Chapter - 2

REVIEW OF LITERATURE

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2.1 CANCER

Cancer is one of the most dreaded diseases in the world today, second only to the latest terror for mankind - AIDS. Cancer has assumed second place on the list of fatal diseases, preceded only by the disorders of the heart and blood vessels.

The word **cancer** is derived from Latin, meaning 'Crab' and this word was coined by Hippocrates to suggest its capacity to reach out and cling tenaciously to adjacent tissues.[1]

Scientifically, cancer is described as a new tissue growth or neoplasm in which undifferentiated cells proliferate uncontrollably.[2] This is commonly referred to as a malignant tumour. Malignant tumours are characterised by irregular, uncontrolled, rapid growth rate, undifferentiated cells, invasiveness and metastasis, as opposed to the benign tumours which are more or less well differentiated, slowly growing, non-invasive and which do not metastasize.

In normal cells, there are growth regulatory mechanisms which maintain a steady rate of cell division.[3] This regulatory mechanism breaks down in cancerous cells and tissues which start growing uncontrollably. Due to their rapid growth rate, they expend most of

their energy, consequently neglecting their more specialized functions. Thus, they tend to revert back, both structurally and functionally, to a more primitive, undifferentiated state. This process is called de-differentiation.

In their haste to grow and proliferate, the cancer cells encroach upon the territorial boundaries of neighbouring cells, actively invading and penetrating between them, usurping their nutrients and vascular supply and utilizing their resources to support their own growth. This invasiveness is the cause of the ultimate destruction and death of the normal cells. If a malignant tissue invades blood vessels or lymphatics, the cells are carried to other parts of the body away from the site of origin and they may proliferate there to form secondary tumours, the process being known as **metastasis.**

Hence, the objective of cancer treatment is : tumour regression or cure, prevention of metastasis and improving the quality of life by reducing pain and other symptoms.

Conventional methods of cancer treatment are surgery and radiotherapy.[2] Surgery is preferred for cancer of focal origin i.e. which is confined to its site of origin, from where it can be surgically removed e.g. for benign tumours. Radiotherapy is preferred when some cancer cells are more sensitive to radiation than the surrounding normal tissues, so that the tumour is destroyed by a dose of radiation which leaves the normal tissues relatively

unharmed. But both these treatments are able to provide cure or remission in only one-third to one-half of the total cancer patients. The void is tried to be filled by **Cancer Chemotherapy** which involves the use of drugs which act specifically on the cancer cells, irrespective of whether they are localized or dispersed in the body. But cancer chemotherapy is much more difficult because, unlike microbial cells, which are structurally, functionally, chemically and nutritively different from the host cells, the cancer cells are similar to, and in fact, originate from the host cells. Hence, therapeutically effective doses of anticancer drugs are toxic to both, neoplastic as well as the normal cells.[4] In fact, this is the main drawback of cancer chemotherapy; although many anti-neoplastic agents are highly potent, their non-selectivity makes them toxic for the normal host cells; and because most of these drugs act on rapidly proliferating cells, normal cells growing rapidly, like the cells of the bone marrow, intestinal epithelium, hair follicle etc. are also subject to extensive damage by these drugs. Thus, their toxicity, non-selectivity and adverse effects limit their usefulness. In addition to this, the immune mechanism is active and plays a major role in the treatment by antimicrobial chemotherapy, whereas in cancer chemotherapy, the immune defences are suppressed and hence cannot play a major role in the treatment.

2.2 THE CELL CYCLE

The cell cycle is defined as the period from one mitosis to the next. All cells, normal or abnormal, pass through various phases of life cycle [5](Fig.1). They are :

i) G_0 - phase
ii) G_1 - phase
iii) S - phase
iv) G_2 - phase and
v) M - phase.

(i) **G_0 - phase**

This is also called the resting phase or the non-proliferative phase. The cells remain in a dormant or resting state in this phase for variable periods of time.

(ii) **G_1 - phase**

This is also known as the pre-synthetic phase. The cells prepare for DNA synthesis during this phase. A marked increase in RNA synthesis is observed towards the end of this phase. This is the most variable period of the entire cell cycle and hence is the rate limiting phase. Slowly growing cells have longer G_1-periods whereas fast growing cells have shorter G_1 - periods.

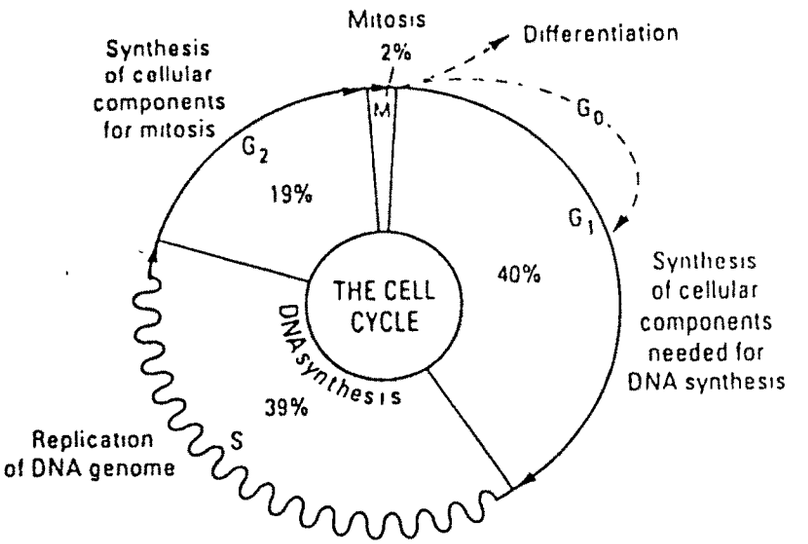


Figure 1 The cell cycle and cancer. A conceptual depiction of the cell cycle phases that all cells—normal and neoplastic—must traverse before and during cell division. The percentages given represent the approximate percentage of time spent in each phase by a typical malignant cell, the duration of G_1, however, can vary markedly

(iii) **S - phase**

It is also known as the synthesis phase. DNA synthesis takes place in this phase.

(iv) **G_2 - phase**

This phase is also called the post-synthetic phase, wherein the cells prepare for cell division or mitosis.

(v) **M - phase**

This phase is commonly referred to as the mitosis phase. In this phase, cell division occurs, resulting in the creation of two daughter cells from each cell.

The tumour cell cycle differs from the normal cell cycle in that it has variable rates or periods for the different cell cycle phases.[6] For example, in a tumour cell, the S - phase may last for 10 to 20 hours, the G_2 - phase may continue for 2 to 10 hours, the M-phase may last upto 1 hour and the G_1 - phase can continue for a few hours or upto a few days. The resting phase or the G_0 - phase in a tumour cell cycle can be zero or it may last for a few hours or even upto a few days.

2.3 CANCER CHEMOTHERAPY

Attempts to cure cancer employ four principle modes : **surgery, radiotherapy, chemotherapy** (including hormonal therapy) and

immunotherapy.[7] Chemotherapy involves the use of drug substances which selectively kill the cancerous cells. Chemotherapeutic agents used in the treatment of cancer are referred to as antineoplastic agents, cytotoxic agents, anticancer agents or carcinostatic agents.

The proper use of cancer chemotherapy needs full understanding of the cell-cycle kinetics. In general, cancer chemotherapeutic drugs are most active against actively cycling cells i.e. cells with a high growth fraction, and least active against resting cells which have a very low growth fraction.

2.3.1 Classification

Cancer chemotherapeutic agents are classified either depending on their activity or on the basis of their physicochemical properties and their source.

Depending on their activity, antineoplastic agents can be broadly classified as :

i) Cell Cycle Specific Agents

ii) Cell Cycle Non-specific Agents.[8]

i) **Cell Cycle Specific Agents (CCS)**

These drugs destroy the cells only during a specific phase of the cell cycle and are more effective on tumours with a high growth fraction e.g. haematologic malignancies. Drugs falling in this category are :

a) Antimetabolites - e.g. cytarabine, 5-fluorouracil, mercaptopurine, methotrexate, thioguanine etc.

b) Antibiotics - e.g. the bleomycin peptide antibiotics.

c) Alkaloids - e.g. etoposide, teniposide, vincristine, vinblastine.

The antimetabolites are more effective during the S - phase of the cell cycle whereas the bleomycin antibiotics and the vinca alkaloids have better action during the G_2 - phase and the podophyllotoxin alkaloids exert their activity during the M - phase of the tumour cell cycle.

ii) **Cell Cycle Non-Specific Agents (CCNS)**

These drugs can destroy the cells during several or all phases of the cell cycle and hence are more effective on tumours with a low growth fraction.

Drugs in this category are :

a) Alkylating agents - e.g. busulfan, cyclophosphamide, melphalan, thiotepa etc.

b) Antibiotics - e.g. dactinomycin, daunorubicin, doxorubicin, mitomycin etc.

c) Nitrosoureas - e.g. carmustine, lomustine, semustine etc.

d) Others - e.g. cisplatin, carboplatin etc.

The Nitrogen mustard alkylating agents and the Nitrosoureas are effective during the G_0 or the resting phase also. Hence, better results can be achieved by combining two or more drugs having the ability to attack the tumour cells at various stages of cell cycle.

