CHAPTER – 2

LITERATURE REVIEW
2. LITERATURE REVIEW

2.1 CANCER - A BRIEF OUTLINE:

Cancer is an english term derived from the Greek word for crab, Karkinos, which was believed to be first used by Hippocrates who attributed this affliction to an excess of black bile. Cancer was known in antiquity, being described in the early writings of Greeks and Romans. Tumours in Egyptian mummies dating back 5000 years represent the first known human malignant growths, although there is a pathologic evidence of bone tumours, occurring in dianosaurs and other prehistoric animals.

Peyton Rous, a Nobel laureate for his pioneering work on viral causes of animal tumours wrote: "Tumours destroy man in an unique and appalling way, as flesh of his own flesh, which had somehow been rendered proliferative, rampant, predatory and ungovernable"(5).

Cancer is not one disease, but a group of diseases affecting different organs and systems of the body. Cancer can be defined very broadly as a disease in which there is uncontrolled multiplication and spread within the body of abnormal forms of the body's own cells(6).
Cancer cells differ from normal cells in behaviour in that they manifest three characteristics not seen in normal cells. They are –

a) Uncontrolled proliferation

b) Invasiveness and
c) Capacity to metastasize.

a) Uncontrolled Proliferation:

Some normal cells (such as neurons) have little or no capacity to divide and proliferate. Others, for example in the bone marrow and the epithelium of the gastrointestinal tract, have the property of continuous rapid division. Some cancer cells multiply slowly and some fast. It is therefore not generally true that cancer cells proliferate faster than the normal cells, the significant difference is that their proliferation is not controlled by the processes which regulate normal tissue or organ growth.

b) Invasiveness:

Normal cells during differentiation and during growth of tissues and organs develop certain spatial relationships with respect to each other, and these are continuously maintained even when the cells are involved in repair processes. Thus although the cells of the normal mucosal epithelium of the rectum proliferate continuously with a turnover time of about 2 hours, they remain as a lining of epithelium. A cancer of the
rectal mucosa on the other hand invades the tissues in the layers of the rectum and may invade the tissues of other pelvic organs.

c) Metastases:
These are secondary tumours formed by the cells which have been released from the initial or primary tumour and have reached other sites through blood vessels or lymphatics or as a result of being shed into body cavities (7).

2.1.1 Etiology:
Many chemicals (benzpyrene, aflatoxin, arsenicals, asbestos), viruses, and physical agents (ionizing radiation, ultraviolet light) can serve as carcinogenic stimuli capable of inducing malignant transformation in animals and humans. Some cancers are atrogenic in origin, as in patients who develop acute leukemia or other cancer years after being cured of systemic cancer by the use of cytotoxic chemotherapeutic drugs, or in patients who receive prolonged immunosuppressive therapy as a part of their renal transplantation program.

Substances that are not themselves carcinogens may serve as co-carcinogens in that they promote tumour formation when given in conjunction with or following exposure to specific carcinogens. One such example is use of tobacco products. In addition to the use of tobacco other aspects of life style appear to be impor-
tant contributors to human carcinogenesis. A high intake of dietary fat increases susceptibility to numerous forms of cancer. Low fiber and low calcium intake appear to increase the risk of colon cancer. High alcohol intake increases oral cancer.

Fundamental mechanisms that govern the etiology of human cancer have recently become enormously exciting as new information about cancer genes, viruses, carcinogens, cell growth, and differentiation is being discovered. Retroviruses (RNA tumour viruses), oncogenes (pieces of cellular DNA found in oncogenic retroviruses) and protooncogenes (DNA sequences in normal cells related to oncogenes) are part of the lexicon of this molecular biology that seeks to explain these essential regulatory processes.

2.1.2 Treatment:
The treatment of cancer with drugs was started by Huggins and Hodges in 1941. Subsequently the poly-functional alkylating agents were developed during world war II. Since then a number of chemotherapeutic agents have become available for the treatment of cancer. In the past, due to a poor therapeutic response, and a high incidence of adverse reactions, chemotherapy was only considered as a last resort after more successful treatments like surgery and radiotherapy had failed. Recent progress holds considerable promise that chemotherapy is now the preferred form of
treatment for leukaemias, lymphomas, choriocarcinomas and certain other tumours. However, even with recent advances, the treatment of cancer continues to be one of the greatest challenges in medicine, as many forms of human cancer still resist effective chemotherapy.

Antineoplastic agents (also known as cytotoxic agents) are used in the treatment of malignant neoplasms when surgery or radiotherapy is not possible or as an adjunct to surgery or radiotherapy or in leukemia as the initial treatment.

The term "Cytotoxic drug" applies in principle to any drug that can damage or kill cells. In practice it is often used more restrictively to mean drugs that inhibit cell division and are potentially useful in cancer chemotherapy.

A. GUIDELINES FOR USE OF CYTOTOXIC DRUGS:

The large number of cytotoxic drugs available today has made it possible to use drug combinations. As many of the drugs differ in their modes of action it is possible to attack malignant cells in several ways at the same time. Originally some drug combinations were employed in a particular type of cancer by trial and error method, and were found to be more effective. Today with increased understanding of drug action and cellular replication it is possible to combine drugs more logically. Today an understanding of the cell cycle
kinetics is essential for the proper use of presently available antineoplastic drugs.

Most of the potent cytotoxic agents act at specific phases of the cell cycle, and therefore, have activity only against cells that are in the process of division. Although differences in the duration of cell cycle occur between cells of various types, all cells display a similar pattern during the division process. This may be characterized as follows:

a) there is a presynthetic phase \(- G_1 \) phase
b) Synthesis of DNA occurs (S phase)
c) an interval follows the termination of DNA synthesis, the post synthetic phase \((G_2)\)
d) Mitosis (M phase) ensues - the \(G_2 \) cell, containing a double complement of DNA, divides into two daughter \(G_1 \) cells.

Each of these may immediately reenter the cell cycle or pass into nonproliferative stage, referred to as \(G_0(8)\).

Figure 1 shows different phases of cell cycle and action of some predominantly phase specific cytotoxic drugs(9).

Most of the antineoplastic drugs act specifically on the processes such as DNA synthesis, transcription, or the mitotic phase, and are labelled as \textbf{cell cycle phase specific drugs} (also known (9).
Figure 1: Phases of Cell-cycle And The Point of Action of Phase-specific Cytotoxic Drugs
as phase-dependent drugs). The phase specific drugs do not act on Go phase. In contrast, there are certain drugs which kill the cells during all or most phases of the cycle labelled as cell cycle phase nonspecific drugs (also known as phase-independent drugs). The phase non specific drugs do possess a slight effect on the Go phase. Table I shows the examples of phase specific and phase non specific drugs.

The clinical implication of cytotoxic drug action on cell cycle can be, that all neoplastic cells are not always in a state of rapid division, and a significant number of cells may be in the Go phase on which phase dependent drugs have no effect. Some evidence indicates that a course of phase independent drug therapy results in the reduction of number of neoplastic cells, followed by a recruitment of resting cells from phase Go into active replication. At this point of time, they are sensitive to phase independent drugs, which may effectively kill them. Thus, there probably is a point in alternating phase dependent and phase independent drug therapy. Cell cycle studies have provided a logical basis for high dosage intermittent combination therapy which possibly is the most effective mode of treatment. Drugs and timings can be selected to allow maximal
# Table I: BRUCE'S CLASSIFICATION OF ANTINEOPLASTIC DRUGS

<table>
<thead>
<tr>
<th>Phase specific drugs (phase dependent)</th>
<th>Phase non specific drugs (phase independent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate</td>
<td>Nitrogen mustard</td>
</tr>
<tr>
<td>6-Mercaptopurine</td>
<td>Cyclophosphamide</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>Melphalan</td>
</tr>
<tr>
<td>Cytosine arabinoside</td>
<td>Busulphan</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>Thio tepa</td>
</tr>
<tr>
<td>Vincristine</td>
<td>Chlorambucil</td>
</tr>
<tr>
<td>Vinblastin</td>
<td>Adriamycin</td>
</tr>
<tr>
<td>Procarbazine</td>
<td>Daunomycin</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>Mitomycin C</td>
</tr>
<tr>
<td>Razoxin</td>
<td></td>
</tr>
</tbody>
</table>

(12)
tumour cell killing, and minimal emergence of drug resistance.

The phase specific (phase dependent) drugs act chiefly on cells, in certain phases, and when in experimental studies a tissue culture is treated with these drugs, the percentage of cells surviving falls rapidly at first with increasing doses, but reaches a plateau when further increase in dose produces no further increase in cell death. Therefore the more rapid the cell turnover the more effective they are. Their dose response curve is initially exponential, but at higher doses a maximum response is reached and the curve becomes asymptotic. The phase specific drugs have proved effective in haematologic malignancies and tumours with a high rate of proliferation or high growth fraction.

