The remarkable development of molecular biology has had its impressive growth on a segment of biology. Knowledge of the biological functions of the trace elements has lagged far behind our understanding of their chemistry. The 19 essential trace elements include the three prominent biologically active metals viz. iron, zinc and copper. The biological activity of one half of the essential trace elements which function in metalloenzyme include Fe, Zn, Cu, Mn, Mo, Co, Ni and Se. However, the physiological role of V, Cr, Cd, Pb, Sn, Li, F, Si, As and B is difficult to explain. Very little concrete information about the actual free concentration of metal occurring within biological fluids is available. Metals control many biological events (Fe$^{2+}$ and Cu$^{2+}$ in oxygen metabolism) and fundamental ones too (eg. M$^{2+}$, Fe$^{2+}$ and Zn$^{2+}$ in DNA biochemistry). They often turn out to be intimately involved in pharmacology of organic drugs either directly eg. Ca$^{2+}$ or amplifier of organic messages. Metallo drugs are well known in medicine and are used to treat a diverse range of complaints and diseases, few have been designed to have a specific therapeutic effect.

The mode of action of chelating agents are as follows:-(i) Transient combination of a chelating agent occurs with the metal of an enzyme without removal of the metal from the enzyme.
(i) Combination of a chelator with metal ions in the cellular environment results in removal of the metal ions.

(ii) Combination of a chelating molecule with metal ions to form a complex which has suitable polarity to transport either a metal ion or the molecule to or through a cell membrane.

(iv) Chelation results in stabilisation of either a chelating molecule or a valence state of metal ion.

(v) Combination of a chelating molecule and metal ions to give an "unsaturated" chelate which exerts a toxic or catalytic effect.

(vi) Combination of a chelator and metal ion may alter the oxidation potential of metal.

The chelating agents in general clinical use today had been introduced by the mid 1960s. The first was 2,3-dimercaptopropanol (BAL) to treat arsenical gas poisoning. Ethylenediaminetetraacetic acid (EDTA) was administered to humans to remove lead (61). Then, through the 1950s and the early part of next decade, there followed a period of intense research into the properties of chelating agents from a chemical and physiological point of view. This resulted in the introduction of penicillamine by Walshe in 1954 (62), the identification of diethylthiocarbamic acid (DDC) as a drug for the specific treatment for nickel carbonyl poisoning in 1958 (63) and the
discovery of desferrioxamine (DFOA) as the iron complex in 1960s. During this period, diethylenetriaminepentaacetic acid (DTPA) was also established as a possible alternative to EDTA, particularly for radionuclide decorporation. With the exception of 2,2,2-triethylenetetramine (TRIEN) (64) and 2,3-dimercaptopropanesulphonic acid (DMPS), no new chelating agents have been accepted into the clinic for nearly 20 years.

In the present investigation,

(1) Folic acid was selected as a ligand as it is nontoxic, conveniently administered, resistant to metabolic degradation and binds the target metal ion very powerfully. Besides, it is structurally similar to 8-Hydroxyquinoline which is a well known chelating agent.

(2) Two groups of divalent metal ions viz. alkaline earth metals like Ca$^{2+}$, Mg$^{2+}$, Ba$^{2+}$, Sr$^{2+}$ and transitional metals like Cu$^{2+}$, Zn$^{2+}$, Fe$^{2+}$, Co$^{2+}$, Mn$^{2+}$ were selected. Ca$^{2+}$ and Mg$^{2+}$ ions as abundant metals in biological systems. Ba$^{2+}$ and Sr$^{2+}$ are known for their cytotoxicity. Cu$^{2+}$, Zn$^{2+}$, Fe$^{2+}$, Co$^{2+}$ and Mn$^{2+}$ are designated as essential metals in biological systems whereas Cd$^{2+}$ is known as toxic metal.

(3) Folic acid when treated with different metal perchlorate in presence of perchloric acid forms folic acid metal ion complex (2:1).
Complex formation at a particular pH is determined by Calvin-Bjerrum pH titrations. The complexation takes place between pH 4.0 and 5.3.

