating with 4 M HCl solution followed by washing with 2 M NaOH solution to remove the both acidic and basic impurities and finally with water until the washings showed neutral pH. The purified resin was filtered, rinsed with ethanol. Then dried in vacuum oven at $60 \text{ }^\circ\text{C}$ and stored in polyethylene bottles for further use. The resin was modified by functionalization with chelating reagents and used as a matrix for separation and preconcentration of metals.

2.7.2 Amberlite XAD-2 resin

The Amberlite XAD-2 resin (Milwaukee, USA, Aldrich) with a particle size, 20-60 mesh, pore diameter, 90 \AA and surface area, $330 \text{ m}^2 \text{ g}^{-1}$ was used. Before use, the resin was washed with methano 1-4 M HCl to remove the metal impurities, washed with deionised doubly distilled water until the pH was neutral and then dried at $40 \text{ }^\circ\text{C}$ for 24 h in a vacuum dessicator. The resin was modified by functionalization with chelating reagents and used as a matrix for separation and preconcentration of metals.

2.8 FUNCTIONALIZATION OF AMBERLITE XAD RESIN WITH LIGANDS

2.8.1 Functionalization of Amberlite XAD-16 with PBHCD

AXAD-16 resin was functionalized by following the procedure described previously [12]. A mixture of concentrated nitric acid (10 ml) and sulphuric acid (25 ml) were added to Amberlite XAD-16 (5 g) and the mixture was stirred at $60 \text{ }^\circ\text{C}$ for 1 h on a water bath. Then the reaction mixture was poured into an ice-water. The nitrated mixture was filtered, washed repeatedly with cold distilled water until free from acid. The nitrated mixture is added to a reaction mixture of 40 g of SnCl_2 , 45 ml of concentrated HCl and 50 ml of ethanol. The system was refluxed for 12 h at 90°C . The precipitated solid was filtered and washed with water and 2 M NaOH, which released amino resin (R-NH_2) from $(\text{R-NH}_3)_2 \text{SnCl}_6$ (R-resin matrix). The amino resin was first washed with 2 M HCl and finally with distilled water to remove the excess of HCl. It was suspended in an ice-water mixture (350 ml) and treated with 1 M NaNO_2 (added in small aliquots of

1 ml) until the reaction mixture showed a permanent dark blue colour with starch-iodide paper. The diazotized resin was filtered, in ice cold condition and washed with ice-cold water and in the ice cold condition 2 % alkaline ethanolic solution of PBHCD was added and continued the reaction at 0-4 °C for 24 h. The resin was filtered, washed with water and dried in air. Schematic representation of reaction sequence of Amberlite XAD-16-PBHCD was shown in Fig. 2.5.