The phase independent drugs act on cells in any phase of the cycle including a slight action on the Go phase. Cell survival in tissue culture falls progressively with increasing doses. Their dose response curve follows first order kinetics, and the cells are killed exponentially with increasing dosage, and the relationship throughout is linear. The phase nonspecific drugs are useful both in low growth fraction tumours and in some high growth fraction tumours.
B. THE LOG "CELL KILL" HYPOTHESIS:

Briefly, the log cell kill hypothesis, which is based on the studies on experimental tumour systems in animals, enunciates that the cell kill caused by cytotoxic drugs follows first order kinetics. This means that a constant percentage rather than a constant number of cells is killed by a given dose of a cytotoxic drug. Precisely cytotoxic drugs kill a constant fraction (99 to 99.999% or 2 to 4 log) of the tumour cell population, but never a 100% cell kill. The malignant cells that remain, cause a relapse in neoplastic disease. There is evidence that immunologic approaches to therapy of cancer may not face this restriction of not eradicating tumour cells completely since the immune system can totally eradicate even small number of tumour cells. However immunotherapy of cancer may be ineffective against large tumour cell masses. In advanced malignancy the body tumour cell burden may be even greater than $10^{12}$ cells and immunotherapy at best can tackle up to $10^{5}$ cells. A logical derivation to this concept has been to attempt and achieve total cell-kill by the use of several cytotoxic agents simultaneously\(9\).
2.1.3 Classification of Cytotoxic Drugs:

They may be grouped into six categories based on chemical and mechanistic properties and others on origins of natural products(10).

A. Alkylating agents

B. Antimetabolites

C. Natural products

D. Hormones and antagonists

E. Radioactive isotopes

F. Miscellaneous agents

A. ALKYLATING AGENTS:
They act by transferring alkyl groups to DNA in the N-7 position of guanine during cell division. They form covalent bonds with DNA and thus impede DNA replication.

B. ANTIMETABOLITES:
They are analogues of normal metabolites and do not act by competition. They block or subvert one or more of the metabolic pathways involved in DNA synthesis.

C. NATURAL PRODUCTS:

(a) Vinca Alkaloids: They are substances of plant origin, which specifically affect microtubule function and hence the formation of mitotic spindle.

(b) Glucosides: They have antimitotic action

(c) Antibiotics: They interfere with DNA/RNA synthesis

(15)
(d) **Enzymes**: Asparginase which starves tumour cells dependent on a supply of amino acid

**D. HORMONES.**

They act by altering the hormonal environment of hormone dependent tumours for example leukaemia, breast cancer and endometrial cancer. They bind with the receptors proteins in the cytoplasm of cancer cells in order to exert their anticancer effects.

The classification of some common antineoplastic agents is summarized in Table II and their mechanisms of action in Figure 2(b).

Two of the main drawbacks of the current chemotherapy of cancer are -

(a) the lack of selectivity of the drugs against the tumour cells as compared to normal cells; and

(b) the fact that, with many tumors, total elimination of malignant cells is not possible with therapeutic doses and the hosts' immune response is often not adequate to deal with the remaining cells.

Attempts are being made to overcome these two problems - the first, by using selective targeting of anticancer compounds and the second, by boosting or augmenting the hosts' immune response to the tumour.
Table II: CHEMOTHERAPEUTIC AGENTS USEFUL IN NEOPLASTIC DISEASE

<table>
<thead>
<tr>
<th>Class</th>
<th>Type of Agent</th>
<th>Non proprietary names (other names)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Alkylating agents</td>
<td>A. Nitrogen Mustards</td>
<td>Mechlorethamine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyclophosphamide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Melphalan</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chlorambucil</td>
</tr>
<tr>
<td></td>
<td>B. Ethyleneimines and methylmelamines</td>
<td>Hexamethylmelamine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thiotepa</td>
</tr>
<tr>
<td></td>
<td>C. Alkyl sulfonates</td>
<td>Busulfan</td>
</tr>
<tr>
<td></td>
<td>D. Nitrosoureas</td>
<td>Carmustine (BCNU)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lomustine (CCNU)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Semustine (methyl CCNU)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Streptozocin/ Streptozotocin</td>
</tr>
<tr>
<td></td>
<td>E. Triazenes</td>
<td>Dacarbazine (DTIC)</td>
</tr>
<tr>
<td>II. Antimetabolites:</td>
<td>A. Folic acid Analogs</td>
<td>Methotrexate</td>
</tr>
<tr>
<td></td>
<td>B. Pyrimidine Analogs</td>
<td>Fluorouracil (5-Fluorouracil) (5-FU)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flouxuridine (Fluorodeoxy uridine) (Fudr)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cytarabine</td>
</tr>
<tr>
<td></td>
<td>C. Purine Analogs and Related inhibitors</td>
<td>Mercaptopurine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thioguanine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pentostatin</td>
</tr>
</tbody>
</table>
(Table II continued)

<table>
<thead>
<tr>
<th>Class</th>
<th>Type of Agent</th>
<th>Non proprietary names (other names)</th>
</tr>
</thead>
<tbody>
<tr>
<td>III. Natural Products</td>
<td>A. Vinca alkaloids</td>
<td>Vincristine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vinblastine</td>
</tr>
<tr>
<td></td>
<td>B. Epipodophyllotoxins</td>
<td>Etoposide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Teniposide</td>
</tr>
<tr>
<td></td>
<td>C. Antibiotics</td>
<td>Dactinomycin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Daunorubicin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Doxorubicin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bleomycin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plicamycin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mitomycin</td>
</tr>
<tr>
<td></td>
<td>D. Enzymes</td>
<td>L- Asparginase</td>
</tr>
<tr>
<td></td>
<td>E. Biological Response modifiers</td>
<td>Interferon alfa</td>
</tr>
<tr>
<td>IV. Miscellaneous agents.</td>
<td>A. Platinum coordination complexes</td>
<td>Cisplatin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carboplatin</td>
</tr>
<tr>
<td></td>
<td>B. Anthracenedione</td>
<td>Mitoxantrone</td>
</tr>
<tr>
<td></td>
<td>C. Substituted Urea</td>
<td>Hydroxyurea</td>
</tr>
<tr>
<td></td>
<td>D. Methyl Hydrazine derivative</td>
<td>Procarbazine</td>
</tr>
<tr>
<td></td>
<td>E. Adrenocortical Suppressant</td>
<td>Mitotane</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amino glutethimide</td>
</tr>
</tbody>
</table>

(18)
<table>
<thead>
<tr>
<th>Class</th>
<th>Type of Agent</th>
<th>Non proprietary names (other names)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. Hormones and Antagonists</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Adreno corticosteroids</td>
<td></td>
<td>Prednisone</td>
</tr>
<tr>
<td>B. Progestins</td>
<td></td>
<td>Hydroxy progesterone caproate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medroxyprogesterone acetate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Megesterol acetate</td>
</tr>
<tr>
<td>C. Estrogens</td>
<td></td>
<td>Diethyl Stilbesterol Ethinyl estradiol</td>
</tr>
<tr>
<td>D. Antiestrogen</td>
<td></td>
<td>Tamoxifen</td>
</tr>
<tr>
<td>E. Androgens</td>
<td></td>
<td>Testosterone propionate</td>
</tr>
<tr>
<td>F. Antiandrogen</td>
<td></td>
<td>Flutamide</td>
</tr>
<tr>
<td>G. Gonadotropin releasing hormone analog</td>
<td></td>
<td>Leuprolide</td>
</tr>
<tr>
<td>VI Radioactive isotopes.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Sodium Phosphate</td>
<td></td>
<td>$^{32}$ Phosphofope</td>
</tr>
<tr>
<td>B. Sodium Iodide</td>
<td></td>
<td>$^{131}$ Iodotope</td>
</tr>
<tr>
<td>C. Radiogold Solution</td>
<td></td>
<td>$^{198}$ Aureotope</td>
</tr>
</tbody>
</table>
Figure 2: Summarization of Mechanisms And Sites of Action of Antineoplastic Agents

PALA = N-Phosphonoacetyl L-Aspartate
2.2 PROFILE OF 5-FLUOROURACIL:

2.2.1 Description:

A. NOMENCLATURE:
   (a) Chemical Names
      (i) 5 Fluoro - 2,4 (1H, 3H) - Pyrimidinedione
      (ii) 2,4 - Dioxo - 5 -fluoro pyrimidine
      (iii) 2,4 (1H, 3H) - pyrimidinedione - 5 -fluoro.
   (b) Generic Name:
      (i) 5-Fluouracil
      (ii) 5-FU

B. FORMULA:
   (a) Empirical \( C_4H_3FN_2O_2 \)
   (b) Structural

\[ \text{\centering}\begin{array}{c}
  \text{H} \\
  \text{N} \\
  \text{O} \\
  \text{F} \\
  \text{NH} \\
  \text{O} \\
\end{array}\]

C. MOLECULAR WEIGHT: 130.08

D. APPEARANCE, COLOR, ODOR:

Fluorouracil is white to practically white practically odorless, crystalline powder.

