Cancer is probably one of the most widely researched areas as it has become the second lethal cause of death since 1960 (1). The treatment of cancer with drug was started by Huggins and Hodges in 1941. Cancer treatment is a task performed by physicians specialized in Oncology, which is a branch of medical science dealing with tumors.

Ideally, cytotoxic drug therapy (2) aims at the elimination of all tumor cells from the body with minimum toxic effects. In actual practice, however, the complete eradication of cancer cells is seldom possible with doses that the patient can tolerate (3). Moreover, these cytotoxic drugs which are employed in chemotherapy are not very selective of tumor cells. Often they are as toxic to normal cells as to tumor cells. Treatment with these drugs requires a careful assessment of the advantage of their use to the patient versus their toxic side-effects.

The antineoplastic agents utilized now a days are generally palliative in their effects. They do not serve to cure cancer but only to control it in some ways. Studies show (4, 5) that about 50% of patients suffering from these diseases, are able to achieve normal life expectancy when treated by modern chemotherapy (6). A judicious combination of several cytotoxic drug is found to be more effective than treatment with a single drug, as the toxic side-effects are thereby reduced and antitumor action is enhanced. (7)

Genetic factors are important in the aetiology of cancer (8), in particular for patients with multiple primary cancer, given the prolonged exposure of these subjects to carcinogens these genetic factors may have a role in modifying carcinogen factors which may be important in persons with environmentally induced cancers (10-11). Recurrence of the tumors is also seen in some cases. (12-14)

Electrochemical studies on anticancer drugs have been reported in some reputed reviews of drugs and chemical substances of biological significance (15-17).

A brief summary of electrochemical studies of the drugs relevant to the present study is given in the following paragraphs:-

The quinone containing antitumor antibiotics mitomycin-B and mitomycin-C, were studied using dc polarography and cyclic voltametry by Rao and others (18-19).

Methotrexate or A-methopterin was studied using dc polarography by Asahi (23). Recently, folic acid (of which methotrexate is an analogue) has been determined by Zhang et al (24) using differential pulse polarography. Sz Cz epaniak and Ren (25) have also successfully used adsorptive stripping voltametry for the determination of folic acid in pharmaceutical preparations. Mechanism of reduction of methotrexate is given in analytical profiles of drug substances (26).

Palecek and others (27) studied the anodic polarographic current given by fluorouracil, while Yan et al (28) carried out the determination of 5-fluorouracil in blood serum using differential pluse cathodic stripping voltametry (DPCSV) following sample pretreatment with trichloroacetic acid.

AC polarography has been used to determine the decomposition kinetics of mercaptopurine by Parrak and Tuckerman (39). According to the literature (30) the oxidation of 6-mercaptopurine at the DME takes place, yielding anodic polarograms. Berek determined 6-mercaptopurine using polarographic and voltametric methods (31).

There are numerous applicability of amperometric method of analysis (32) widely used for the determination of metal ions and organic compounds.

In our laboratory procedures leading to the amperometric determination of some rare earths and transition metal ions have been developed using some reagents of modern analytical importance, some antibiotics and sulfa drugs (33-40).

Agrawal (41) determined arsenic with 2-mercapto and 3-mercaptopropanoic acid amperometrically. Recently amperometric studies of complexes of dysprosium (III) with rhodamin B, has been reported by Rathore (42).

Halbert and Baldwin (43) reported an amperometric determination of 6-mercaptopurine in blood plasma with a cobalt phthalocyanine chemically modified electrode after liquid chromatography.

Various other methods have also been reported in the literature for the estimation of anticancer drugs (44) in thier pharmaceutical formulation and in different forms.
Assadullahi et al (45) determined methotrexate in serum by HPLC. Brand steterova et al (46) determined it in clinical samples by micro HPLC. Gandhi et al (47) determined methotrexate in pharmaceutical formulation by spectrophotometric method.

Banerjee et al (48) reported colorimetric method for the determination of 5-fluorouracil in different forms.