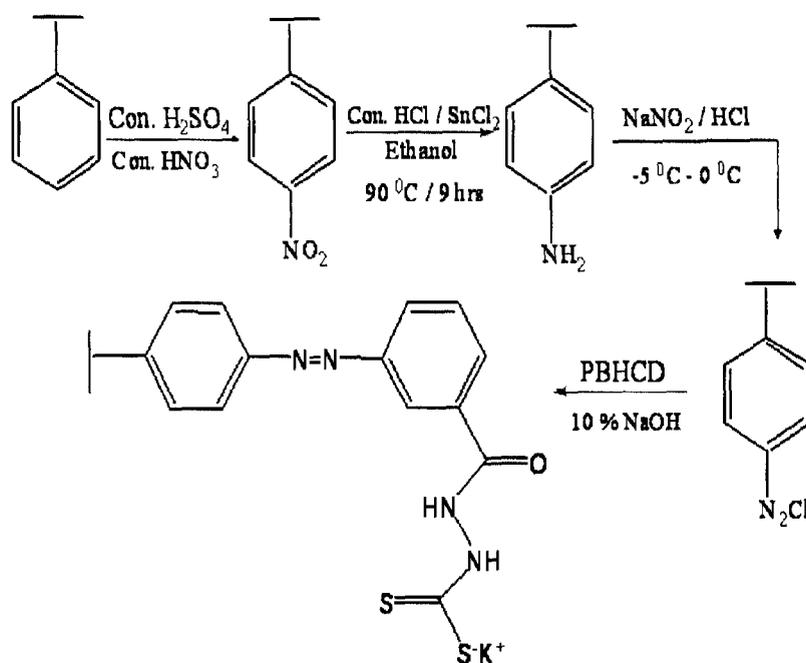


Fig. 2.5: Functionalization of Amberlite XAD-16 with PBHCD

2.8.2 Functionalization of Amberlite XAD-2 with NPTT

5g of Amberlite XAD-2 was treated with a nitrating mixture, containing 10 mL of concentrated HNO_3 and 25 mL of concentrated H_2SO_4 and stirred for 1 h at 60 °C on water bath. The reaction mixture was poured into ice-water and the nitrated resin then filtered off, and washed with water until the effluents were pH neutral and reduced with a mixture of SnCl_2 (40 g) in the presence of concentrated HCl (45 mL), and ethanol (50 mL), and refluxed for 12 h at 90 °C. The amino polymer was filtered off and washed with distilled water and 2 M NaOH so as to get the free amino polymer. The amino polymer was treated with 100 mL of 2 M HCl for 30 min, washed with distilled water to remove excess of HCl , and suspended in 250 mL of ice water. It was then diazotized with a equimolar mixture of 1 M HCl and NaNO_2 by adding 1 mL each time with constant stirring until the reaction mixture showed a permanent blue color with starch-iodide paper. The diazotized Amberlite XAD-2 was filtered at -5 to 0 °C to avoid its disintegration at higher temperature and treated with NPTT (3 g taken in a mixture of 100 mL water and 25 mL of 4 % sodium hydroxide) at 0-5 °C for 24 h. The dark brownish crimson colored beads were filtered, washed with 4M HCl and doubly distilled water successively and finally vacuum-dried. Schematic representation of reaction sequence of Amberlite XAD-2-NPTT was shown in Fig. 2.6.

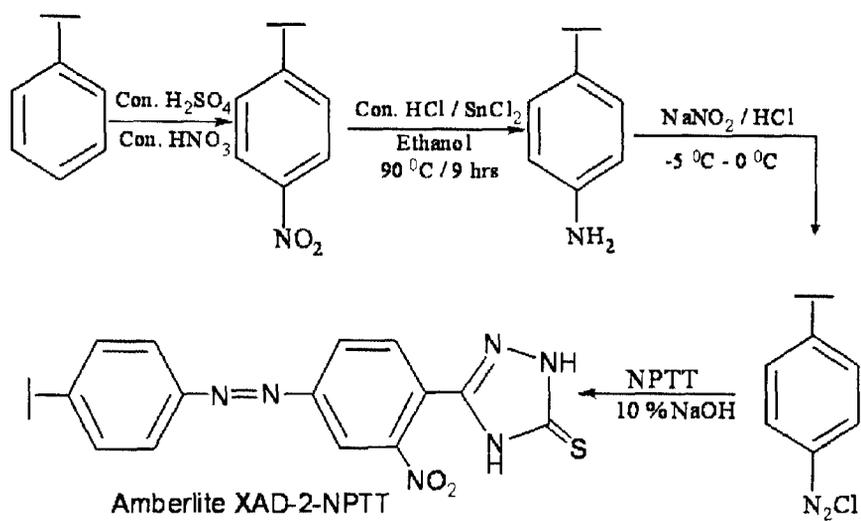


Fig. 2.6: Functionalization of Amberlite XAD-2 with NPTT

2.8.3 Functionalization of Amberlite XAD-16 with HPTT

5g sample of Amberlite XAD-16 was treated with a nitrating mixture, containing 10 mL of concentrated HNO_3 and 25 mL of concentrated H_2SO_4 and stirred for 1 h at 60°C on water bath. The reaction mixture was poured into ice-water and the nitrated resin then filtered off, and washed with water until the effluents were pH neutral and reduced with a mixture of SnCl_2 (40 g) in the presence of concentrated HCl (45 mL), and ethanol (50 mL), and refluxed for 12 h at 90°C . The amino polymer was filtered off and washed with distilled water and 2 M NaOH so as to get the free amino polymer. The amino polymer was treated with 100 mL of 2 M HCl for 30 min, washed with distilled water to remove excess of HCl , and suspended in 250 mL of ice water. It was then diazotized with a equimolar mixture of 1 M HCl and NaNO_2 by adding 1 mL each time with constant stirring until the reaction mixture showed a permanent blue color with starch-iodide paper. The diazotized Amberlite XAD-16 was filtered at -5 to 0°C to avoid its disintegration at higher temperature and treated with HPTT (3 g taken in a mixture of 100 mL water and 25 mL of 4 % sodium hydroxide) at $0-5^\circ\text{C}$ for 24 h. The dark brownish crimson colored beads were filtered, washed with 4M HCl and doubly distilled water successively and finally vacuum-dried. Schematic representation of reaction sequence of Amberlite XAD-16-HPTT was shown in Fig. 2.7.