2.2.2 Physical Properties:

A. MELTING POINT:

Lies between 282 to 283°C with decomposition

(21)
B. SOLUBILITY:

The solubility data obtained at $25^\circ C$ for reference standard 5-fluorouracil is listed in Table III.

Table III: SOLUBILITY DATA OF 5-FLUOROURACIL

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>3.44</td>
</tr>
<tr>
<td>Benzene</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Chloroform</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>95% ethanol</td>
<td>5.54</td>
</tr>
<tr>
<td>Ethyl ether</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>2.15</td>
</tr>
<tr>
<td>Methanol</td>
<td>9.37</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Water</td>
<td>12.20</td>
</tr>
</tbody>
</table>

C. ULTRAVIOLET SPECTRUM:

(a) The ultraviolet spectrum of 5-fluorouracil in acetate buffer pH 4.7 in the region 350 to 220 nm exhibits one maximum at 266 nm ($E = 7.07 \times 10^4$) and one minimum at 232 nm.

(b) Infrared spectrum

The infrared spectrum of 5-fluorouracil in a potassium bromide disc is presented as follows:

<table>
<thead>
<tr>
<th>IR spectral Assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
</tr>
<tr>
<td>3124</td>
</tr>
<tr>
<td>1716 and 1657</td>
</tr>
<tr>
<td>1245</td>
</tr>
<tr>
<td>813</td>
</tr>
</tbody>
</table>
(c) **Others**

Characteristic peaks that 5-fluorouracil exhibits with regard to F, NMR, PMR and mass-spectra and x-ray diffraction have been discussed by Rudy and Senkowski\(^{(11)}\).

### 2.2.3 Dissociation Constant\(^{(11)}\):

The \(pK_a\) values for fluorouracil have been determined spectrophotometrically to be 8.01 ± 0.1 and 13.0 ± 0.1.

### 2.2.4 Stability\(^{(12)}\):

It is stable in solution which are not strongly basic (pH less than 9). 5-fluorouracil undergoes hydrolysis in strong alkaline solutions, with the probable formation of barbituric acid which rapidly degrades to other products. The hydrolysis is enhanced by increased pH and temperature. The shelf life of aqueous solution is about 3 years at pH 9 and room temperature.

### 2.2.5 Methods of Analysis:

The methods of analysis reported in I.P. \(^{(13)}\), B.P. \(^{(14)}\), USP \(^{(15)}\) are titrimetry for pure fluorouracil and ultraviolet spectrophotometric method for 5-fluorouracil cream and injection.

Other methods like colorimetry, \(^{(16)}\) thin layer chromatography \(^{(17)}\), gas chromatography \(^{(18}, 19, 20, 21, 22\)}, isotachophoresis \(^{(23)}\) and high pressure liquid chromatography \(^{(24}, 25, 26, 27\)}, have been widely
reported in literature for analysis of 5-fluorouracil in biological fluids like plasma and serum.

2.2.6 Incompatibility:

Preparations of fluorouracil are alkaline and compatibility problems may be expected with acidic drugs and preparations containing alkali sensitive drugs. 5-Fluorouracil is also reported to be incompatible with cytarabine, diazepam, doxorubicin and other anthracyclines (28).

2.2.7 Pharmacokinetics (28):

A. ABSORPTION:

5-Fluorouracil is most commonly administered intravenously. Oral preparations as tablets, syrups and solutions have been used, although in most cases absorption is unpredictable by this route. Responses after oral dosing appear to be shorter and fewer when compared with intravenous dosing.

After oral doses of 5-fluorouracil 15 to 20 mg/kg/day, bioavailability may range from 50 to 80%. Absorption can be increased by buffering the solution with 0.2 mol/L bicarbonate buffer (pH=9) along with administration of 100 ml of water. After a 200 mg oral dose of 5-Fluorouracil peak serum concentrations may range from 0.5 to 1.0 mg/L within 15 to 30 minutes, depending on oral preparation used and the presence and absence of food in the stomach.
B. DISTRIBUTION:
After intravenous injection, 5-fluorouracil is cleared rapidly from the plasma and is distributed throughout the body tissues and fluids including the cerebrospinal fluids and malignant effusions by passive diffusion, giving a volume of distribution of about 8 to 54 litres. It disappears from the plasma within 3 hours. After intravenous injection 5-fluorouracil persists for longer periods in the malignant effusions than in plasma.

C. METABOLISM:
5-Fluorouracil is metabolized extensively in the liver and its concentration decline rapidly to undetectable level within 2 hours.

The metabolic pathway of fluorouracil is presented schematically in Figure 3.

Fluorouracil is converted to fluorouridine and then to mono, di and triphosphates of fluorouridine. This is then incorporated into the fraudulent RNA. Fluoridine monophosphate is also reduced to fluoro-2'-deoxyuridine monophosphate. There is no further metabolism to the di and triphosphate nucleotides of fluoro-2'-deoxyuridine. Therefore the formation of fluoro-2'-deoxyuridine monophosphate is considered to be the basis for the antineoplastic action of fluorouracil since it inhibits DNA synthesis by blocking the enzyme thymidylate synthetase. Fluorouracil is catabolized in an ana-
Figure 3: Metabolic pathway of fluorouracil

\[ \alpha\text{-Fluoro-}\beta\text{-Alanine} + \text{Urea} + \text{Co} \]

\[ \alpha\text{-Fluoro-}\beta\text{-Ureidopropionic acid} + \alpha\text{-Fluoro-}\beta\text{-Guanidopropionic acid} \]

\[ \text{Dihydrofluorouracil} \]

\[ 5\text{-Fluorouracil} \rightarrow 5\text{-Fluorouridine} \rightarrow 5\text{-Fluorouridine monophosphate} \]

\[ 5\text{Fluoro-2'}\text{-Deoxyuridine} \rightarrow 5\text{Fluoro-2'}\text{-Deoxyuridine monophosphate} \]

\[ \text{RNA} \rightarrow 5\text{-Fluorouridine triphosphate} \]

\[ \text{DNA} \rightarrow \text{Thymidilic acid} \]

\[ \text{Deoxyuridilic acid} \rightarrow \text{Uridylic acid} \]
logous manner to uracil, forming the following degradative products: dihydro fluorouracil, α-fluoro-β-ureidopropionic acid, α-fluoro-β-guanido-propionic acid, α-fluro-β-alanine, urea and Co.

D. **ELIMINATION** (28): 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 a respiratory carbon dioxide. The total body clearance of 5-fluorouracil after intravenous injection is 0.6 to 1.9 litre/minute. When given as a continuous infusion, its urinary excretion is only 4%.

E. **CLINICAL TOXICITY**: The earliest untoward symptoms during a course of therapy are anorexia and nausea; and these are followed shortly after by stomatitis and diarrhoea, which constitute reliable warning signs that a sufficient dose has been administered. The major toxic effects, resulting from the myelosuppressive action of these drugs includes leukopenia, thrombocytopenia, anemia, gastrointestinal ulceration, loss of hair, nail changes, dermatitis and increased pigmentation and atrophy of the skin. Local inflammatory and photosensitivity reactions can also occur following topical use.
2.2.8 Mechanism of Action:
Fluorouracil, a pyrimidine analogue is an antineoplastic agent which acts as an antimetabolite by interfering in DNA synthesis. 5-Fluorouracil is biotransformed to 5-Fluorodeoxyuridylate which inhibits thymidylate synthetase, an enzyme that promotes the conversion of deoxyuridine into deoxythymidine. Since thymidine is essential for DNA synthesis, its lack halts cell proliferation. In addition fluorouracil is incorporated as the nucleotide into RNA thereby depressing RNA synthesis.

Fluorouracil biotransformed
to
5-Fluorodeoxyuridylate
inhibits
thymidylate synthetase

Uridine monophosphate \(\rightarrow\) Thymidine monophosphate
\(\downarrow\)
DNA

2.2.9 Uses:
Fluorouracil is used alone or in combination in the adjuvant treatment of breast and gastrointestinal cancer and palliation of inoperable malignant neoplasms, especially those of the gastrointestinal tract, breast, head and neck, genitourinary system and pancreas. It is often used with cyclophosphamide and

(28)
methotrexate in the combination chemotherapy of breast cancer.