Bates et al (49) analysed 5-fluorouracil in human plasma by chromatographic methods. Sawant et al (50) analysed 5-fluorouracil in microcapsules.

Gorog determined 6-mercaptopurine by difference enzyme spectrophotometry (51). Das et al (52) studied dacarbazine and lomustine electrochemically. Taneja et al (53) analysed 6-mercaptopurine content in dosage form using difference spectrophotometric method.

The capacity of incomplete d-subshell of the transition metals has introduced millions of compounds in the literature. Many new complexes are prepared and characterised in the world of science. As a result of this fact a number of research journals mainly devoted to the subject are published regularly. Recently the subject has been beautifully reviewed in "Metal ligand interaction : Structure and reactivity" (54). Overall coverage of this book is based on the transition metal species, several articles are based on theoretical modelling of interactions. In the context of bioinorganic chemistry modelling for metal-ligand interactions is presented for Zn(II), its activity of relevance to the metalloenzyme, carboxypeptidase.

Use of metal coordinate complexes in cancer chemotherapy has been known. These compounds have been identified as highly cytotoxic and tumor reducing (55). Cisplatin was found to be the most active of compounds in this category (56) and it is capable of cross-linking with DNA (57). Cisplatin is useful in carcinoma of the bladder and carcinoma of head and neck (58).

Petering had proved that Cu (II) alone is neither cancer toxic nor has any antitumor activity (59). It has been reported that the chelate could be active agent against the tumor growth (60, 61).

Transition metal complexes with various organic ligands have been studied by a number of workers (62-70).

Ternary complexes of Zn (II) with some L - amino acids and nicotinic acid have been studied polarographically by A. K. Jain et al (71). Study of Zn (II) reduction at the Hg electrode from water, ethanol and water, acetone mixture in the presence of thiourea has also been studied (72).
Sharma (73) studied the complexes of azo derivatives of transition metal by polarographic method. Polarographic determination of structural aspects and formation constants between Zn(II) and amino acids and propionic acid have been reported (74).

Polarographic estimation of Cu(II), Pb(II) and Zn(II) in the presence of 2-amino, 3-hydroxypyridine as complexing agent has been reported by Singh et al (75) Polarographic behaviour of Ternary Cd(II) complexes with L-amino acids and picoline has also been reported (76).

Rao, et al (77) determined cadmium after adsorption of its piperazine, dithiocarbonate complex onto microcrystalline naphthalene by differential pluse polarographic method. Ternary complexes of some sulphadrugs and salicylaldehyde with Cu(II) and Fe(III) have also been reported (78).

Metal complexes are well known for their biological activity (79). The importance of sodium, potassium, calcium, iron etc. has been well recognised. In blood haemoglobin, an iron complex of protein is present (80). A number of metal ions are essential for life process, some are present in the structural units of our body while others are used in metabolic activities in the form of enzymes. Many enzymes contain metals and removal of metal inactivate the enzyme. Trace amounts of Cu, Zn, Co, Ni etc are necessary for life processes (81). The chemistry of life involves in an essential way, some chemical elements including metals (82, 83). Investigation on the chelation reaction of anticancer compounds, Riboflavin (84) Folic acid (85) Thioquinone (86), Adenine (87) and carcinogenic compounds, amino and napthol (88) have been reported and chelating properties have been discussed in the light of thier anticancer activities.

Sobathiya et al (89) have investigated the nitrogen, oxygen and sulphur containing heterocyclic rings and suggested that they may be potent antibacterial substance. N, S and O containing heterocyclic compounds were tested in-vitro for biological activity against gram positive and gram negative bacteria (90, 91). Most of the Cu (II) complexes were found to be active and their activities were more than those of the free Cu (II) and ligand against gram positive and gram negative bacteria (92, 93).

Mishra et al (94) reported antibacterial study of some 3d-metal complexes with thiophene 2-aldehyde-4chloro/bromoaniline. Microbial studies on complexes of heavy metals ( particularly those belonging to the transition series ) have also been studied by some scientist (95,96).
In recent years considerable attention has been focused on the development of new drug delivery systems. This is evidenced by the spate of books and reviews published on new systems (97-105).