Antineoplastic agents are more commonly classified on the basis of their chemical structure and origin as under :

i) Alkylating agents,

ii) Antimetabolites,

iii) Natural products,

iv) Hormonal agents and

v) Miscellaneous agents. [2]

i) **Alkylating Agents**

These agents affix an alkyl group to cellular DNA, causing crosslinking of DNA strands that triggers cell death. They are cell cycle non-specific and kill dividing as well as non-dividing cells. They are further subdivided into five groups :

a) Nitrogen mustards e.g. chlorambucil, cyclophosphamide, ifosfamide, mechlorethamine, melphalan and uracil mustard

b) Alkyl sulfonates e.g. busulfan

c) Nitrosoureas e.g. carmustine, lomustine, streptozocin

d) Triazenes e.g. dacarbazine

e) Ethylenimines e.g. thiotepa

ii) **Antimetabolites**

They are cell cycle specific agents and are most effective during the S - phase of the cell cycle. These agents impair the synthesis of purine and pyrimidine bases by interfering with folic acid metabolism or prevent incorporation of the bases into nucleic acids.[9] These agents include the following categories :

a) Folic acid analogues e.g. methotrexate

b) Purine analogues e.g. mercaptopurine, thioguanine

c) Pyrimidine analogues e.g. cytarabine, floxuridine, 5-fluorouracil

iii) **Natural Products**

This group includes compounds of natural origin, all having different mechanisms of action :

a) Vinca alkaloids e.g. vincristine, vinblastine

b) Epipodophyllotoxins e.g. etoposide, teniposide

c) Antibiotics e.g. bleomycin, dactinomycin, daunorubicin, doxorubicin, mitomycin, plicamycin etc.

d) Enzymes e.g. L-asparaginase

iv) **Hormonal Agents**

They act mainly by altering the hormonal environment of hormone-dependent tumours e.g. leukemia, breast cancer and endometrial cancer. These agents include the following categories :

a) Adrenocorticosteriods e.g. prednisone, prednisolone, dexamethasone etc.

b) Progestins e.g. hydroxyprogesterone, medroxyprogesterone, megestrol

c) Androgens e.g. testosterone, fluoxymestrone

d) Estrogens e.g. estradiol, diethylstilbesterol

e) Antiestrogens e.g. tamoxifen

f) Antiandrogens e.g. flutamide

g) Gonadotropin - Releasing Hormone analogues e.g. leuprolide

v) **Miscellaneous Agents**

These include :

a) cisplatin, carboplatin - they cross-link DNA strands

b) hydroxyurea - it inhibits ribonucleoside diphosphate reductase enzyme, thus interfering with DNA synthesis

c) interferon alfa (2a & 2b) - these agents trigger a series of immunologic responses leading to suppression of viral replication and cell proliferation.

d) procarbazine - it damages DNA and inhibits RNA, DNA and protein synthesis

e) immunostimulants - These are substances which stimulate immune mechanisms e.g. levamisole, vaccines, specific antigens and antibodies, serum or bone marrow lymphocytes etc.

f) angiogenesis inhibitors - e.g. methotrexate, and cytokines, like, transforming growth factor β , tumour necrosis factor, α -interferon, human platelet factor 4 etc. These agents inhibit angiogenesis or neovascu-

lanization which is responsible for supplying nutrients and oxygen for tumour growth.[10]

Nowadays, polyanionic polymers are widely being tested as anti-tumour agents because they have been shown to demonstrate interferon-inducing ability.[11] Examples are natural polymers like polysulphates, polyphosphates, polynucleotides, some polysaccharides, synthetic polymers like copolymers of maleic anhydride, polyacrylic acid, polyethacrylic acid and their various copolymers.

The mechanisms and sites of action of some common antineoplastic agents are summarized in Fig. 2.

2.3.2 Log-Cell Kill Hypothesis

The cytotoxic agents kill by first order kinetics i.e. they achieve a log-cell kill. In other words, a given dose of the anticancer agent will kill a fixed fraction or percentage of the total tumour cell mass and not a fixed number. For example, a drug dose that would result in three-log-cell kill of a tumour mass would reduce the tumour cell burden from, suppose, 1×10^8 cells to 1×10^5 cells, eventhough its cytotoxicity is 99.9%. Although this will mean 'complete remission' clinically, the remaining 1×10^5 surviving cells are capable of reproducing again in a short time span and may cause a relapse.

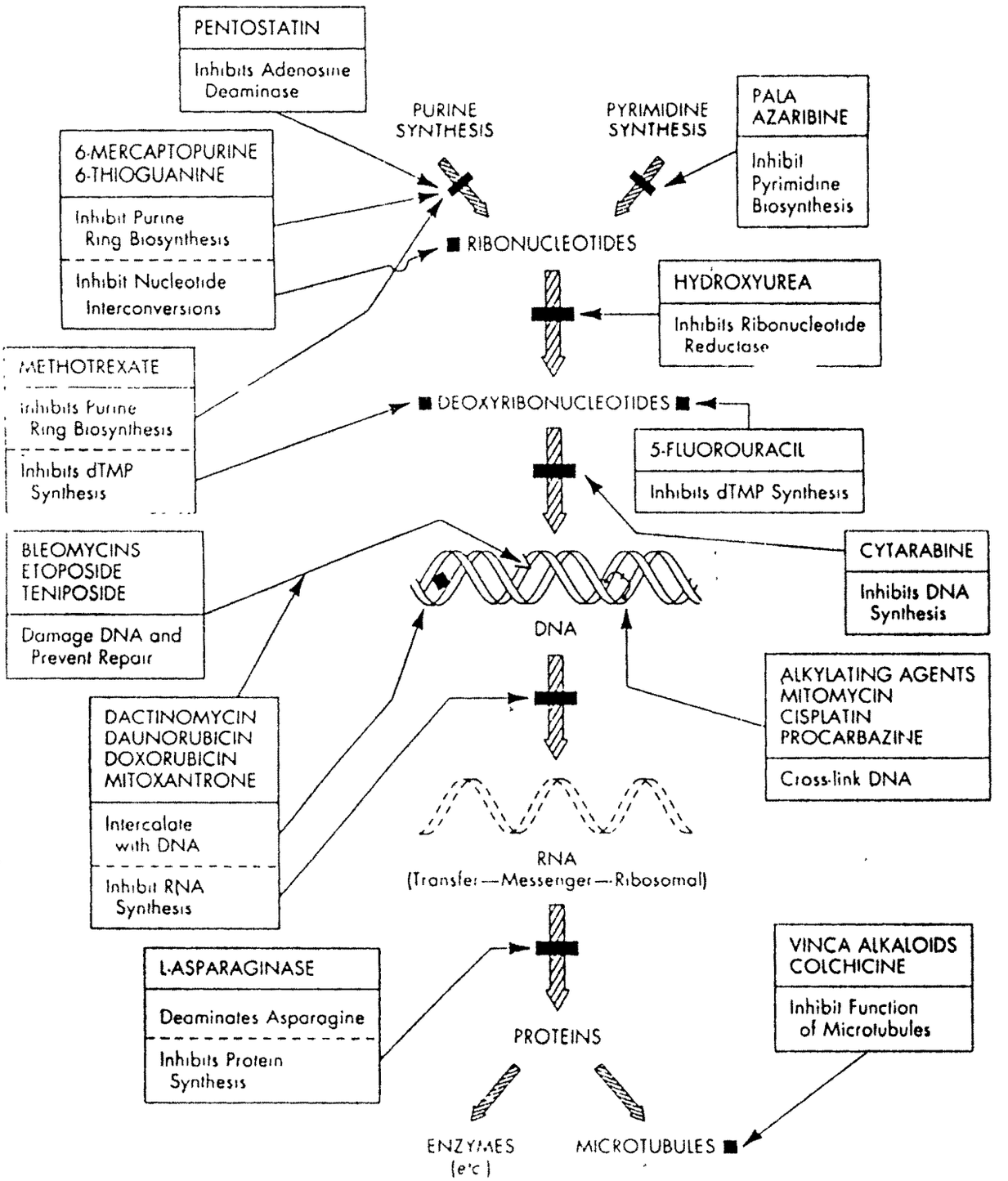


Figure 2 : *Summary of the mechanisms and sites of action of chemotherapeutic agents useful in neoplastic disease.*

PALA = N-phosphonoacetyl-L-aspartate

2.4 DRUG TARGETING

2.4.1 Importance

The concept of drug targeting was first introduced by Paul Ehrlich (1909) when he reported about a 'Magic Bullet' which can deliver a drug selectively to the desired site of action without harming the non-target organs or tissues. This can be achieved by associating the drug with a pharmacologically inactive carrier capable of conveying the drug selectively towards its target cells. This concept of drug targeting was based on the fact that most drugs are non-selective and therefore present various undesirable side effects.[12]

Drug targeting can be defined as the ability to direct a therapeutic agent specifically to the desired site of action with little or no interaction with non-target tissues.[13]

The rationale for drug targeting includes :[14]

i) The ability to reach specific cells or diseased sites in the body with concomitant reduction in the dose and side effects.

ii) To reach previously inaccessible sites or areas.

iii) To control the rate and frequency of drug dosing to the pharmaceutical receptor.

iv) To protect the drug and the body from one another until the desired site of action is reached.

The search for methods for selectively targeting antineoplastic agents has increased tremendously during the past decade because of the lack of tumour specificity, common toxic side effects and a low therapeutic index of these drugs.

Hence, the goal of a site specific drug delivery system for an anticancer agent is not only to increase the selectivity and therapeutic index, but also to reduce the toxicity of the drug.[15] While past approaches towards achieving drug specificity at cellular sites have been centred around changes in the drug structure or alteration in the route of administration, present research is oriented towards the development of improved and unique drug carriers which act like 'guided missiles'[16] or 'magic bullets'[17] that can deliver a drug to a specific site in the body.