It is used topically in the treatment of solar (actinic) keratoses and other tumours and premalignant conditions of the skin including Bowens' disease and superficial basal cell carcinomas. It is usually applied as a 1 or 5% cream or ointment or as a 1 to 5% solution in propylene glycol.

2.2.10 Dose and Dosage Schedule:
A usual dose by intravenous injection is 12 mg per kg body weight daily to a maximum of 0.8 to 1 gram daily for 3 or 4 days. If there is no evidence of toxicity this may be followed after 1 day by 6 mg per kg on alternate days for 3 or 4 further doses.

An alternative schedule is to give 15 mg per kg intravenously once a week throughout the course. The course may be repeated after 1 month or maintenance doses of 5 to 15 mg per kg may be given weekly.

Fluorouracil may also be given by intravenous infusion, usual doses of 15 mg per kg daily to a maximum of 1 gram daily being infused in 500 ml of 0.9% NaCl or 5% glucose injection over four hours and repeated on successive days until toxicity occurs or a total of 12 to 15 gram has been given. Continuous infusion may also be used. The course may be repeated after 4-6 weeks. Flurouracil has been given by

(29)
intra-arterial infusions in doses of 5 to 7.5 mg per kg daily and by mouth.

2.2.11 Preparations(30):

A. FLUOROURACIL CREAM (U.S.P.):
A cream containing fluorouracil. It may contain sodium hydroxide to adjust the pH.

B. FLUOROURACIL INJECTION (USP):
A sterile solution in water for injections prepared with the aid of sodium hydroxide. It contains 45 to 55 mg of fluorouracil in each ml, pH 8.6 to 9 and stored at 15-30°.

C. FLUOROURACIL TOPICAL SOLUTION:
A solution of fluorouracil. It may contain sodium hydroxide to adjust the pH.

2.3 DRUG TARGETING:
The object of drug targeting is to achieve a desired pharmacological response at a selected site without undesirable interactions at other sites. This is especially important in cancer chemotherapy and enzyme replacement therapy. At present drug targeting is achieved by one of the two approaches.

The first approach involves chemical modification of the parent compound to a derivative which is activated only at target site.

The second approach utilizes carriers such as liposomes, microspheres, nanoparticles, antibodies, cellular
carriers, (erythrocytes and lymphocytes) and macromolecules to direct the drug at its site of action(31).

2.3.1 Chemical Modification:

There are a variety of strategies to modify the chemical structure of drug molecules, the most common being the pro drug approach and the most sophisticated being the chemical delivery system approach. A prodrug is an inactive chemical derivative of a parent compound that is activated predictably invivo to the active drug species.

A Chemical delivery system involves transformation of the active drug by synthetic means into an inactive derivative which, when placed in the body will undergo several predictable enzymatic transformations principally at its site of action. This approach has been proven successfully for local delivery of drugs to the eye, brain and testes(31).

2.3.2 Use of Carrier systems:

A wide variety of drug carriers like microspheres, liposomes, nanoparticles have been reported in literature for site specific delivery. A drug delivery system employed for cancer chemotherapy, must be preferentially distributed within a target area where the tumour resides, and then release the drug in an active form at a suitable rate.

Widder et al (31a) has reported three stages of targeting using drug carrier systems.

(31)
A. **FIRST ORDER TARGETING**:

It involves the restricted distribution of a delivery system to the capillary beds of a predetermined target site, organ or tissue. For a solid tumour, this includes transendothelial migration of either the drug carrier complex or drug alone.

B. **SECOND ORDER TARGETING**:

It refers to selective direction of the drug carrier complex or the drug alone to tumour cells Vs. normal cells. For solid tumour, this can occur only after the drug carrier complex or the drug itself has gained access to the tissue parenchyma.

C. **THIRD ORDER TARGETING**:

It involves the carrier directed release of drug at selected intracellular sites. This infers that the drug carrier complex enters the tumour cells by either phagocytosis or cell fusion.

2.3.3 Ideal Attributes of a Drug Delivery System For Drug Targeting(32):

(i) It must accumulate or remain at the desired target area, organ or tissue

(ii) It must make the drug readily accessible to tissue parenchyma in the target site.

(iii) It must deliver the drug preferentially to tumour cells.

(iv) The carrier material must be nontoxic and bio-compatible.
It must be pharmaceutically as well as clinically acceptable with regard to stability, sterilization, cost of manufacture and ease of administration.

If a drug delivery system satisfies all of the above mentioned criteria, then it would be given through intravenous or oral routes leading to markedly enhanced therapeutic effects without any severe systemic toxicity. Because of impermeability of the drug carrier complex in the hostile environment of the GI tract, administration of high molecular weight carrier complex is restricted to intra-venous or intra-arterial administration or to direct injection into the target tissue.

2.3.4 Obstacles Encountered in Drug Targeting Using Macromolecular and Particulate Carrier(32-34)

At present the major obstacle of drug targeting using macromolecular and particulate carriers is rapid sequestration of intravascularly administered drug carriers by mononuclear phagocytes of the reticulo endothelial system. Because of rapid clearance, only a small fraction of the injected carrier ultimately reaches the target if at all.

Secondly, a drug carrier to be able to recognise the target, must first extravasate (pass through the capillary endothelium). The vascular endothelium of most tissues and organs being continuous with an
The effective pore diameter of about 2 nm is essentially impermeable to molecular assemblages such as liposomes (0.025-5 micron) and nanoparticles (<1 micron). Significant extravasation of the structures in this size range is only possible at those sites where a discontinuous endothelium exists, notably in the sinusoids of the liver and spleen, where effective pore diameter is 100 nm. Thus most particulate matter is confined to general circulation.

Several strategies have been adopted in achieving drug targeting and overcoming the above mentioned problems. They are:

a) The first approach involves blocking of RES by administration of a placebo colloid prior to the administration of the drug carrier. However paralysis of RES is undesirable especially in cancer patients. Without the first line defense mechanism of the RES against infectious agents, these cancer patients will be at risk to infections.

b) A second approach is to impart specificity to the drug carrier by coupling specific ligands on its external surface. These include desialylated fetalin, erythrocyte membrane glycoproteins, heat aggregated immunoglobulins, monoclonal antibodies, and native immunoglobulins. Such type of targeting is known as active (ligand) targeting.
This strategy is yet to be proved successful in preserving the recognition ability invivo and avoid triggering any immunological response.

c) The localisation of a drug delivery system to specific anatomical compartments can be achieved by intra arterial or by local administration into joint, lung and peritoneal cavity. Such targeting is known as compartmental targeting. The microcapsules can be readily infused into tumour supplying arteries via percutaneous catheterisation and distributed within the arteriolar beds in the tumour tissue, where they are entrapped and where they gradually release the drug through the semipermeable capsular membrane. They act as long acting drugs and as an embolic material. Infarction itself caused by embolization produces a considerable range of histological damage in the tumour tissue, but it also increases the diffusion of drug into the surrounding parenchyma at an early stage of oedematous change. Additionally, the reduction in topical blood circulation by embolization impedes the rapid clearance of the drug from the target tissue into systemic circulation thus leading to a decrease in systemic drug toxicity.

This approach to first order targeting is simple and practical and has proved to be clinically useful.
Microspheres can be used for passive targeting or natural targeting to organs such as liver, spleen, lung and kidney, by judicious choice of particle size and to a lesser extent the nature of carrier system. This can take place due to interaction between colloidal particles and physiological processes.

The first organ site in which significant number of particles can accumulate following intravenous administration is the lungs. Large particles greater than 7 micron will be trapped by mechanical filtration in the capillary beds. Consequently, highly specific targeting of colloidal particles to the lungs can be achieved by the use of large particles. Particles less than 7 micron will normally pass through the lungs without being trapped. The liver and spleen are normally the primary sites for the deposition of small colloidal particles. These organs are supplied with fixed macrophages that will engulf foreign particles. Such uptake into liver and spleen can be extremely efficient and provides a simple means of selective delivery of the drugs to the liver. Particles less than 100nm have the possibility of leaving the systemic circulation through fenestrations in the cells lining the blood vessels. Such colloidal carriers can lead
to first order targeting of liver and kidney and second order targeting to tumour bearing organs.

e) Physical Targeting:
It involves the use of influence such as temperature, pH or magnetic field to direct particles to desired site. Both the temperature and pH effects are based respectively on the fact that the tumour cells have a local hyperthermia and lower ambient pH than normal tissues.

Ferromagnetic microcapsules containing zinc ferrite particles can be guided to target sites by external magnetic force and exert a potential antineoplastic effects. First order targeting can be well developed using magnetic guidance system.