Recognition of the possibility of repatenting successful drug by applying the concepts and technique of controlled release drug delivery systems in bringing new drug entities to market has encouraged the development of new delivery systems.

Azmin (106) investigated the effect of niosomal entrapment on the pharmacokinetics of methotrexate in mice in comparison to 6% polysorbate to 80% solution of drug. Pharmacokinetic parameters of methotrexate increased but it seemed dependent on methotrexate concentration.

R. Jeyanthi (107) reported three potent anticancer chemotherapeutic agents namely, 5-fluorouracil, bleomycin and mitomycin which are entrapped in the collagen-poly (Hema) hydrogel matrix. The entrapment efficiency of these drugs varied in the order: mitomycin > 5-fluorouracil > bleomycin. In vitro release studies of these drugs from the freshly prepared hydrogel containing 40% of water were carried out in phosphate buffer, pH = 7.4 at 37°C. The release rates were found to be independent of time and the release profiles followed zero-order methotrexate drug has been studies in different delivery systems from different views (108-115).

Controlled release of 5-fluorouracil from linear or ortho esters has been studied by Maa et al (116).

Takakura (117) reported physicochemical properties and antitumor activities of polymeric prodrugs of mitomycin with different regeneration rates.

Hydrolytic and enzymatic stability of macromolecules of mitomycin derivatives (118). Synthesis and evaluation of macromolecules prodrugs of mitomycin have also been reported (119).
MATERIALS:

All the chemicals used to prepare experimental sets were of Analar / BDH grade. Sulphate of Zn (II), Nitrate of Ni(II), Chlorides of Co(II) and Fe(III) were used and their 0.1M stock solutions were prepared by dissolving a requistic amount of the respective salts in conductivity water. The stock solutions of these metal ions where standarised by known methods(120). The stock solutions of 2M potassium chloride was prepared in conductivity water. The maximum suppressor, gelatin (0.1%) was prepared in hot distilled water. The gelatin solution was prepared afresh every third day.

The drugs, 6-Mercaptopurine [ M/S Burrough's Wellicom (India) Ltd.], Methotrexate [ Lederle Laboratories Division, American Cynamid Co. New York, U.S.A.], Mitomycin [Bristol Laboratories EVANSVILLE U.S.A.] and 5-Fluorouracil [ National Institute of Health, U.S.A.] were obtained from their concerns [ mentioned in brakets]. The stock solutions of these drugs were prepared by the following methods:

1. **6-Mercaptopurine** \([C_5\,H_4\,N_4\,S\cdot H_2O, \text{purine-6-thiol mono hydrate}, \text{Mol. Wt.}=170.2]\)
   Solution (121) : 0.01M stock solution of 6-mercaptopurine was prepared by dissolving the weighed amount of the drug in conductivity water, adding 0.2N NaOH solution till the turbid solution becomes fairly clear and making upto the volume with conductivity water.

2. **Methotrexate** \([C_{20}\,H_{22}\,N_8\,O_5, \text{N-[4-[(2,4-Diamo no-6- pteridinyl )methyl] amino]benzoyl]} - L-glutamic acid, 4-amino 10-methylic acid; \text{Mol wt} = 454.56]\) solution (121) : 0.01M stock solution of methotrexate was prepared by dissolving the weighed amount of the drug in minimum quantity of 0.1N Na_2CO_3 solution and then making upto the volume with conductivity water.