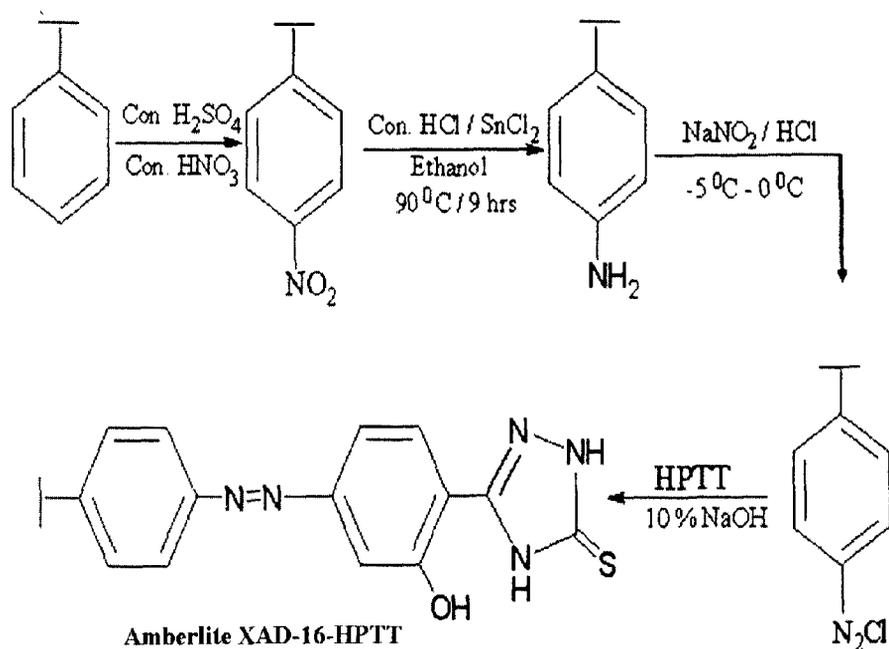


Fig. 2.7: Functionalization of Amberlite XAD-16 with HPTT

2.9 GENERAL SPE PROCEDURE

A glass column (1.0 cm i.d., length of 10 cm) with a stopcock was taken and required quantity of functionalized resin slurried in doubly distilled water was poured in the column. A small amount of glass wool was placed on the disk to prevent loss of resin beads during sample loading. Then treated with 2.0 M HCl and washed with doubly distilled water until free from acid. Sample containing metal ions was passed through the column at a flow rate of 2.0 mL min⁻¹ after adjusting the pH to desired value. The bound metal ions were eluted from the resin bed by using 10 mL of HNO₃. The concentration of metal ions in elutes were determined by ICP-AES. The parameters described in Table 2.1.

2.9.1 Procedure for the determination of trace metal ions in natural water samples

The water samples were filtered through a cellulose membrane filter of pore size 0.45 µm and the pH of the sample (500 mL) was adjusted to suitable pH with HCl (1+1) or dilute ammonia (1+1) and 2 mL of buffer solution solution was added. The solution was passed through the column containing functionalized resins at the desired flow rate and then washed with 5mL of doubly distilled water. The adsorbed metal ions were eluted with 10 mL of HNO₃ and determined as described in general procedure (section 2.9).

2.9.2 Procedure for the determination of metal ions in leafy vegetable and vegetable samples

Sample preparation for leafy vegetables and vegetable samples were carried out by the following procedure. Green leaves and vegetable samples were air dried for three days then cleaned with double distilled water followed by drying at 105 °C in hot air oven for 2 h and then powdered. One gram of each sample was taken in borosilicate flasks and 5 ml of concentrated nitric acid was added to each flask. After standing for 8 h, the samples were digested in microwave system for 10 min at 106 °C. The clear solution was obtained. The digested samples were further made up to 25 mL using double distilled water and determined as described in recommended procedure.

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