2.3.5 Carriers Associated With Anticancer Drugs(32):
The recent rapid growth of polymer science and technology has provided novel carrier materials for drug delivery.

A variety of carrier materials have been reported to be associated with anticancer drugs as summarized in the Table IV.

2.4 EFFORTS TOWARDS TARGETING OF 5-FLUOROURACIL:
Targeted drug delivery systems of 5-fluorouracil are reported by many researchers. They include the use of microspheres, nanoparticles, liposomes, emulsions and other miscellaneous systems. These reports are briefly summarised as under.
Table IV: CARRIERS ASSOCIATED WITH ANTICANCER DRUGS

<table>
<thead>
<tr>
<th>CARRIERS</th>
<th>DRUGS</th>
<th>REFERENCE NUMBER</th>
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</thead>
<tbody>
<tr>
<td>Macromolecules</td>
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<tr>
<td>Immunoglobulins</td>
<td>Chlorambucil</td>
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<tr>
<td></td>
<td>Methotrexate</td>
<td>(36)</td>
</tr>
<tr>
<td></td>
<td>Daunomycin, Adriamycin</td>
<td>(37)</td>
</tr>
<tr>
<td>Desoxyribonucleic acid</td>
<td>Daunomycin</td>
<td>(38)</td>
</tr>
<tr>
<td></td>
<td>Adriamycin</td>
<td>(39)</td>
</tr>
<tr>
<td>Cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>Methotrexate, Adriamycin</td>
<td>(40)</td>
</tr>
<tr>
<td></td>
<td>Methotrexate</td>
<td>(41)</td>
</tr>
<tr>
<td>Synthetic System</td>
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<td></td>
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<tr>
<td>Liposomes</td>
<td>Actinomycin D</td>
<td>(42)</td>
</tr>
<tr>
<td></td>
<td>5-Fluorouracil</td>
<td>(43)</td>
</tr>
<tr>
<td></td>
<td>Neuraminidase</td>
<td>(44, 44a)</td>
</tr>
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<td></td>
<td>Methotrexate</td>
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<tr>
<td></td>
<td>Bleomycin</td>
<td>(46)</td>
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<tr>
<td></td>
<td>Cytosine arabinoside</td>
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<tr>
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<td>Colchicine</td>
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<tr>
<td></td>
<td>Asparginase</td>
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<td></td>
<td>Bichloroethyl nitrosourea</td>
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<tr>
<td></td>
<td>Vinblastin</td>
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<tr>
<td>Fat emulsion</td>
<td>5-Fluorouracil</td>
<td>(52)</td>
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<tr>
<td></td>
<td>Bleomycin, Mitomycin</td>
<td>(53)</td>
</tr>
<tr>
<td></td>
<td>Bleomycin</td>
<td>(54)</td>
</tr>
<tr>
<td>Albumin microspheres</td>
<td>6-Mercaptopurine</td>
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<td></td>
<td>5-Fluorouracil</td>
<td>(56)</td>
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<tr>
<td></td>
<td>Adriamycin</td>
<td>(57)</td>
</tr>
<tr>
<td>Poly (glutamic acid)</td>
<td>Phenylalanine mustard</td>
<td>(58)</td>
</tr>
<tr>
<td></td>
<td>Cyclophosphamide</td>
<td>(59)</td>
</tr>
<tr>
<td>Agarose beads</td>
<td>Mitomycin C</td>
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<td></td>
<td>Cytosine arabinoside</td>
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<tr>
<td>Ethyl cellulose microcapsules</td>
<td>Mitomycin C</td>
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<td></td>
<td>Adriamycin, 5FU, Bleomycin</td>
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<tr>
<td></td>
<td>Peplomycin</td>
<td>(64)</td>
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</table>

(38)
<table>
<thead>
<tr>
<th>CARRIERS</th>
<th>DRUGS</th>
<th>REFERENCE NUMBER</th>
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</thead>
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<td>Ferromagnetic ethylcellulose</td>
<td>Mitomycin C</td>
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<tr>
<td>microcapsules</td>
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<td></td>
</tr>
<tr>
<td>Magnetic Albumin microspheres</td>
<td>Adriamycin</td>
<td>(66)</td>
</tr>
<tr>
<td>Poly (alkyl 2-cyanoacrylate)</td>
<td>Dactinomycin, Vinblastin, Methotrexate</td>
<td>(67)</td>
</tr>
<tr>
<td>Poly (L-lysine)</td>
<td>Methotrexate</td>
<td>(68)</td>
</tr>
<tr>
<td>Dextran</td>
<td>Mitomycin C</td>
<td>(69)</td>
</tr>
<tr>
<td>Gelatin microspheres</td>
<td>Mitomycin C</td>
<td>(70)</td>
</tr>
</tbody>
</table>
2.4.1 Microspheres And Nanoparticles:

Sugibayashi et al (71,72) reported the preparation and tissue distribution of albumin microsphere entrapped 5-fluorouracil in mice. They reported that 5-fluorouracil entrapped in albumin microsphere localized mainly in the liver and disappearance of the drug from the tissue was very slow in comparison with that of free drug. Morimoto et al (73) studied albumin microspheres containing 5-fluorouracil as a drug carrier and studied the anti-tumour activity of albumin microsphere entrapped 5-fluorouracil against Ehrlich-ascites carcinoma and solid tumour in mice. They reported that after intraperitoneal and intratumoural injection of microspheres entrapped 5-fluorouracil into Ehrlich ascites bearing mice, the 5-fluorouracil level in ascites was high compared with that after injection of free drug.

Miyazaki et al (74) studied antitumour activity of fibrinogen microspheres containing 5-fluorouracil in Ehrlich ascites carcinoma in mice. They also reported that fibrinogen microspheres can be used as a novel carrier in injectable delivery systems for antitumour drugs.

Benita et al (75) reported the preparation and properties of carnuba wax microspheres containing 5-fluorouracil for arterial chemoembolization.
Kenneth et al (76) prepared microspheres of monoglyceride, monodiglyceride, lactic acid, cellulose and natural wax for chemoembolization of 5-fluorouridine.

Teder et al (77) reported the influence of degradable starch microspheres on liver uptake of 5-fluorouracil after hepatic artery injection in the rat and reported that these microspheres might be of value in increasing the drug concentration in tumour tissue and reducing systemic toxicity.

Jeyanthi et al (78, 79) incorporated 5-fluorouracil in gelatin microspheres and studied its in vitro release rate. They also incorporated 5-fluorouracil in implantable collagen Poly (HEMA) hydrogel carriers and reported better antitumour activity of hydrogel formulation over free 5-fluorouracil when evaluated against a solid tumour fibrosarcoma in wistar rats.

Mukherji et al (80, 81, 82) encapsulated 5-fluorouracil in gelatin microspheres polygluteraldehyde nanoparticles and ethylcellulose microspheres and reported the tissue distribution of 5-fluorouracil from these drug delivery systems in liver, lungs and intestine when injected intravenously in rats.

Ghorab et al (83) reported the preparation of controlled release 5-fluorouracil ethylcellulose microspheres as a promising formulation for the treatment of skin lesions.

Yalabik-Kas et al (84) studied the sorption of 5-fluorouracil onto polymethylmethacrylate nanoparticles.
and found a linear sorption isotherm, fitting the van't Hoff's equations.

Kreuter et al(85) reported a comparative study on the cytostatic effects and the tissue distribution of 5-fluorouracil in a free form and bound to poly (butyl cyanoacrylate) nanoparticles in sarcoma 180 bearing mice. They reported an enhanced efficacy and higher toxicity of the drug bound to nanoparticles.

Sawant et al(86) reported the in vivo evaluation of poly isobutylcyanoacrylate microparticles containing 5-fluorouracil and reported preferential accumulation of 5-fluorouracil in lungs and spleen as compared to free drug solution.

The same co-workers(87) also reported maximum drug accumulation of 5-fluorouracil in lungs, followed by liver and kidneys from polyacrylamide microcapsules containing 5-fluorouracil.

They evaluated polyterepthalamide microcapsules and crosslinked haemoglobin microcapsules(88) containing 5-fluorouracil for in vivo organ distribution studies and reported preferential distribution of 5-fluorouracil in lungs and liver as compared to free 5-fluorouracil solution.

2.4.2 Liposomes:

Ozer et al(89) and Tsukada et al(90) and Fresta et al(91) reported the formulation, characterization, stability and in vitro drug release of liposomes entra-
pped 5-fluorouracil. Tao et al (92) entrapped 5-fluorouracil and cyclophosphamide in polyphase liposome and found two of the preparations devoid of any adverse effects on phagocytosis and leukocyte counts. Simmons et al (93) found that the liposomal entrapment of floxuridine is better than the parent compound, 5-fluorouracil.