Characteristics of an ideal drug carrier for site specific drug delivery are :[16]

i) It should be able to deliver the drug selectively to the desired site of action from the site of administration.

ii) The drug-carrier conjugate should remain inactive during transport but should be able to activate the drug on reaching the target site.

iii) The carrier should interact with specific binding sites on the surface of the target cells.

iv) The carrier should not be able to penetrate through the cell membranes.

v) The carrier should be non-immunogenic and biocompatible.

Hence, the objective of any cancer treatment should be :

i) total-cell kill,

ii) to induce treatment regression or complete cure,

iii) to improve the quality of life by reducing pain and other related symptoms,

iv) to prevent metastasis.

2.4.2 Combination Therapy

In order to achieve total-cell kill using single drug therapy, a relatively higher dose of the drug will be needed at a more frequent dosing interval. But this will in turn increase its toxicity beyond the limit of patient tolerance. At the same time, repeated administration of high doses of the drug will lead to the development of drug resistance. Hence, it is better to combine two or more drugs which will not only improve the chances of achieving total-cell kill, thereby increasing the efficacy of the treatment, but will also reduce the chances of development of resistance to a particular drug. Generally, the drugs used in combination therapy should have different mechanisms of action so that a synergistic or an additive effect is achieved; e.g. for gastrointestinal carcinomas,

the FAM regimen is widely recommended (FAM - Fluorouracil, Adriamycin (doxorubicin) and Mitomycin) whereas the MOPP (Mechloroethamine, Oncovine (vincristine), Procarbazine and Prednisone) regimen is widely used for the treatment of Hodgkin's disease. For better results, sometimes adjuvant therapy like surgery, radiation and/or immunotherapy are also used, especially for tumours which have a propensity to spread e.g. breast cancer.

In spite of all this, chemotherapy can cure only 17% of the patients suffering from cancers. It is effective in the treatment of cancers like testicular cancer, choriocarcinoma, Burkitt's lymphoma, Wilm's tumour, acute lymphoblastic leukemia, prostatic cancer and Ewing's sarcoma. However, chemotherapy is ineffective by conventional means in cervical and uterine carcinomas, melanoma, small cell lung cancer, gastrointestinal carcinomas, hepatoma, renal carcinoma etc., and specialized systems for selective or site specific delivery of drugs is needed in order to improve the chances of remission in such cases.

2.4.3 Methods for Drug Targeting

The development of effective drug targeting systems is dependent on a proper understanding of exploitable mechanisms that will give rise to selectivity.

There are five different approaches to drug targeting :

i) The Chemical Approach or the Prodrug Concept,

ii) Compartmental Delivery,

iii) Natural or Passive Targeting,

iv) Ligand Mediated or Active Targeting, and

v) The Physical Approach.[18]

i) **The Chemical or Prodrug Approach**

A prodrug is a drug derivative with no intrinsic pharmacological activity but which can be transformed by a chemical or enzymatic process to yield the pharmacologically active drug at the appropriate time or site. This is a suitable mode of designing drugs which undergo excretion or metabolism at an inadequate or very rapid rate or when it is desired to improve the selectivity and therapeutic index and/or reduce the toxicity of the drug.[13]

ii) **Compartmental Targeting**

The localization of the drug in specific anatomical compartments can be achieved by intraarterial, intraperitoneal or intrathecal injection of the dosage form into the blood vessels supplying blood to the target tissue or organ or by local administration at or near the desired site e.g.

peritoneum, lymphs, lungs, joints etc.[19] But these routes are advantageous only when the total body clearance (CL_B) is greater than the blood flow rate at the particular site.

Another approach to compartmental targeting is obstruction or chemoembolization of the blood vessels supplying to the target tissue by administration of large particles (100-300 μm).[20]

iii) **Natural or Passive Targeting**

There are a number of instances where a physiological process can be exploited to provide selective delivery of drugs. For example, using **colloidal carriers** by intravenous route, greater than 90% of the system can be delivered either to the lung or the liver by judicious choice of the particle size and to a lesser extent, the nature of the carrier system.[18] Particles greater than 7 μm will be removed by mechanical filtration as they lodge in the smallest capillaries of the lungs. Particles of lesser size will pass into the general circulation where they can be trapped by the phagocytic cells of the reticulo-endothelial system (RES) i.e. liver, bone marrow and spleen, with the liver having the maximum number of macrophages. Hence, colloidal carriers can be effectively used to target drugs readily to the organs of the RES.

iv) **Active or Ligand Mediated Targeting**

Particulate carriers cannot be effectively used for targeting drugs to sites other than the RES. This is achieved by active targeting where the macrophages are tricked into ignoring the administered colloid which is then specifically directed to chosen sites by exploiting natural recognition processes in the body. For this purpose, appropriate ligands are attached to the drugs through a suitable spacer moiety e.g. covalently attaching antitumour agents to tumour-specific antibodies, antigens, immunoglobulins, glycoproteins, glycolipids and lectins.

The limitation of this approach is that the drug may not internalize into the target cell after reaching its surface, its recognition ability may be lost due to enzymatic degradation or the covalent binding may be too strong to release the drug upon reaching the site of action.[13]

v) **Physical Targeting**

This form of targeting involves the use of external influence such as temperature, pH or magnetic field to direct particles to desired sites. Both the temperature and the pH approaches are based respectively on the fact that the tumour cells have a local hyperthermia and lower ambient pH than normal tissues.

Targeting can also be defined as Carrier-dependent or Carrier-independent.[20] In the former case, the drug carrier is localized in the target tissue, taken up by the target cells and the drug is released intracellularly in a controlled manner. But in the latter case, the drug is released from the carrier extracellularly and its intracellular activity is not influenced by the carrier.

Nowadays, targeting is also classified as Total or Absolute Targeting and Partial Targeting. In total or absolute targeting, maximum fraction of the drug reacts exclusively with the cancer cells, without any harmful effects on normal cells. In partial targeting, the drug is distributed preferentially to the cancer cells and hence complete eradication of the cancer cells is accompanied by some degree of destruction of the normal cells.[19]

2.4.4 Natural or Passive Targeting

Natural or passive targeting is achieved through the use of specific carriers like **liposomes, microspheres, nanospheres, antibodies, erythrocyte ghosts, macromolecular proteins, polymers** etc. The carrier can be soluble or particulate. Soluble carriers include synthetic polymers like polylysine, polyglumatic acid, polyaspartic acid, polyethylene glycol (P.E.G.), antibodies, DNA, proteins, enzymes, hormones, lectins, albumins etc. Particulate drug carriers are colloidal systems having diameters ranging from 5 nm to 2000 nm.[21] They may be monolithic or capsular and include liposomes,

emulsions or microspheres made up of albumins, carbohydrates, gelatin and polymers viz. polyamides, polyacrylamide, poly-alkylcyanoacrylates, polymethylmethacrylate etc.

The major advantages of particulate carriers in the delivery of antineoplastic agents include low toxicity, low immunogenicity, versatility in formulation, high drug payload capacity, high potential for drug stability enhancement, relative ease of sterilization and controlled release of drug over a desired period of time.[20] The most encouraging prospect of the macromolecular carriers is that they can be effectively used to increase the therapeutic index of antitumour drugs by encapsulating them inside these carriers which can then localize with high specificity at the tumour sites.[15]

The main drawback of these systems is that they are readily recognized by the body's immune system and are captured by the macrophages of the RES, they cannot extravascate from the circulation except at the discontinuous or damaged endothelia, they are mostly non-biodegradable, they have limited access to non-phagocytic cells and they have to be administered parenterally.[14]

Encouraging results have been observed by a number of investigators using different types of drug delivery systems for targeting of antineoplastic agents :

Takahashi et al[22] reported the usefulness of a sesame oil/water emulsion (using 5% Span 80) and a multiple water-oil-water emulsion (using 5% Pluronic F 68) for delivery of bleomycin and mitomycin C in patients suffering from squamous cell carcinoma of the skin and adenocarcinoma of the breast.

Sells et al[23] entrapped adriamycin in liposomes and observed that the treatment response compared well with that of the free drug schedule but the liposomal preparation was associated with fewer side effects.

Selective delivery of 1,3-bis (2-chloroethyl) -1-nitrosourea (BCNU)[24] and mitomycin C have been successfully reported by chemoembolization of the hepatic arteries in patients with hepatic metastasis using starch microspheres.

Albumin microspheres have also been successfully used to improve the delivery of mitomycin C in case of hepatocellular cancer by using the arterial chemoembolization technique.[26]

Rolland[27] demonstrated elevated drug levels in the plasma after administration of polymethylmeth acrylic nanoparticle-bound adriamycin in hepatoma patients as compared to the free drug injection.

Use of monoclonal antibodies reportedly[28] improved the antitumour response in mice transplanted with MM46 tumours when actinomycin containing liposomes coated with the antibodies were administered intraperitoneally.

Bissery et al[29,30] tested poly (DL-lactide) microspheres of CCNU (lomustine) intraperitoneally in mice suffering from Lewis lung carcinoma and leukemia and found that the drug uptake in the lungs was greatly increased and the response by the leukemic cells was better as compared to the conventional drug therapy in both the cases.

2.5 5-FLUOROURACIL

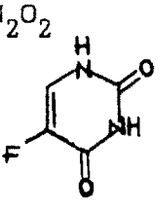
2.5.1 General Information

5-Fluorouracil (5-FU)[31-35] belongs to the class of antimetabolites, which are effective during the S - phase of the cell cycle.