Complex formation was confirmed by (a) determination of metal ion content by chemical method
(b) determination of molecular weight and elemental analysis
(c) UV, IR and X-ray study.

The complex formation takes place in pteridine moiety. The 4-OH and 5-N of pteridine form the most stable 5-membered ring with the metal ion.

The stability constants of folic acid metal complexes were determined and discussed on the basis of (a) Variation with the atomic no. of metals. In case of transition metals the stability constant increases with the increase in atomic no. whereas the stability constant decreases with increase in atomic no. for alkali earth metals.
(b) Order of stability constants: alkali earth metals Mg$^{2+}$ > Ca$^{2+}$ > Sr$^{2+}$ > Ba$^{2+}$, transition metals Cu$^{2+}$ > Zn$^{2+}$ > Co$^{2+}$ > Fe$^{2+}$ > Mn$^{2+}$. (c) Variation with ionic radii. The stability constant increases with the increase in the value of $e^2/r$ where $e$ = ionic charge and $r$ = ionic radius.
(8) The physico-chemical properties of folic acid metal complexes were studied. The melting points (Decomposition) were in the range of 200-235°C.

(9) The folic acid metal complexes were analysed for metal ion content and folic acid. Folic acid was determined by official method B.P.'88 and metal ions volumetrically by EDTA titrations.

(10) The UV spectra of complexes show a bathochromic shift. IR spectra show absence of absorption peak around 3300 cm\(^{-1}\) for \(-\text{OH}\) group and prominent peaks at 825 cm\(^{-1}\), 525 cm\(^{-1}\), 450 cm\(^{-1}\) for metal ions. This indicates that the complex formation involves \(-\text{OH}\) group in position 4 of folic acid molecule.

(11) Determination of molecular weight and elemental analysis confirm the formation of 2:1 folic acid : metal ion complexes.

(12) X-ray analysis indicates that the folic acid metal complexes formed have a stable cubic structure.

(13) Folic acid metal complexes were also determined microbiologically. This, in turn, indicates non-toxic effect of complexes on streptococcus faecalis.
The effect of pH and temperature on solubility of folic acid metal complexes was studied; solubility increases with increase in pH and temperature. The complexes are more soluble than folic acid.

The complexes are more soluble than folic acid in propylene glycol and Tween 80. Tween 80 does not show appreciable increase in solubility.

The analytical procedure involves the estimation of aromatic amine PABG before and after the reduction of folic acid with Zinc and hydrochloric acid. The PABG (free) content of unreduced sample corresponds to the amount of folic acid which has undergone decomposition.

The stability of folic acid in its metal complexes was tested at different pH (2 to 9) using McIlvain's buffer and Clark and Lubs buffer at 6°, 30° and 45°C after 21 days storage. At all the pH levels folic acid metal complexes are more stable than folic acid. Stability increases with the decrease in temperature.

The stability of folic acid metal complexes was studied in the presence of Riboflavin, Thiamine HCl and Ascorbic acid individually as well as in multivitamin...
liquid preparations. The decomposition was greater at pH 6.0 than at pH 4.0. The order of decomposition was Riboflavin > Thiamine > Ascorbic acid.

(19) The effect of light was studied under three experimental conditions at 30° for 21 days.
(a) Amber glass vials stored in subdued light. (b) Flint glass vials stored in subdued light. (c) Flint, UV-light cabinet. The order of decomposition found was c > b > a.

(20) The Pharmacological screening of folic acid metal complexes showed that copper, magnesium and manganese complexes possess good anti-inflammatory activity as detected by Rat paw method.

(21) Acute toxicity of folic acid metal complexes was determined. The LD₅₀ of manganese, magnesium and copper complexes of folic acid are 370, 450 and 500 mg/kg body weight respectively.

(22) The absorption of metal ions by folic acid in simulated gastric juice indicates the formation of metal complexes. Hence, folic acid can be used in the treatment of acute metal poisoning.