2.4.3 Emulsions:

Hashida et al (94) evaluated the efficiency of w/o and gelatin microspheres in oil (s/o) emulsion as a drug delivery system of delivering 5-fluorouracil specifically into lymphatics of rat following intragastric and intra muscular injection. They compared the distribution of 5-fluorouracil in plasma, regional lymph nodes, thoracic lymph and injection site with that of intravenous injection of 5-fluorouracil and reported superiority of w/o and s/o emulsions in cancer chemotherapy.

Fukushima et al (95) reported the preparation and drug release from w/o/w type double emulsions containing cytarabine and 5-fluorouracil. They reported a prolonged release of 5-fluorouracil from these emulsions as a function of pH.

Omotosho et al (96-98) incorporated 5-fluorouracil in w/o/w multiple emulsions and reported that the drug absorption was increased and prolonged as compared to free drug solution when administered intramuscularly.
and also reported lymphatic absorption and accumulation of 5-fluorouracil in rats following oral administration.

2.4.4 Miscellaneous systems:

Miyazaki et al. (99-101) reported the factors affecting release pattern and anti-tumour effect of 5-fluorouracil incorporated in ethylene vinyl acetate copolymer matrices. They also reported a decreased release of 5-fluorouracil and prolongation of life span of tumour bearing mice from ethyl vinyl alcohol copolymer matrices.

Jones et al. (102) reported the use of polystyrene based ion exchange resins as a carrier system for sustained delivery of 5-fluorouracil and floxouridine.

Maa and Heller (103) reported the use of bio-erodible matrices of linear poly orthoesters for controlled release of 5-fluorouracil. Similarly Seymour et al. (104) reported the preparation and evaluation of poly ortho ester matrices containing 5-fluorouracil against a human colorectal carcinoma model in vivo.

Ouchi et al. (105) synthesized conjugates of poly (α-malic acid) and 5-fluorouracil bound via ester, amide and carbamoyl bonds and studied their anti-tumour activity.

Mukherji et al. (80, 106) 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 rats. They also synthesized the prodrugs of 5-fluorouracil containing cysteine and glutamic acid ethyl ester. When entrapped in ethylcellulose particles these 3-carbamoyl derivatives of 5-fluorouracil were found to accumulate predominantly in the lungs, followed by liver.

Chung et al(107) studied the pharmacokinetics of 5-fluorouracil in rabbits after intravenous infusions of 5-fluorouracil acetic acid-human serum albumin conjugates as compared to free 5-fluorouracil and reported a slow release of 5-fluorouracil from the conjugates.

Miyazaki et al(108) evaluated the use of pluronic F-127 gel as a vehicle for topical administration of 5-fluorouracil.

2.5 NIOSOMES:

Niosomes or non ionic surfactant vesicles are microscopic lamellar structures formed on admixture of a nonionic surfactant and cholesterol with subsequent hydration in aqueous media. Stable vesicles can also be formed in absence of cholesterol.

Many synthetic amphiphiles form vesicles but as most are ionic and relatively toxic they are generally unsuitable for use as drug carriers. Vesicle formation by some members of dialkyl polyoxyethylene ether nonionic surfactant series was reported by Okahata et al(109). Handjani Vila et al(110) reported that vesicular systems were formed when a
mixture of cholesterol and a single alkyl chain nonionic surfactant was hydrated. The resultant vesicles which have been termed "Niosomes" can entrap solutes, are osmotically active and stable(111). In addition handling and storage of the surfactant requires no special conditions. Niosomes behave in-vivo like liposomes, prolonging the circulation of entrapped drug and altering its organ distribution and metabolic stability(112).

The properties of niosomes depend both on the composition of bilayer(113) and on the method of their production(114). Niosomes are now widely studied as an alternative to liposomes as liposomes exhibit certain disadvantages. Disadvantages of liposomes:

(a) Expensive
(b) Phospholipids are chemically unstable because of their predisposition to oxidative degradation.
(c) They require special storage and handling.
(d) Purity of natural phospholipids is variable.

On the other hand niosomes possess several advantages(115) which include:

(a) Surfactant does not require special handling and storage condition.
(b) Inexpensive
(c) Niosomes are biodegradable, bio compatible and non-immunogenic.
(d) Niosomes can entrap a wide range of compounds ranging from small solute ions to complex macromolecules.

(e) They possess a great deal of flexibility in their structural characteristics.

(f) They offer their surface for the attachment of target oriented ligands.

(g) They can be made to reach the site of action by oral, parenteral and topical route.

(h) Niosomes can enhance the skin penetration of drugs.

Niosomes like liposomes can be expected to have two beneficial effects:

(a) As a vehicle for drug formulations, they may reduce the systemic toxicity of clinically important antineoplastic agents.

(b) As carriers for enhanced delivery to specific cells, niosomes may improve the therapeutic index by restricting drug effects to target cells.

2.5.1 Materials used for Niosome preparation:

Many synthetic non-ionic surfactants are reported to be used in the formulation of niosomes. These include:

- Single alkylchain non-ionic surfactants, dialkyl polyoxyethylene ethers, monoalkyl glycerol ethers, sorbitan monoesters (including sorbitan mono laurate, mono palmitate, mono stearate, mono oleate), sorbitan triesters (sorbitan trioleate), Polyoxy ethylene sorbitan
esters (Tween 40, 60, 80), Brij 35, Hexadecyl di-glyceryl ether, etc.

The major disadvantage with colloidal carriers is that when they are administered intravenously, they are rapidly removed from the circulation by the cells of mononuclear phagocytic system (MPS). Modification of the surface properties by coating the carriers with hydrophilic polymers is known to alter the rate of uptake by MPS. Hence a vesicle forming non ionic surfactant, which contained as its hydrophilic portion a long polyoxyethylene chain, was synthesized, namely poly oxyethylene glycerol 1, 2 distearoyl ether (116) and used in niosome formulation. Another component which has been used along with most surfactants in formulation of niosomes is cholesterol. Cholesterol can regulate the permeability of biological membranes by affecting the internal viscosity and molecular motion of the lipids in the membrane. The decrease in permeability is generally proportional to the concentration of the cholesterol which may be explained due to increased packing and decreased mobility of the hydrocarbon chains. The stability and drug release from the niosomes in vitro and in vivo may be controlled by varying the cholesterol content of the vesicle membrane (115).

Cholesterol content has also been reported to increase the entrapment efficiency of niosomes prepared from Span surfactants. X-ray diffraction methods have demonstrated that cholesterol increases the width of
phospholipid bilayers. The increased drug entrapment is most likely to be the result of increased vesicle size (117). Cholesterol also reduces leakage rate of entrapped drug and drug release rate from niosomes (111, 118).

Dicetyl phosphate has been reported to decrease the size of vesicles since the introduction of charges in the bilayers might increase the membrane curvature (119).

Water soluble drugs get accumulated in the aqueous compartment of niosomes. Their entrapment is low and on storage, leakage of the drug is a problem. Further separation of drug entrapped vesicles from that of unentrapped drug requires another step. However such problems are not countered if the drug is lipid soluble. The drug remains lipid membrane bound and such preparation can be stored in a dry form, meant to be rehydrated prior to use. Therefore selection of the lipid soluble drug can almost result in 100% drug encapsulation irrespective of non ionic surfactant type and composition (115).

2.5.2 Methods of Preparation:

Several methods have been reported for the preparation of niosomes, which include -

(A) Ether injection method

(B) Hand-shaking method
A. **ETHER INJECTION**

This method provides a means of making niosomes by slowly introducing a solution of surfactants dissolved in diethyl ether into warm water maintained at 60°C. Typically the surfactant mixture is injected through a 14 gauge needle into an aqueous solution of the material to be encapsulated at 15°C or 60°C. Vaporization of ether leads to formation of single layered vesicles. Depending on the conditions used, the diameters of the resulting vesicles range from 50 to 1000 nm.

B. **HAND SHAKING METHOD**

The mixture of vesicle forming ingredients like surfactant and cholesterol are dissolved in a volatile organic solvent (diethyl ether, chloroform) in a round bottom flask. The organic solvent is removed at room temperature (20°C) using a rotary evaporator, leaving a thin layer of solid mixture deposited on the wall of a round bottom flask. The dried surfactant film can be dehydrated with aqueous phase at 50-60°C with gentle agitation. By this process typical multilamellar niosomes are formed.
C. **SONICATION (120):**
The surfactant/cholesterol mixture is taken in a glass vial and aqueous phase is added to it and the mixture probe sonicated. Probe sonication leads to more rapid size reduction, heat production, metal particle shedding from the probe tip and aerosol generation which may present problems.