Chemical Name : 5-fluoropyrimidine, 2,4 (1H, 3H) dione or 2,4 (1H, 3H), pyrimidine dione, 5-fluoro

Chemical Formula : $C_4H_3FN_2O_2$

Structural Formula :



Molecular Weight : 130.08

Melting Point : 280°C - 284°C

Description	:	White to practically white, practically odourless, crystalline powder.
Solubility	:	1 g in : 80 ml water, 170 ml alcohol, 55 ml methanol. Practically insoluble in chloroform, ether, benzene. Solubility in aqueous solutions increases with increasing pH of the solution.
Partition Coefficient	:	log $P_{octanol/water(pH\ 7.4)}$ = -1.0
Dissociation Constant	:	pKa_1 = 7.71, pKa_2 = 11.3 (at 25°C)
Standard	:	It contains not less than 98.5% and not more than the equivalent of 101.0% of $C_4H_3FN_2O_2$ calculated with reference to the dried substance.
Preparations	:	1) Injection (50mg/ml solution), having pH 8.6 to 9.0, adjusted with sodium hydroxide. 2) Topical solution or cream containing 1 to 5% of $C_4H_3FN_2O_2$.
Storage	:	Store in airtight containers, protected from light. Avoid freezing.
Stability	:	5-FU undergoes hydrolysis in alkaline solution, with probable formation of barbituric acid which rapidly degrades to other products. The shelf life of aqueous solutions of 5-FU stored at room temperature at pH 9.0 is about 3 years.

2.5.2 Physical Properties

i) Infrared spectrum of mineral oil suspension of 5-FU between cesium iodide discs measured on Perkin-Elmer 621 spectrophotometer shows characteristic peaks at 3124, 1716, 1657, 1245 and 813 cm^{-1}.

ii) Ultraviolet spectrum of 5-FU in acetate buffer (pH 4.7) exhibits a maxima at 266 nm ($E = 7.07 \times 10^4$) and a minima at 232 nm ($A^{1\%}_{1cm} = 552$)

2.5.3 Pharmacokinetics of 5-fluorouracil

(i) Absorption

The oral route is too unpredictable to be of clinical value because of variations in peak levels attained and overall bioavailability (0 to 74%, mean 28%) as 5-FU undergoes extensive first pass metabolism.[36]

The preferred route of administration for 5-FU is by rapid intravenous injection. Rapid intravenous administration of 10 mg/kg dose produces plasma concentration of 0.1 to 1.0 mM with a plasma half life of 10 to 20 minutes. Generally, the plasma levels of 5-FU decline monoexponentially between 5 and 45 minutes and return to baseline values by 1 to 2 hours.

When 5-FU is administered as a continuous intravenous infusion for 24 hours, the plasma concentrations range from 0.5 to 3.0 μM.[37,38]

(ii) **Distribution**

After intravenous injection, 5-FU is cleared rapidly from the plasma and is distributed throughout the body tissues and fluids, including the cerebrospinal fluid (CSF) and malignant effusions[39] by passive diffusion, giving a volume of distribution equivalent to the total body water. The volume of distribution (V_d) ranges from 8 to 54 litres,[40] whereas the CSF levels reach 6 to 8 x 10^{-6} M within 30 minutes, persist upto 3 hours and then decline to 1 x 10^{-8} M after 9 to 12 hours. After intravenous injection, 5-FU persists for longer periods in the malignant effusions than in the plasma.

(iii) **Metabolism**

The metabolism of 5-fluorouracil is shown schematically in Fig. 3.[41-43] The degradation of 5-FU occurs principally in the liver by a series of enzymes which first reduce and then hydrolyze the pyrimidine ring.[14] Intracellularly, the active phosphorylated metabolites of 5-FU are apparently catabolized by phosphatases.

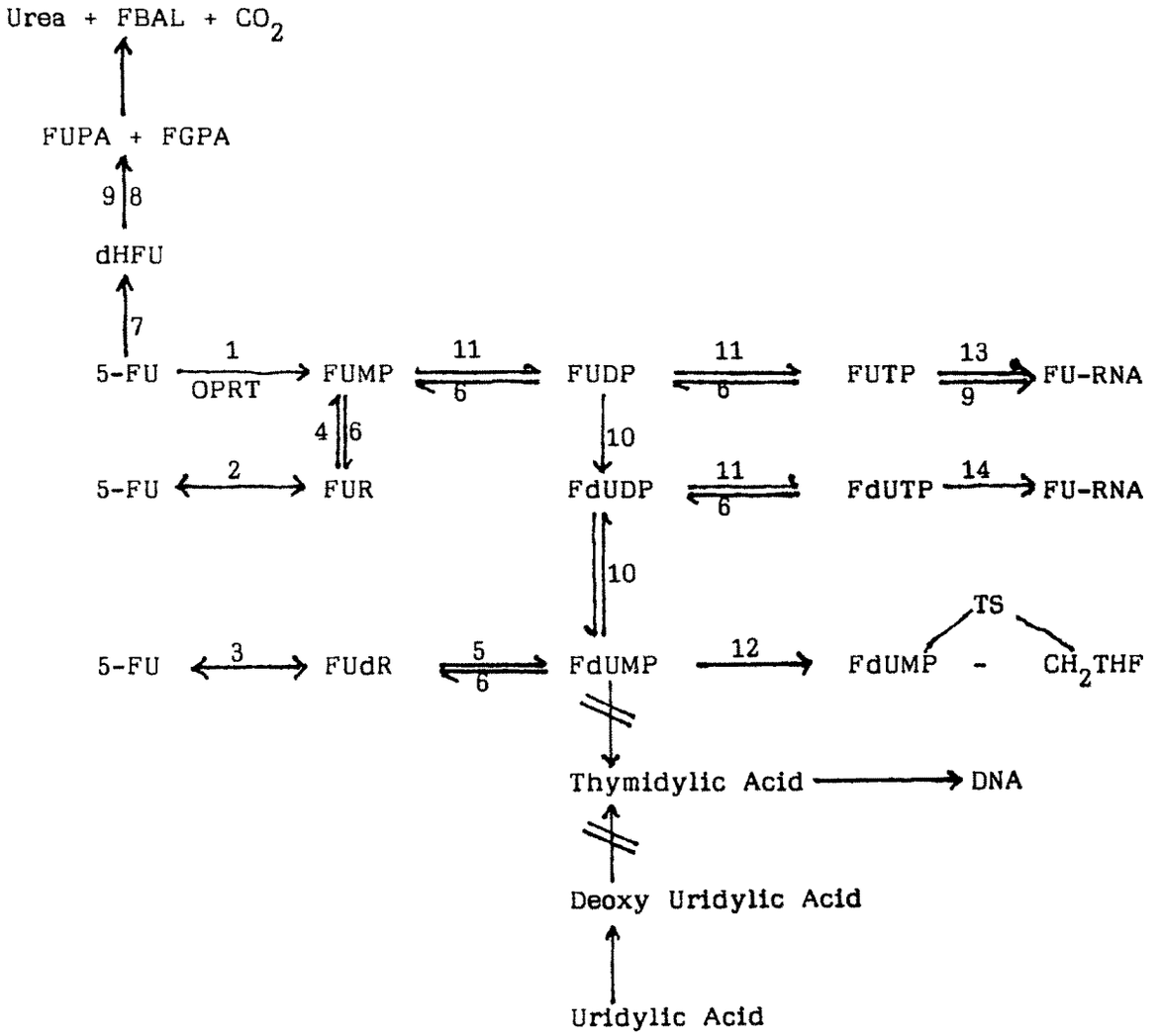


Fig. 3 : Schematic flow diagram of the pathways of 5-FU metabolism

(iv) **Elimination**

Less than 10 to 15% of the drug is excreted unchanged in the urine. The remainder is catabolized in a manner similar to uracil into urea, ammonia and carbon dioxide. 60 to 80% is eliminated as respiratory carbon dioxide. The total body clearance of 5-FU after intravenous injection is 0.6 to 1.9 lit/min. When given as a continuous infusion, its urinary excretion is only 4%.

2.5.4 Mechanism of Action

The formation of 5-fluoro 2' deoxy uridine monophosphate (FdUMP) is the basis of the antineoplastic action of 5-FU.[37,38] FdUMP inhibits DNA synthesis by blocking thymidylate synthase enzyme. It also interferes with RNA synthesis by getting incorporated into RNA as the ribonucleotides, FUMP, FUDP and FUTP and altering the synthesis, function and stability of the RNA (Fig.3).

Thus, 5-FU requires enzymatic conversion to the nucleotide, FdUMP and FUMP in order to exert its cytotoxic effects. FdUMP and the folate cofactor, $N^{5,10}$ methylene tetrahydrofolate, form a covalently bound stable ternery complex with the enzyme thymidylate synthase, which is required for the incorporation of thymine into the DNA strands. 5-fluorouracil therefore exerts its Cytotoxic effect by bringing about a 'thymine-less' death. As DNA synthesis occurs only

during the S - phase of the cell cycle, 5-FU is a S - phase specific drug and is ineffective against resting or non-proliferating cells.

2.5.5 Uses of 5-Fluorouracil

Though 5-fluorouracil is not curative, it may bring about regression in a number of neoplasms.[39] It is the drug of choice in the treatment of carcinoma of pancreas. In combination with other drugs, it is effective in the treatment of breast cancer, islet cell tumours, colorectal cancer and gastric carcinoma. It is the second choice in the chemotherapy of primary hepatic carcinoma, prostatic carcinoma, bladder tumour and tumours of cervix, ovary, head and neck. Topically, it can be used in the treatment of precancerous, keratotic dermatoses like actinic keratosis.

Resistance to 5-Fluorouracil

Resistance can occur to 5-FU due to :[45]

i) loss or decreased activity of the enzymes necessary for the activation of 5-fluorouracil.

ii) decreased pyrimidine monophosphate kinase levels, leading to decreased incorporation into RNA.

iii) amplification of thymidylate synthase.

iv) presence of altered thymidylate synthase that is not inhibited by FdUMP.

v) insufficient concentration of N^{5-10}, methylene tetrahydro folate.