3. **Mitomycin** \([C_{15}\,H_{18}\,N_4\,O_5 [1\alpha - (1\alpha\alpha, 8\beta, 8\alpha\alpha, 8\beta\alpha)]-6- \text{amino -8-}[ (aminocarbonyl) oxy] \text{methyl}]) - 1, 1a, 2, 8, 8a, 8b, hexahydro -8a- methoxy -5-methylazirinol [ 2', 3' : 3, 4] pyrrolo [ 1,2 - a] idole -4, 7 - dione; \text{Mol. wt} = 334 ] solution (121) : 0.01M stock solution of mitomycin was prepared by dissolving the weighed amount of mitomycin in conductivity water.
(4) 5-Fluorouracil [C₄H₅N₂O₂, 2, 4 (1H, 3H) pyrimidinedione, 5-fluoro, Mol wt=130.08 ] solution (121): 0.01M stock solution of 5-fluorouracil was prepared by dissolving a weighed amount in conductivity water and minimum quantity of ethyl alcohol and then making up to the volume with conductivity water.

ELECTROCHEMICAL STUDY:

EQUIPMENTS FOR POLAROGRAPHIC STUDY:

All the polarograms were recorded on an Elico (India) plus e polarograph model CL-90, which was coupled with X-Y polarocardiograph model LR-180. The polarographic cell comprising of three electrode system i.e. a dropping mercury electrode (dme) used as working electrode, a saturated calomel electrode (SCE) used as a reference electrode and the auxiliary electrode which was coiled platinum wire. The dropping mercury electrode had the following characteristics, a mercury flow rate = 2.377 mg / sec (m²/sec t¹/⁶ = 2.136 mg²/³ sec⁻¹/²) at 140 cm effective height of the mercury column. In polarographic measurements triple distilled mercury was used.

All the pH-measurements were carried out on an Elico digital pH-meter, model L I-120. The pH of experimental sets were adjusted to the desired value by the addition of necessary amount of dilute Hydrochloric acid/dilute Sodium Hydroxide solution. All the measurements were carried out at room temperature. Purified Hydrogen gas was bubbled through the test solution for 5 minutes. pH was rechecked before recording polarograms.

POLAROGRAPHIC STUDY OF METAL-DRUG COMPLEXATION EQUILIBRIA:

Experimental sets were prepared by keeping overall metal ion concentration fixed at 1.0mM with varying concentrations of drug (0.5, 1.0, 1.5, 2.0, 5, 10mM) in 1.0M KCl, supporting electrolyte +0.001% solution used as maximum suppressor. The pH of the experimental sets was adjusted as required by the addition of necessary amount of dilute Hydrochloric acid / Dilute Sodium Hydroxide solution. The ionic strength of the test solution was adjusted to μ = 1.0, with potassium chloride solution.
EQUIPMENTS FOR AMPEROMETRIC STUDY:
Amperometric titrations of Zn(II), Co(II), Ni(II) and Fe(III) with anticancer drugs i.e. 6-mercaptopurine, methotrexate, mitomycin and 5-fluorouracil were separately performed on a manually operated assembly comprising, of an AJCO Vernier potentiometer attached to a polyflex galvanometer (sensitivity 8.10 x 10^{-6} amp / div). A dropping mercury electrode (dme) was used as working electrode and a saturated calomel electrode (SCE) served as reference electrode. The capillary characteristic of dme was \( m^{2/3} t^{1/6} = 2.136 \text{ mg}^{2/3} \text{ sec}^{-1/2} \) at 140 cm effective height of mercury column. Triple distilled mercury was used in amperometric measurements.

AMPEROMETRIC STUDY ON METAL-DRUG COMPLEXATION EQUILIBRIA
For amperometric titration of metal ions with anticancer drugs, experimental sets containing varying concentrations of the metal ions (overall, 0.5, 1.0, 1.5 and 2.0 mM) in 1.0 M potassium chloride as supporting electrolyte and 0.001% gelatin as maximum suppressor, were prepared pH of the test solution was adjusted, with the help of dilute Hydrochloric acid / dilute Sodium Hydroxide solution, to the desired value. The test analyte deaerated using purified hydrogen gas and its pH was recheked before performing amperometric titrations. The plateau potential of each metal ion was fixed on the potentiometer and current was read on the polyflex galvanometer. Amperometric titrations were then performed by gradually adding definite aliquots of the titrant drug of the same pH as that of titrate, the current changes were read on the galvanometer with each addition. The current so obtained after volume correction \( (V+v / V) \) was plotted against the volume of titrant added. The end point of the titration was determined by the point of intersection of the lines. For the estimation of some drug content of pharmaceutical formulation viz. Purie-nethol, Biotrexate, Mutamycin and Fluracil injection. The first three were dissolved in conductivity water. Whereas fluracil injection was dissolved in 20% alcoholic conductivity water. These drug solutions were converted into analyte for amperometric titration following the procedure as mentioned above.
SYNTHESIS AND CHARACTERISATION OF SOLID COMPLEXES:

SYNTHESIS PROCEDURE:

As per literature the complexes may be synthesised by one of the following procedures:

(1) Metal Sulphate / Nitrate / Chloride and drug solution in alcohol were mixed in 1:1 or 1:2 (as the case may be) molar ratio. The mixed solution was transferred to the flask of refluxing assembly. The mixture of the two solutions was then refluxed for one-two hours. The precipitate formed was filtered and washed thoroughly to remove any unreacted materials and the complex was dried at 40°C and stored over P₄O₁₀.

(2) Metal sulphate / Nitrate / Chloride and drug solutions were mixed in 1:1 or 1:2 (as the case may be) molar ratio. The solution was transferred in a beaker and kept over water bath for an appropriate time so that the volume was reduced to one fourth or less, of the initial volume. The mixture was kept standing over night. Precipitate so obtain was filtered, washed and dried over P₄O₁₀.

(3) Metal Sulphate / Nitrate / Chloride and drug were dissolved separately in distilled water. The metal and drug solutions were mixed in 1:1 or 1:2 (as the case may be) molar ratio and the solution was transferred in a beaker and pH of the solution was adjusted to appropriate value with dilute NH₄OH solution. The solution on standing for a few days resulted in a precipitate which was washed, dried and stored over P₄O₁₀. The author has chosen procedure (2) for the synthesis of solid complexes in the present study.

ANALYTICAL STUDIES ON COMPLEXES:

The refluxed metal-drug complexes were characterised by elemental analysis and physical measurements.

(1) Estimation of Metal ions: A weighed amount of complex was repeatedly evaporated to dryness with small amount of concentrated Nitric acid. The digestion is continued till the residue gave transparent clear solutions with Hydrochloric acid. In this solution metal was estimated complexometrically by EDTA titration (122)
using appropriate buffer to maintain the pH as desired and specific indicator to determine the accurate end point.

(2) Estimation of Non-metals: Carbon, hydrogen, nitrogen and sulphur in the complex compounds were estimated with the help of elemental analyser mode 29/33 at Central Drug Research Institute, Lucknow (U.P.). The results of which were furnished to the author by the institute.

SPECTROPHOTOMETRIC STUDY:

In spectrophotometric analysis of each anticancer drug and its metal-drug complexes were recorded in KBr phase using Perkin-Elmer, ir spectrophotometer model-397.

Preparation of KBr Pallets: A small amount of finely grounded solid sample was intimately mixed with about 100 times or more than its weight of potassium bromide powder. The finely grounded mixture was then pressed under very high pressure in a press (about 10/cm²) to form a small pallet (about 1 - 2 mm thick and 1cm in diameter).

ANTIMICROBIAL SCREENING:

The main intention of these investigation was to study the change in the toxicity of drug when they form complexes with life essential metals viz Zn (II), Co (II), Ni (II), Fe (III) etc. Raper’s (123) method was used to evaluate the activity of complex compounds. Raper’s paper disc method consists of the following steps:

1. Preparation of the medium.
2. Treatment of the glass apparatus and its sterilisation.
3. Pouring of the needed medium into sterilised petridishes.
4. Preparation of the required concentration of complexes and their pouring into sterilised filter paper discs (diameter 6mm).
5. Incubation at a particular temperature.
6. Measurement of the zone of Inhibition.

Some factors that affect the toxicity test are as under:

1) The kind and condition of the test organism.
2) The concentration of the drug solution and the site of action.
3) Environment factors which may counteract the interaction of the drug and the parasite.
4) pH of the medium for bacteria which is usually in the range of 7.2-7.6.