D. **MICROFLUIDIZATION (115):**
Microfluidization is the recent technique used to prepare unilamellar vesicles of a defined size distribution. This method is based on submerged jet principle in which two fluidized streams interact at ultra high velocities in precisely defined microchannels within the interaction chamber. The impingement of thin liquid sheets along a common front is arranged such that the energy supplied to the system remains within the area of niosome formation. The result is greater uniformity, smaller size and better reproducibility in a device scaleable to commerical production.

E. **MULTIPLE MEMBRANE EXTRUSION METHOD (121):**
The mixtures of surfactant and cholesterol and dicetyl phosphate are formed into thin film by evaporation from chloroform. The film is hydrated with aqueous drug solution and the resultant suspension extruded through two 0.1 micron polycarbonate membrane, in series, for upto 8
passages. Extrusion is a good method for controlling niosome size and for reducing polydispersity.

2.5.3 Separation of unentrapped drug:

The removal of unentrapped solute from the vesicles can be accomplished by various techniques which include:

A. **DIALYSIS** ([112, 116, 120, 121, 122]):

The aqueous dispersions are dialyzed in a dialysis tubing against pH 7.4 phosphate buffer or normal saline or glucose solution.

B. **GEL FILTRATION** ([117, 123, 124]):

The unentrapped drug is removed by gel filtration of the niosomal dispersion through a Sephadex-G-50 and elution with phosphate buffer saline or normal saline.

C. **CENTRIFUGATION** ([118]):

The niosome suspension is centrifuged and the supernatant is discarded. The pellet is washed and then resuspended to obtain a niosomal suspension free from unentrapped drug.

2.5.4 Characterization of niosomes:

A. **ENTRAPMENT EFFICIENCY**:

The amount of drug entrapped is determined by disruption of vesicles with 0.025% Triton-X-100 or propanol and analysing the resultant solution by appropriate assay method for the drug.
Entrapment efficiencies are expressed as a percentage of total amount of drug added initially (118, 122, 117).

An indirect method to determine drug entrapment efficiency has also been reported (123). The niosomal suspension is separated from unentrapped drug by gel filtration through Sephadex G-50 column. The eluant after separation of niosomal fraction is collected and analysed for amount of drug unentrapped by suitable assay method. The amount of drug entrapped is determined from the difference between amount loaded initially and amount unentrapped.

B. VESICLE SIZE:
Methods used for vesicle size determination which are reported include photon correlation spectroscopy and microscopy. Freeze fracture electron microscopy has been used to visualize the niosome vesicles.

C. IN VITRO RELEASE:
A method for an in vitro release rate study reported recently includes use of dialysis (Cuprophan) tubing. Dialysis tubing is washed with distilled water and left soaking in distilled water. The vesicle suspension is pipetted into a bag made up of the tubing and sealed. The bag containing the vesicles is placed in 250 ml of buffer solution in a 300 ml conical flask with constant shaking at (53).
25° or 37° C. At various time intervals, the buffer is analysed for the drug content by appropriate assay method (117).

D. STABILITY:
The stability of niosomes has been studied with respect to the amount of leakage/efflux of drug from the niosomes. The niosomes are stored in a suitable buffer and amount of drug effluxing is determined at various time intervals after Sephadex elution (119, 120).

2.5.3 Niosomes as carriers for various drugs:
A number of workers have reported the preparation characterization and use of niosomes for site specific delivery of various drugs.

Baillie (111, 120) et al have investigated the encapsulation and retention of entrapped solute 5, 6 carboxy fluorescein in niosomes, and have also compared various methods of niosome production as a prelude to further biological investigations. They have also described various aspects of characterization of niosomes. The same workers have reported the effect of cholesterol on entrapment efficiency and drug retention property of niosomes. They have also reported that vesicular structures could be formed even in absence of cholesterol.
Baillie et al\textsuperscript{(125)} have also reported the use of niosome as a delivery system for antileishmanial drug sodium stibogluconate.

Hunter et al\textsuperscript{(122)} reported the efficacy of niosomel loaded sodium stibogluconate in treatment of experimental murine visceral leishmaniasis and reported an improved anti parasitic activity as compared to the free drug.

Stafford et al\textsuperscript{(121)} reported the influence of entrapped drug (sodium stibogluconate or calcein) on the size of niosomes prepared by a membrane extrusion method. They indicated that entrapped drug generally increased niosome size which may be due to solute interaction with surfactant head groups.

Dolan et al\textsuperscript{(126)} studied the factors affecting the outcome of carrier mediated therapy of experimental visceral leishmaniasis. They reported that the type of vesicular carrier did not influence the therapeutic outcome. But manipulation of vesicles size by sonication and use of multiple dosage schedule achieved a decrease in parasite numbers in liver, spleen and bone marrow.

Carter et al\textsuperscript{(127)} reported that multiple dosing with sodium stibogluconate loaded niosomes was found to be effective against parasites in the liver, spleen and bone marrow as compared to sodium stibogluconate solution.
Chauhan et al. (116) reported the preparation of nonionic surfactant vesicles containing sodium stibogluconate by incorporation of a long polyoxyethylene chain in the surfactant in order to reduce the uptake of niosomes by cells of mononuclear phagocytic system which otherwise are responsible for removal of colloidal particles from the circulation. They found that incorporation of polyoxyethylene chain lead to formation of smaller vesicles.

Azmin et al. (112, 128) investigated the effect of niosome entrapment on the absorption and distribution of methotrexate in mice. They reported that intravenous administration of niosomes containing methotrexate prolonged the level of methotrexate in blood. Large amount of drug was taken up by the liver and brain on intravenous as well as oral administration. The same co-workers also reported that following oral and intravenous administration of niosome encapsulated methotrexate in mice, its excretion was reduced in urine and bile and it is protected from rapid metabolism as compared to co-administration of free drug and polysorbate 80.

Chandraprakash et al. (129, 130) and Udupa et al. (131) reported the formulation and pharmacokinetic evaluation of niosome entrapped methotrexate in tumour bearing mice. They reported that entrapment efficiency increased with increase in lipophilicity of surfactant and its pharmacokinetics in mice transplanted with
S-180 tumour was markedly different in comparison to unentrapped methotrexate.

Rogerson et al(132) reported the microencapsulation of adriamycin in niosomes and studied its entrapment efficiency and stability. They showed that efficiency of adriamycin entrapment was dependent on surfactant adriamycin association. Light induced drug degradation was reduced by niosome encapsulation and efflux of niosome was decreased by inclusion of cholesterol.

Rogerson et al(133) also reported the distribution of doxorubicin (adriamycin) encapsulated in niosomes by bolus injection in S-180 tumour bearing mice. The biological half life of the drug was found to be prolonged as compared to free solution and tumour levels of drug were higher following administration of cholesterol containing niosomes and resulted in more effective reduction in tumour growth.

Khana et al(118) studied the effect of cholesterol on release of doxorubicin from niosomes. They reported that rate of drug release decreased as cholesterol concentration increased till a maximal effective concentration. Further increase in cholesterol content caused minimal alteration in release rate.

Cable et al(134) modified the surface of niosomes by incorporating a polyethylene alkyl ether into the bilayer structure. Modified niosomes containing
cholesteryl poly oxyethylene ether (solulan C-24) were prepared by hand shaking method and the effect of its incorporation on niosomes diameter was observed. doxorubicin was incorporated in a similar formulation and it was injected into mice.

It showed sustained and higher plasma levels of doxorubicin as compared to free drug solution and empty niosomes without solulan C-24, indicating that solulan C-24 enhanced the absorption of Doxorubicin.

Uchegbu et al (135, 136) reported the in vitro activity of doxorubicin niosomes against a resistant human ovarian cancer line and showed that they were slightly more active against a model ovarian cancer cell with acquired resistance but they were unable to reverse the resistance to an appreciable extent. The same authors reported that doxorubicin loaded Span 60 niosomes were more active than doxorubicin solution against a MAC 13 tumour in male NMRI mice.

Raja Naresh et al (123) reported that the anti-inflammatory activity of niosome encapsulated diclofenac sodium in rats was higher as compared to free drug solution when administered by oral, intraperitoneal and transdermal route.

Yoshioka et al (117, 124, 137) have reported the preparation of niosomes from Span surfactants containing 5, 6 carboxy fluorescein (CF) as model solute to investigate the entrapment efficiency and release. They found that entrapment efficiency increased with
cholesterol content, mean size showed a regular increase with increasing HLB and the release rate of CF was dependent on the surfactant used in the preparation of vesicles.

They also reported a new vesicular formulation in which niosomes containing CF were dispersed in aqueous phase and then emulsified in an non-aqueous continuous phase to produce a vesicle in water in oil (V/W/O) system. They reported the invitro release rate of CF and showed a decrease in release rate in the order: Free solution > Vesicle suspension > W/o emulsion > V/w/o emulsion.