2.5.7 Toxicity of 5-Fluorouracil

As 5-FU acts by affecting the DNA synthesis of rapidly growing cells, alongwith the malignant cells, the rapidly proliferating normal cells like those of the haemopoetic tissues, gastrointestinal epithelium, hair follicle etc. are also seriously damaged. This is the main cause of the toxicity of 5-FU. About two-third of the patients show signs of toxicity to 5-FU.[32]

Serious toxic manifestations of 5-FU include myelosuppression, gastrointestinal mucositis, leukopenia, thrombocytopenia, aphthous ulceration and septicemia.

Common adverse effects include diarrhea, vomitting, nausea, gastrointestinal ulceration, alopecia, dermatitis, hyper-pigmentation, pharyngitis, esophagitis, cerebellar ataxia, lassitude etc.

Topically, 5-FU may induce photosensitization and always causes erythema, necrosis and re-epithelization.

The toxic side effects are often dose-related and disappear when therapy is discontinued.

2.5.8 Dose and Dosing Schedule

5-Fluorouracil is usually administered by rapid intravenous injection. For normal, low risk, healthy patients, the usual dose is 12 mg/kg/ day for four days, followed by 6 mg/kg for four more alternate days. Poor risk patients should be given half the normal dose. The schedule should be repeated every month, if the toxicity from the first schedule was minimum; Otherwise, a dose of 10 to 15 mg/kg should be administered weekly. When used in combination, only 50 to 75% of the initial dose should be used.

5-Fluorouracil is also administered intraarterially or intraperitoneally.

Nowadays, special ambulatory infusion pumps like the Traveno Infusor, capable of delivering 48 ml/day have also been successfully used for the long term infusion of 5-fluorouracil.[46]

2.5.9 Methods of Analysis of 5-Fluorouracil

Both the I.P.[33] and the U.S.P.[34] prescribe a titrimetric method for assay of 5-fluorouracil in bulk and a ultraviolet spectrophotometric measurement of a sample in acetate buffer (pH 4.7) at 266 nm for the estimation of 5-FU in injections.

Apart from a few methods for estimating 5-FU in bulk and injectable solution forms like colorimetry,[47] spectrophotometry,[48-49] potentiometry,[50] voltametry,[51-52] and isotachophoresis,[53] chromatographic methods are widely used for the analysis of 5-FU from biological samples. Although thin layer chromatography (TLC)[54-55] and gas liquid chromatography (GLC)[56-58] have been reported, the most widely used method for estimating 5-FU and its derivatives in biological tissues and fluids is the high pressure liquid chromatography (HPLC).[59-67]

2.5.10 Efforts towards Targeting of 5-Fluorouracil

Out of the various techniques tried towards improving the specificity for 5-fluorouracil, few have met with success:

Umrigar *et al*[68] copolymerized 1-(2-carbomethoxy acryloyl) - 5-FU with divinyl ether, styrene and β-chloro vinyl ether and demonstrated that the copolymers provided a sustained release of 5-FU.

Hashida *et al*[69-70] improved the lymphatic delivery of 5-FU by incorporating it in water-in-oil (w/o) and gelatin microspheres-in-oil (s/o) emulsions. The emulsions were injected via intravenous, intramuscular and intragastric routes in rats and the intragastric route showed most promising results.

Miyazaki et al[71] reported an improved life expectancy in mice bearing Ehrlich ascites carcinoma when intraperitoneal implantation of 5-fluorouracil incorporated into ethylene-vinyl alcohol copolymer matrices was carried out.

Yalabik-Kas et al[72] studied the sorption of 5-FU onto poly methylmethacrylate (PMMA) nanoparticles and found a linear adsorption isotherm, fitting the van't Hoff's equation.

Benita et al[73] reported the preparation of carnauba wax microspheres containing 5-fluorouracil for arterial chemoembolization, while Kenneth et al[74] prepared microcapsules of monoglyceride, polylactide, cellulose polymer, sterol derivatives and various waxes for chemoembolization of floxuridine (FUDR).

Sugibayashi and Morimoto et al[75-77] prepared bovine serum albumin (BSA) microspheres containing 5-FU and studied their tissue distribution in healthy mice and Ehrlich ascites and solid tumour bearing mice. They reported a higher 5-FU level in the tumour tissue and a 50% increase in the life expectancy as compared to the free drug treatment.

Omotosho et al[78-80] incorporated 5-FU into w/o/w multiple emulsions and showed that the drug absorption was increased and prolonged as compared to the free drug solution, when administered orally as well as intramuscularly, highlighting its potential as a lymphotropic carrier.

Jeyanthi et al[81-82] incorporated 5-FU in gelatin microspheres and in implantable collagen - poly (HEMA) hydrogel carriers for various routes of drug delivery. The implantable hydrogel formulation showed an improved antitumour activity over the free drug when tested on solid tumour fibrosarcoma in Wistar rats.

Itoh and Nakano[83] encapsulated coprecipitates of 5-FU with cellulose acetate in ethyl cellulose by a non-solvent addition phase-separation coacervation method for providing sustained release.

Mukherji et al encapsulated 5-FU in gelatin,[84] ethylcellulose, methylcellulose[85] and polyglutaraldehyde[86] microspheres and found that upon intravenous injection in healthy rats, the drug distributed preferentially into the liver, lungs and intestine.

Liposomal entrapped formulation of 5-FU has been reported by Ozer[87] and Ishii et al.[88] The latter used magnetic liposomes as the site specific delivery system.

Maa and Heller[89] reported the preparation of bioerodable linear poly (orthoesters) for the controlled release of 5-FU.

Efforts to improve the transdermal delivery of 5-FU have been carried out by Sasaki et al[90] by using pyrrolidone derivatives as penetration enhancers.

Ligand mediated active targeting of 5-FU has also been tried by several workers :

Mukherji et al[84] reported a 5-fluorouracil - lectin complex using Concanavalin A and showed that maximum distribution of the drug took place in the liver, followed by lungs and intestine, when administered intravenously in healthy rats. They also synthesized prodrugs of 5-FU containing cysteine and glutamic acid ethyl esters.[91] When entrapped in ethyl cellulose particles, these 3-carbamoyl derivatives of 5-FU were found to accumulate predominantly in the lungs, followed by the liver.

Ouchi et al[92] synthesised conjugates of poly (α - malic acid) and 5-fluorouracil via ester, amide and carbomoyl bonds and showed that the antitumour activity of the methyl ester conjugates was best when tested against P - 388 lymphocytic leukemia in mice.

Chung et al[93] reported a sustained and higher in vivo release of 5-FU from intravenous infusion of 5-fluorouracil - acetic acid - human serum albumin conjugates to rabbits as compared to infusion of free 5-fluorouracil.

Polystyrene based ion exchange resins were investigated by Jones et al[94] as carriers for sustained delivery of various cytotoxic agents including 5-fluorouracil and floxuridine.

Silicone capsules containing cyclophosphamide, mechloroethamine, triethylene melamine and 5-fluorouracil were implanted interstitially into the tumour mass in Syrian hamsters infected with lymphosarcoma tumour and a marked improvement was observed in the therapy as compared to the conventional treatment.[95]

The present work was undertaken to develop site specific delivery systems for 5-fluorouracil. Three different polymeric carriers of different characteristics were chosen for the purpose. 5-fluorouracil was incorporated in microcapsules of :

(1) Polyamides, which are supposed to be slowly biodegradable,
(2) Polyacrylamide, which is nonbiodegradable but is thought to be eliminated after slow bioerosion, and
(3) Polyalkylcyanoacrylate, which are rapidly biodegradable.

The microcapsules were evaluated for various properties like size and size distribution, drug entrapment efficiency, drug release and drug leaching studies and in vivo studies.The pharmacokinetic properties of 5-FU from the microcapsules were studied by injecting a suspension of the microcapsules in healthy rats and estimating the drug distribution in major organs like lungs, liver, kidneys, spleen and intestine.

2.6 POLYAMIDE MICROCAPSULES

Polyamides are the condensation products containing recurring amide groups as integral parts of the main polymer chains.[96] Self condensing linear polyamides prepared from amino acids are termed as AB type polyamides, where 'A' represents the amine group and 'B' represents the carboxyl group. Polymers formed from condensation of diamines and dibasic acids are termed 'AABB' type and have the general formula : $H_2NRNH\text{-}\left(COR'CONHRNH\right)_n COR'COOH$. They are frequently called **Nylons.**

Polyamides are prepared by various methods including direct amidation, melt polymerisation, solution polymerisation, ring opening polymerisation and interfacial polymerisation.

For the purpose of this work, interfacial polymerisation which was the method of choice for preparing the microcapsules, will be discussed.

2.6.1 Interfacial Polycondensation

The interfacial polycondensation process involves polymer formation at or near the interface between two immiscible monomer solutions under very mild reaction conditions. This is a step-growth polymerisation reaction based on the Schotten-Baumann reaction between a diacid chloride and a substance with a functional group

containing active hydrogen, like amine, alcohol or thiol (-NH, -OH, -SH). In general, this type of reaction is particularly useful when the polymer formed would normally decompose, isomerize or crosslink at the elevated temperature in the melt or solution polymerisation procedures.