5) Temperature of the incubator fixed (because, for each bacteria there is an optimal temperature for most of the pathogenic bacteria.

The nutrient agar medium with the following composition was used for preparing the slants and agar plates:

- Peptone: 5.0gm
- Beef extract: 1.0gm
- Sodium chloride: 5.0gm
- Dextrose: 10.0gm
- Yeast extract: 2.0gm
- Agar-Agar: 20.0gm
- Distilled Water: 1000ml
- pH: 7.2 - 7.6

For the preparation of the medium all above ingredients except agar-agar were weighed and dissolved in water (500ml) with gently heating. When all the ingredients were dissolved completely, more distilled water (500ml) was added to this solution, a weighed quantity of agar-agar was added and the mixture autoclaved for half an hour at a pressure of 1.05kg / cm² and temperature of 121°C. The hot medium was filtered through cotton to obtain a clear solution. All the glass apparatus was cleaned with chromic acid and then sterilised by keeping in an oven. Medium was cooled to 40°C and homogeneous suspension was prepared by transferring aseptically micro-organism from fresh sub-culture into the agar medium, followed by vigorous shaking. 20ml of this medium was poured into each sterilised petridish under aseptic condition and allowed to gel. After gelling of medium the paper disc (6mm diameter) is fixed in seeded agar plates. 0.1ml of test solution of 0.1M concentration was dropped onto the filter paper disc. Petriplates were incubated at the foresaid temperature for 24 hrs. The inhibition zone for each test solution was than measured in mm.

**PHARMACOLOGICAL STUDY:**

The influence of the complex on the anticancer response of pure drug is assessed by the two methods:

(i) In-Vitro Pharmacological Screening.

(ii) In-Vivo Pharmacological Screening.
IN - VITRO:

Cell viability is measured by trypan blue exclusion test (124) which is based on the ability of trypan blue to stain dead cells. A drop of culture is added on haemocytometer and the number of stained, non-stained and total number of cells were counted and the percentage inhibition is calculated as under:

\[
\text{Percentage Inhibition} = \frac{\text{Total number of viable cells} - \text{Total number of viable cell after treatment}}{\text{Total number of viable cells}}
\]

Sarcoma-180 cells were purchased from the National Centre for Cell Science (NCCS), Pune, maintained in DMEM medium (Dulbecco’s modified Eagle’s medium) supplemented with 10% v/v foetal calf Serum, penicillin 100 IU / ml and streptomycin 100 mg / ml. Cells were obtained as monolayer culture in plastic Roux bottels (Corning plastics).

Cells were harvested using Trysin Versin Glucose in the exponential growth phase from the Dulbecco's modified egles medium preincubated at 37°C for 24 hrs. The cells were centrifuged to adjust starting cell concentration to 2 x 10^5 cell / ml. One ml of concentrated cell suspension was seeded into multiwell culture plate (corning plastics) (2 x 10^5 cells / well). A 0.5 ml of DMEM was added to each well and incubated with metal-drug complex containing varying concentrations. It was compared with cells without complex containing similar supplements the plate was incubated at 37°C in incubator at 95% RH and 5% CO2 atmosphere, cell counts were made on haemocytometer (Neubour Chamber) before the start of experiment and after 2, 4, 6 hrs of the begining the experiment.

IN - VIVO:

Inbred male BALB / m mice 6-8 weeks of age, weighing about 18-20g, are selected and incubated with sarcoma-180 tumor cell line. The mice then divided into two groups (10 mice in each group). One week after tumor incubation the first group of mice is administered dose of pure drug 2 / kg (125) intravenously, second group of mice was administrsted the same dose of metal-drug complex.
The mice are observed for one month after drug treatment for macroscopic sign of tumor and toxicity. The tumor volume is measured using vernier calipers. The tumor volumes thus measured are plotted against time and the test control ratio (T/C ratio) of the tumor volume is also compared. In the present study only in-vitro pharmacological test have been carried out.
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