In the interfacial polymerisation process, the polymerisation occurs very close to the organic side of the interface between an aqueous solution of one monomer and an immiscible organic solvent solution of the other. The adjacent aqueous phase generally also contains a basic reagent capable of neutralising the hydrochloric acid liberated in the reaction. Some degree of crosslinking is obtained with the carboxyl groups of the diacid chloride reacting to form an anhydride linkage, thus strengthening the capsule walls.[97]

Usually, the first monomer is dissolved in the aqueous phase containing the core material which is then emulsified in the organic phase in presence of a suitable emulgent to form a water-in-oil (w/o) emulsion and then, the second monomer, dissolved in the same organic phase, is added to the preformed emulsion.[98] The monomers diffuse together and rapidly polymerize at the interface to form a thin coating around the core material.

The polymerisation reaction appears to be bimolecular with the second order rate constant ranging from 10^2 to 10^6 L/mol. s.[99]

The degree of polymerisation is controlled by the reactivity of the monomers chosen, their concentration, the composition of the two phases and the temperature of the system. The reaction between the two monomers is quenched by depletion of the monomer, which is frequently accomplished by adding excess continuous phase vehicle to the emulsion.

The newly formed microcapsules should be removed from the nonpolar vehicle immediately after quenching the reaction in order to reduce the degradation of the core material and the chances of further polymerisation. This is achieved by gentle centrifugation, decanting the supernatant and dispersing the microcapsules in a 50% solution of Tween 20 in water. The resultant dispersion is centrifuged, the microcapsules are repeatedly washed with saline to free them from residual Tween and resuspended in saline or lyophilized.

The aqueous phase usually is sodium hydroxide, sodium carbonate or sodium bicarbonate solution or buffer.

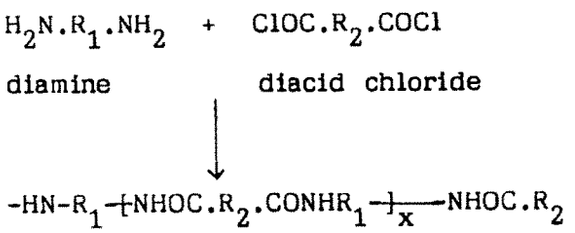
The organic phase usually consists of benzene, toluene, chloroform, cyclohexane or carbon tetrachloride, either alone or preferrably in various combinations to match the density of the microcapsules formed.

The water soluble monomer is an amine, diamine, amino acid, diamino acid or a protein, whereas the acid chlorides used are sebacoyl chloride, azeloyl chloride, terephthaloyl chloride, dimer acid chloride, trimer acid chloride, trimesoyl chloride, adipoyl chloride, phosgene, succinyl chloride etc.

A suitable core material should form a suspension or a solution of macromolecules in the aqueous phase because a very low molecular weight substance can easily permeate the nylon wall formed.

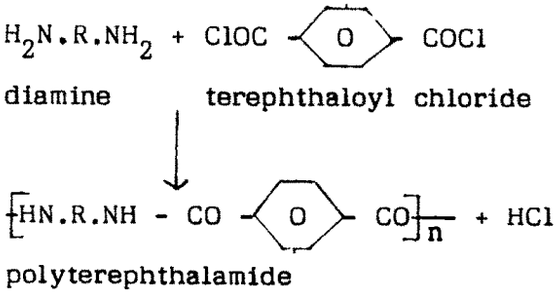
The emulgent of choice for this process is Span 85 (sorbitan trioleate).

The general chemical reaction for polyamide formation is :



Polyamide

Polyamides formed by reaction of a diamine with terephthaloyl chloride are called polyterephthalamides. Such a polymerisation is generally represented as :



diamine terephthaloyl chloride

polyterephthalamide

A number of workers have reviewed the factors affecting the interfacial polymerisation procedures.[100-106]

Chang[107] was the first to report the use of semipermeable nylon microcapsules as protective carriers for erythrocyte hemolysate for use as artificial red cells.

Later, Shiba et al[108] incorporated bovine serum albumin into various polyamide microcapsules and found that the protein was chemically incorporated as a constituent element in the nylon membrane and helped to strengthen the microcapsular wall.

Shigeri et al[109] reported the preparation and characterisation of polyphthalamide microcapsules using various diamino acids instead of a diamine. The advantage of such microcapsules are that they are biodegradable and biocompatible. Also, the crosslinking between the carboxylic acid group of the amino acid with the carboxyl chloride moiety of the diacid chloride strengthens the wall material and makes it more resistant to centrifugation, hypertonic solutions and lyophilization.

Several workers have used polyamides for microencapsulating various enzymes like carbonic anhydrase[110] L-asparaginase[111-113] urease[114-116], invertase[117], arginase[118], secretin[119] etc., with a view to increase their stability and activity.

Another group of workers have used the interfacial polymerisation procedure for encapsulating various proteins like hemolysate[120], albumin and fibrinogen[121], which were reported to have a protective effect on the stability and stress-durability of the microcapsules.

McGinity et al[122] prepared nylon microcapsules containing formalinized gelatin, calcium alginate and calcium sulfate for entrapping a wide range of drugs - anionic, cationic, nonionic and amphoteric.

Luzzi et al[123] tried nylon microcapsules as a prolonged release carrier for sodium pentobarbital by incorporating methylcellulose as the release retarding agent.

Desoize & Levy et al[124] showed that nylon microcapsules prepared by crosslinking various proteins like lysozyme, casein, human serum albumin, heamoglobin and pepsin exhibited a cytotoxic activity in vitro towards leukemic cells by inhibiting cell division.

Interfacial nylon polymerisation has also been used to encapsulate a complex formed by the anion-exchange resin, Dowex-7 and sodium fluoresceinate, as a sustained release drug delivery system.[125]

2.6.2 Crosslinked Haemoglobin Microcapsules

Levy and Rambourg[126] observed that incorporation of proteins helped to improve the stability of microcapsule - incorporated invertase by crosslinking with the acyl chloride. This prompted them to develop the emulsification-reticulation procedure in which no diamine was used but the protein itself acted as the crosslinking/ polymerizing agent with terephthaloyl chloride. Of the various proteins tested, haemoglobin, serum albumin and egg albumin successfully encapsulated 87 to 97% of the enzyme.

Thereafter, they studied the preparation of crosslinked haemoglobin microcapsules with various diacylchlorides like terephthaloyl chloride, sebacoyl chloride and succinyl chloride.[127] Only terephthaloyl chloride gave highly crosslinked and stable microcapsules. The strength and resistance to centrifugation, freezing and melting cycles, lyophilization, deformation as well as to protease enzymes increased when the microcapsules were stabilized by further crosslinking with glutaraldehyde. Cross-linked haemoglobin microcapsules were suggested as potentially stable and biodegradable carriers.

Recently, Levy et al[108] reported the preparation of crosslinked human serum albumin microcapsules and evaluated them using Fourier Transform Infrared Spectroscopy (FT-IR) and concluded that anhydride and ester bonds were involved in protein crosslinking.

In the present study, various polyterephthalamide microcapsules were prepared by reacting a number of diamino acids with terephthaloyl chloride. Crosslinked haemoglobin microcapsules were also prepared using terephthaloyl chloride as the crosslinking agent.

2.7 POLYACRYLAMIDE MICROCAPSULES

Acrylamide polymers, popularly known as Polyacrylamides are being widely used in water treatment, mining and paper-manufacturing industries since the past 30 to 40 years. Modification of the water soluble polyacrylamide by crosslinking has resulted in a large expansion of its applicability in plastic, gum and pharmaceutical industries also.[129]

In absence of oxygen, acrylamide undergoes vinyl polymerisation to give a water soluble polymer in presence of free radical sources like peroxides, persulfates, redox couples, azo compounds, ultrasonic energy, ultra violet light, ionizing radiation etc.

The chemical reaction is :

$$CH_2 = CH\ CONH_2 \longrightarrow -\left[CH_2 - \underset{\underset{CONH_2}{|}}{CH} - CH_2 - \underset{\underset{CONH_2}{|}}{CH}\right]_x-$$

acrylamide polyacrylamide

where x is 20,000 to 30,000

Crosslinked polyacrylamide also includes $-[CH_2.CH_2.CONH]_n$ chains.

Copolymerisation of acrylamide with a polyfunctional monomer gives an insoluble, covalently cross-linked gel with a three dimensional network structure. This crosslinked polymer has been thoroughly investigated as a carrier for a number of enzymes and macromolecules as it is able to entrap the core material into its porous matrix, thus protecting it from degradative or denaturing forces and are thought to prolong the release of the entrapped moiety by acting as a slowly metabolizable carrier.

Raymond and Werntraub[130] reported a pharmaceutical application of a covalent acrylamide gel. They used stiff polyacrylamide gels as supporting media for zone electrophoresis.

Bernfeld and Wan[131] used crosslinked polyacrylamide matrices for entrapping antigens and enzymes and showed that the core material not only retained its biological activity but also remained protected from degradative forces.

Polyacrylamide gel was investigated as a high capacity immunosorbent for entrapping human immunoglobulin and as a carrier for haemoglobin by Carrel et al.[132]

These acrylic polymers proved useful as carriers for immobilizing enzymes due to their chemical and mechanical stability, non-

susceptibility to microbial attack and high payload capacity.[133] Johansson and Mosbach[134] used polyacrylamide beads for entrapping various enzymes and concluded that the enzyme activity had a direct relation with the molecular weight of the polymer.

Ekman and Sjoholm[135-137] first tried the entrapment of macromolecules in polyacrylamide microparticles with diameter less than 10 μm as against the gel matrix and large beads reported by the earlier workers. For this purpose, they used an emulsion bead polymerisation procedure wherein the core material (albumin) was dissolved in the aqueous phase of a w/o emulsion before polymerisation. Pluronic F 68 was used as the emulgent, ammonium persulphate as the catalyst and tetra ethyl methylene diamine (temed) as the initiator. They could successfully entrap 30 to 35% of the protein inside the microparticles. Later on, they entrapped lactate dehydrogenase[138], antihuman globulin[139] lymphocyte, L -asparaginase and L-glutaminase.

Birrenbach and Speiser[141-142] reported the preparation of nanoparticles of polyacrylamide using a micelle polymerisation procedure for the parenteral delivery of the entrapped core materials, urease, tetanus toxoid and human immunoglobulin.

Sjoholm and Edman[143-144] reported the <u>in vivo</u> fate of polyacrylamide and polyacryldextran microcapsules by injecting them in mice and rats. The autoradiographs indicated that the reticuloendothelial

system was mainly responsible for the capture of the microparticles and that their distribution into various organs was size-dependent. In another paper,[145] they described a prolongation of the effect of asparaginase activity by subcutaneous implanatation of the enzyme - containing microparticles in rats.

Intramuscular administration of L-asparaginase immobilized in the microparticles produced partial regression of 6C3HED lymphoma in mice.[146]

Before being recommended for human trials, the effect of polyacrylamide microparticles was studied on cultured mice macrophages.[147,148] Very low doses (0.02 mg/ml) had no significant toxicity. As the dose increased, the survival rate decreased and at the highest dose (0.1 mg/ml), 75% of the cells were destroyed. No signs of acute toxicity and tissue incompatibility were observed with low doses in mice. Only massive doses (100-200 mg/kg) produced transient hepatosplenomegaly which normalized after 4 to 8 weeks.

An earlier report[149] showed that the LD_{50} of polyacrylamide was 1750 ± 218.5 mg/kg for mice and 3000 ± 91.3 mg/kg for rats. Death reportedly occured due to the failure of the nervous system. Ingestion of 1/10th of the LD_{50} dose was not fatal but reduced the weight of the test animals. The toxicity was thought to be due to the presence of residual monomer which is a neurotoxin and is reported to damage both the central as well as the peripheral nervous systems.

Encouraged by the biocompatibility and safety of acrylamide microparticles, Edman et al[150] continued their extensive research on the in vivo disposition of acrylic microspheres. Though the microparticles promised to be very good tools for the 'active targeting' of drugs to the organs of the RES, the same can be a major drawback for targeting to organs and tissues other than the RES. Hence, they tried to modify the organ distribution of the microparticles by incorporating various albumins but found that all the particles localized in the RES.

El-Samaligy and Rohdewald[151] prepared gelatin-crosslinked polyacrylamide microbeads by a w/o emulsion polymerization technique as carriers for sustained release of tetracycline hydrochloride and theophylline.

In the present study, polyacrylamide microparticles have been prepared using the emulsion polymerisation procedure and their potential as site specific carriers for 5-fluorouracil has been tested.

2.8 POLY (ALKYL CYANO ACRYLATE) MICROCAPSULES

Poly (alkyl 2-cyano acrylate) analogues are used as carriers for a wide range of materials like enzymes, cytotoxic agents, antibiotics, antimitotics etc. They are biodegradable, biocompatible and their rate of degradation can be varied by varying the alkyl chain length of the monomer. They can be used as carriers to incorporate a wide

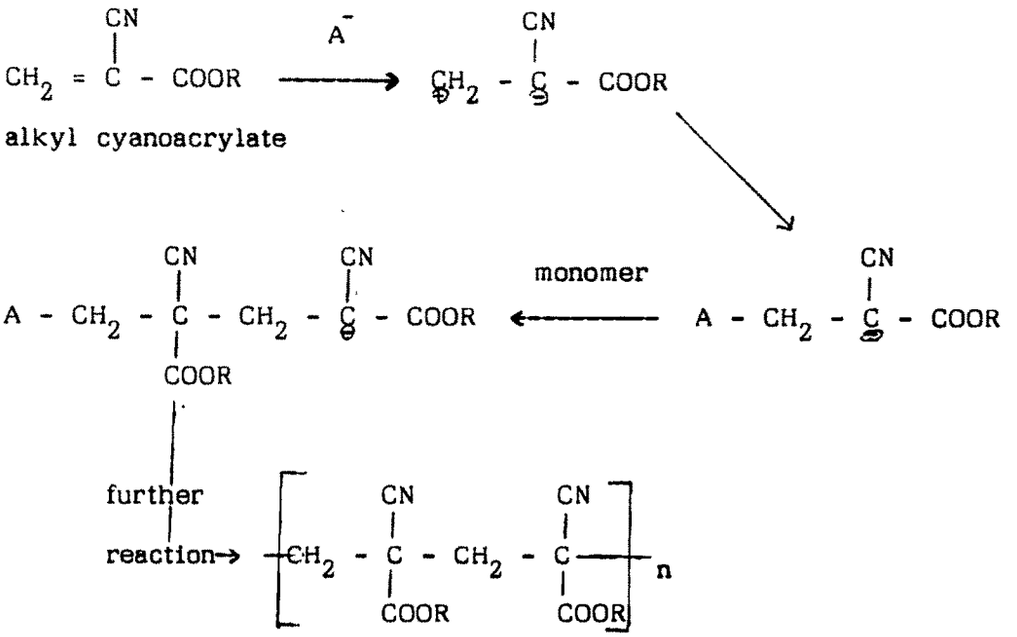
variety of drugs to sustain or modify their release or distribution pattern. Polyalkylcyanoacrylate (PACA) microcapsules are comparatively easy to prepare with a high degree of reproducibility and have a good stability.

The applicability of 2-cyanoacrylic ester polymers as excellent adhesives was accidentally discovered in 1951 when its spontaneous polymerisation property was observed by the chemist measuring its refractive index.[152] Thereafter, their applicability was extended as tissue adhesives in nonsuture closure of wounds during surgery and as haemostasis inducing compounds, owing to their ability to polymerize spontaneously even on wet surfaces.

However, the adhesive properties of the polyalkylcyanoacrylates are afffected by moisture, heat, pH and the solvents used.

Cyanoacrylate polymers degrade by hydrolytic scission of the polymer chain, the end products being formaldehyde and an alkyl cyano acetate.[153] The rate of breakdown decreases with increasing chain length of the monomer. The tissue tolerance to the monomers and their corresponding polymer is also increased with increase in the homologous series. Hence, the lower analogues like the methyl and ethyl esters are not preferred while the higher analogues like the butyl and hexyl derivates are preferred because they are safer, better tolerated and are slowly biodegradable. The choice of the analogue can be made depending on the desired drug release rate.

The alkylcyanoacrylates undergo stepgrowth anionic polymerisation according to the following mechanism :



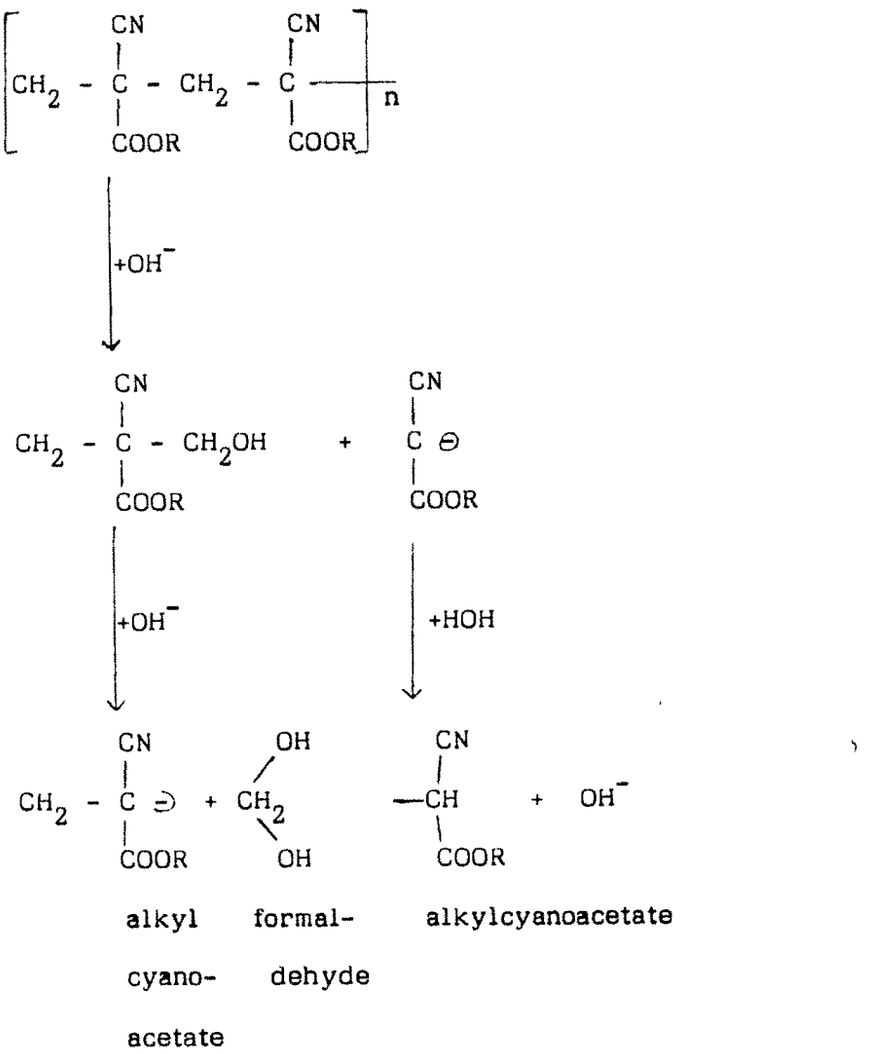
Polyalkylcyanoacrylate

Formation of formaldehyde is responsible for the toxicity of the alkylcyanoacrylates. In a special study,[154] it was demonstrated that the cytotoxic effect of the poly(butylcyanoacrylate) particles on hepatocytes manifested only at very high concentration i.e. 0.5 to 1.0%, corresponding to 1-2 x 10^4 particles per hepatocyte cell. Moreover, poly (methylcyanoacrylate) was found to be more mutagenic to S. typhimurium at the dose of 1500 ug/ plate, slightly toxic at 300 ug/plate and safe at 150 ug/plate.

Toxicity studies on mice showed that low doses of poly(isobutyl

cyano acrylate) [PiBCA] nanoparticles were safe and did not cause damage to cells and tissues. The LD_{50} for PiBCA particles was found to be 196 mg/kg whereas that for poly(butylcyanoacrylate) was 230 mg/kg.

The polyalkylcyanoacrylates undergo degradation according to the following mechanism :



Leonard et al[155] studied the interfacial polymerisation of various alkylcyanoacrylate homologues while investigating their applicability as tissue adhesives.

Florence et al[156] investigated the feasibility of using them as biodegradable carriers for various core materials. Using the interfacial emulsion polymerisation procedure, they successfully encapsulated 30-40% of albumin in poly(butyl2-cyanoacrylate) [PBCA] and stored them as an aqueous suspension for 18 months without substantial degradation.[157]

Vezin et al[158] studied the diffusion of small molecules viz. salicylic acid, p-nitro aniline, trinitrophenol, procaine and fluphenazine through polyalkylcyanoacrylate films and concluded that the diffusivity increased with an increase in the length of the polymer side chain.

Illum et al[159] studied the conditions for improving the carrier capacity of PiBCA nanoparticles for rose bengal and adriamycin. Although 90% rose bengal could be incorporated, only 25% adriamycin could be loaded into the nanoparticles.

Samaligy et al[160] incorporated 55 to 74% of fluorescein and doxorubicin in polymethylcyanoacrylate (PMCA), polyethylcyano-acrylate (PECA) and polybutylcyanoacrylate (PBCA) nanocapsules using the interfacial polymerisation procedure.

Henry et al[161] encapsulated 60 to 75% ampicillin and 75 to 82% gentamicin into polyisobutylcyanoacrylate (PiBCA) and polyisohexyl-cyanoacrylate (PiHCA) nanoparticles while Grangier et al[162] used polyalkylcyanoacrylate nanoparticles as carriers for the growth hormone releasing factor, somatoliberin.

Indomethacin was encapsulated in PiBCA nanoparticles to improve its stability whereas hematoporphyrin was adsorbed onto PiBCA nano paritcles for improving its tumour distribution[164] by Gursoy et al and Brassuer et al, respectively.

Gasco and Trotta[165] reported an in situ anionic emulsion polymerisation procedure for the preparation of PiBCA nano particles. They claimed this procedure as being superior to interfacial polymerisation procedure.

Rollot et al[166] compared a w/o emulsion polymersion procedure with the interfacial o/w polymerisation procedure for preparing PiBCA nanocapsules. They observed that the earlier method gave nano particles with solid core whereas the latter method yielded micro capsules wherein a polymeric wall surrounded the internal oil phase droplet.

Lenaerts et al[167-168] successfully prepared very small polyalkyl-cyanoacrylate particles having diameter less than 10 nm by bubbling sulfur dioxide through the monomer solution prior to polymerisation.

Krause et al[169] claimed that the interfacial polymerisation procedure was superior to the micelle polymerisation procedure for preparing PMCA nanoparticles.

Couvreur et al[170,171] reported the use of polymethyl- and polyethyl- cyano acrylates as carriers for dactinomycin, methotrexate and fluorescein using an anionic polymerisation procedure in a mildly acidic medium. While 85 to 90% of dactinomycin and 50 to 65% of fluorescein was adsorbed, only 15 to 40% of methotrexate could be incorporated into the nanoparticles.

In other papers,[172-173] the same workers encapsulated actinomycin, dactinomycin and vinblastine in PMCA and PECA nanoparticles. They observed that the drug entrapment was higher when the drug was incorporated before polymerisation (86-93%) rather than adsorbing the drug onto preformed nanoparticles (65%). Tissue distribution of intravenously injected drug-loaded nanoparticles in rats showed a significantly higher uptake of dactinomycin in the small intestine and lungs as compared to the free drug, whereas it was markedly higher in organs of the RES in case of vinblastine and actinomycin.

Toxicity studies of doxorubicin-loaded PiBCA nanoparticles in female mice showed that a dose of 7.5 mg/kg/day was safe and toxicity appeared only at a higher dose of 15 mg/kg/day. The weight loss and mortality of nanoparticle-treated groups was found to be less than that of the free drug-treated groups, indicating that the PiBCA

nanoparticles helped in reducing the toxicity of doxorubicin.[174]

Ibrahim et al[175] prepared magnetic actinomycin - loaded PiBCA nanoparticles and observed that 10 minutes after intravenous injection in rats, the magnetic nano-particles produced a three times higher concentration in the kidney bearing a magnet, as compared to the nonmagnetic particles, without reducing the payload capacity or increasing the acute toxicity.

Ratcliffe et al[176] investigated the potential of PBCA nanoparticles as carriers for intraarterial delivery of drugs but found that the particles caused a severe, localized inflammatory response in rabbits, which they attributed to the presence of formaldehyde, the breakdown product of the alkylcyanoacrylate.

Gipps et al[177] studied the in vitro cytotoxic effects of polyalkyl-cyanoacrylate nano-particles on normal human foetal lung fibroblasts and malignant mesenchymal cells. They found that PECA and PBCA nanoparticles were more toxic than PHCA (polyhexylcyanoacrylate) nanoparticles. Death was found to be associated with shrinkage and disorganisation of the cell cytoplasm and nucleus.

Grislain et al[178] studied the influence of the route of administration on the pharmacokinetics, distribution and tissue localization of PiBCA nanoparticles. They found that after intravenous injection in mice, the nanoparticles were rapidly cleared from the blood stream within

30 minutes and concentrated in the lungs, spleen and liver, from where they were slowly eliminated over a period of 7 days. The nanoparticles also showed a preferential distribution into the tumoral tissues as compared to the normal tissues.

In a separate study on the uptake of polyalkylcyanoacrylate nanoparticles by liver, it was found that about 70% of the nanoparticles taken up by the liver were associated with the Kupffer cells.[179]

In a comparative biodistribution study of different types of colloidal microparticles in mice, Waser et al[180] reported that all the carrier systems accumulated in the organs of the RES. Polyhexylcyanoacrylate (PHCA) nanoparticles were found to be incorporated in the hepatic and pulmonary cells 5 minutes after intravenous injection and persisted there upto 10 days.

Douglas et al[181] tried to modify the biodistribution of PBCA nanoparticles in rabbits by coating them with Poloxamer 338 and Poloxamine 908 but found that all particles localized mainly in the liver and spleen.

Fallouh et al[182] prepared PiBCA nanoparticles using a novel oil-in-water (o/w) interfacial polymerisation procedure suitable for encapsulating lipophilic drugs with comparable entrapment efficiency (90%) as that of the water-in-oil (w/o) method. In vivo studies of

the nanoparticles in rabbits showed that they got localized in the liver and spleen regions.

Gipps et al[183-184] studied the distribution of intravenously injected PHCA nanoparticles in nude mice bearing human osteosarcoma but found no significant localisation of the radioactivity in the tumour tissue. They noted that repeated injection increased the redistribution of the nanoparticles into the spleen, lungs and other organs of the RES.

Maincent et al[185] compared the bioavailability of vincamine after intravenous and oral administration of vincamine-loaded polyalkyl-cyanoacrylate nanoparticles in rabbits. They reported that the oral absorption and bioavailability was considerably higher from the encapsulated drug as compared to that from the free drug solution.

Kreuter et al[186] reported a higher transport rate and activity from PBCA nanoparticles-encapsulated norchlesterol as compared to its micellar solution when injected into rabbits.

In another report[187] they observed that the radioactivity accumulated mainly in the ileaco-cecal region when radiolabelled polyhexylcyanoacrylate nanoparticles were administered perorally to mice.

Damge et al[188-189] reported a marked reduction in glycemia in both

normal and diabetic rats when insulin-loaded PiBCA nanoparticles were administered orally to them.

Gasper et al[190] incorporated 80 to 90% primaquine in PiHCA nanoparticles and observed a marked reduction in acute toxicity of the carrier-associated drug as compared to the free drug.

Simeonova et al[191] investigated the tissue distribution of PBCA nanoparticles carrying spin-labelled nitrosourea in healthy and B-16 melanoma-bearing black mice and found an increased drug accumulation in the tumour tissue and a decreased drug accumulation in the lungs, liver, brain and kidneys.

Kubaik et al[192] coated PiHCA and PiBCA nanoparticles with tumour specific monoclonal antibodies in an attempt to minimize their in vivo uptake by the organs of the RES.

A few workers have also tried polyalkylcyanoacrylate nanoparticles as carriers for non-parenteral administration of drugs:

Harmia et al[193-194] encapsulated pilocarpine nitrate in PBCA and observed that there was an enhancement of the miotic response to pilocarpine only when the drug was adsorbed onto empty nanoparticles and not when it was incorporated into them.

Heussler et al[195] adsorbed upto 70% of betaxolol chlorohydrate and

observed a highly variable therapeutic response in glaucamatous rabbits as compared to that of the free drug.

Alonso and Losa et al[196-197] also reported the incorporation of amikacin sulphate onto PBCA nanoparticles for improving its ocular penetration.

In the present study, formulation, evaluation and in vivo organ distribution of polyisobutylcyanoacrylate microcapsules has been described.