Yoshida et al(119) reported the preparation and characterization of niosomes for oral delivery of peptide drugs. They incorporated the octapeptide 9 -des-glycinamide, 8-arginine vasopressin (DGAVP) in to niosomes and reported increased stability of peptide in niosomes and increased absorption of DGAVP in vitro as compared to free DGAVP.

2.6 MICROSPHERES USING COPOLYMERS OF ACRYLIC ACID AND METHACRYLIC ACID ESTERS:

Copolymers of acrylic acid and methacrylic esters popularly known as Eudragit RL 100 and Eudragit RS 100 are widely used for delayed release permeable film coatings.

Both, Eudragit RL 100 and Eudragit RS 100 are copolymers of acrylic and methacrylic esters with a low content of quaternary ammonium groups, which produce water
insoluble films with defined permeability to water and to dissolved drugs, independent of pH values. By the virtue of the content of quaternary ammonium groups Eudragit RL 100 films are freely permeable so that drug release is relatively modestly retarded, whereas Eudragit RS 100 films are only slightly permeable so that drug release is relatively retarded (138).

2.6.1 Methods of preparation of microspheres using Eudragit resins:

Preparation of microspheres of Eudragit resins by various methods have been widely reported by a number of workers. These can be grouped as follows:

A. SOLVENT EVAPORATION TECHNIQUE:

Goto et al (139, 140, 141) reported a solvent evaporation process for the preparation of Eudragit RS 100, Eudragit RL 100 and Eudragit S 100 microcapsules containing ketoprofen. They used acetone as the solvent for Eudragit, aluminium stearate as dispersing agent and liquid paraffin as the non-solvent.

Benita et al (142) also reported a solvent evaporation for preparation of Eudragit microspheres containing nifedipine and also reported their in vitro release kinetic behaviour. They used methylene chloride as a solvent for Eudragit and an aqueous phase consisting of polyvinyl alcohol as the non-solvent.
Bogataj et al (143) reported the preparation and evaluation of Eudragit E microspheres containing bacampicillin by a solvent evaporation method. They reported the use of methanol, acetone and methyl acetate as a solvent for Eudragit, magnesium stearate as dispersing agent and liquid paraffin as the non solvent.

B. EMULSIFICATION SOLVENT EVAPORATION TECHNIQUE:
Watts et al (144, 145) reported an emulsification solvent evaporation technique to produce microspheres containing 5-amino salicylic acid and sulphasalazine using acrylic polymer Eudragit RS 100. They used methylene chloride as a solvent for Eudragit and aqueous solution containing Tween 20 or sodium dodecyl sulphate as the external phase. The same authors (146, 147, 148) also reported the effect of incorporation of samarium oxide on properties of Eudragit RS 100 sulphasalazine, sulphapyridine microspheres when prepared by a similar procedure.

Muhuri et al (149) reported kinetics of drug release from isonicotinic acid hydrazide microcapsules prepared using Eudragit RS 100 by an emulsification solvent evaporation technique.

A similar technique was reported by Bhanja et al (150) to encapsulate salbutamol sulphate with Eudragit RS 100 and Eudragit RL 100. They used...
methylene chloride as solvent for Eudragit, aluminium stearate as dispersing agent and heavy liquid paraffin containing Span 20 as the external phase.

Kim et al (151) reported a similar method for preparation of sustained release microspheres of terbutaline sulphate using Eudragit RS 100. They used Span 80 as emulsifier instead of Span 20.

Kawashima et al (152, 153, 154) reported a novel quasi emulsion solvent diffusion method to prepare microspheres of ibuprofen with acrylic polymers Eudragit S 100, Eudragit L 100-55, Eudragit RS 100 and Eudragit RL 100. They used ethanol as solvent for Eudragit and water containing emulsifier sucrose fatty acid ester DK-F 70 as the external phase.

They also reported the effect of pH of aqueous dispersion medium on the size of microspheres when prepared with a similar technique, indicating that the average diameter decreased with decreasing pH.

Bodmeier et al (155) prepared nanoparticles containing ibuprofen, indomethacin and propranolol using Eudragit RS 100 and Eudragit RL 100 by two processes. They reported that nanoparticles could be formed spontaneously after the addition of solution of drugs and acrylic polymers in acetone or ethanol to water without sonication. They also
described a process of microfluidization, for preparation of microspheres from Eudragit polymers. They reported that the colloidal dispersions were stabilized by quaternary ammonium groups and did not require addition of surfactants or polymeric stabilizers.

C. PHASE - SEPARATION TECHNIQUE:

Benita et al(156, 157) also reported a phase separation technique for preparation of microspheres with Eudragit resins for micro encapsulation of water soluble drugs. They used chloroform as solvent for Eudragit and cyclohexane containing polyisobutylene as the non-solvent.

Chattaraj et al(158) reported a similar phase separation method of preparing Eudragit RS 100 microspheres containing theophylline with only one change that phase separation was achieved by chilled n-hexane instead of cyclohexane as reported by Benita et al(156, 157).

D. MISCELLANEOUS:

Li et al(159) reported the preparation of Eudragit acrylic resin beadlets containing Pseudoephedrine hydrochloride for controlled release. They devised a new method of spray drying using a Wurster column process, where a slurry of the drug, Eudragit S 100, dibutyl sebacate and alcohol was sprayed on to non pareil seeds in the Wurster column.
Bodmeier et al (160) reported the microencapsulation of ibuprofen, theophylline, guaifenesin and Pseudoephedrine hydrochloride with Eudragit polymer dispersions NE 30D, L 30D, RS 30D, RL 30D in a completely aqueous environment as an alternative to conventional microencapsulation technique. Spherical particles were prepared by spraying or dropping dilute sodium alginate solutions containing the dissolved or dispersed drug and colloidal polymer particles into calcium chloride solution. The gelled beads could be separated by filtration.

Kawashima et al (161) reported the preparation of controlled release microspheres of ibuprofen with Eudragit by spherical crystallization technique which involved the dissolution of Eudragit in a mixture of methylene chloride : alcohol (1:1) and pouring 0.1N HCl solution with constant stirring till organic solvent evaporated and microspheres were produced.

Akuabaga et al (162) also used the same technique to prepare microspheres of furosemide with Eudragit L100, Eudragit RS 100 and Eudragit RL 100.

2.7 POLYAMIDE MICROSPHERES:
Polyamides are the condensation products containing recurring amide groups as integral parts of the main polymer
chains. Self condensing linear polyamides prepared from amino acids are termed 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 polyamides.(163)

2.7.1 General Method of Preparation by Interfacial Polymerization:

Polyamide microspheres can be prepared by various methods. For the purpose of this study, interfacial polymerization which has been the method of choice for preparing the polyamide microspheres will be discussed in detail.

Interfacial polycondensation involves the reaction of various monomers at the interface between two immiscible liquid phases to form a film of polymer that encapsulates the disperse phase. Usually two reactive monomers are employed, one dissolved in the aqueous disperse phase containing a solution or dispersion of the core material, and the other dissolved after the emulsification step in the nonaqueous continuous phase. The water in oil emulsion formed requires the addition of a suitable emulgent as stabilizer. The monomers diffuse together and rapidly polymerize at the interface between the phases to form a thin coating, and the by product of the reaction is neutralized by added material such as an alkaline buffer. The degree of polymerization can be controlled by the reactivity of monomers chosen, their concentration, the composition

(65)
of either phase, vehicle and by the temperature of the system. Variation in particle size of the disperse phase controls the particle size of the product. The reaction between the monomers is quenched by depletion of the monomer, which is frequently accomplished by adding excess continuous phase vehicle to the emulsion.

After quenching the polymerization reaction by addition of further non aqueous solvent, the newly formed microcapsules must be quickly removed from the nonpolar vehicle to lessen degradation of core material and further slight polymerization. This is accomplished by gently centrifuging the microcapsules, decanting the supernatant and dispersing them in a 50% v/v solution of polysorbate 20 (Tween 20) in water. The resultant system is then gently centrifuged to separate the microcapsules, which are rapidly resuspended in normal saline and repeatedly washed if necessary to free them from residual Tween 20. Microcapsules suspended in normal saline are normally stored at 2 to 4°C prior to use.

The aqueous phase normally contains sodium bicarbonate or sodium hydroxide to neutralise the hydrogen chloride formed in the condensation. The non aqueous phase consists of organic liquids such as chloroform, cyclohexane and carbon tetra chloride in suitable combinations that can be matched to the density of the microcapsules so as to reduce their rate of
sedimentation. Emulsification of the aqueous and non aqueous phase is aided by inclusion of suitable non ionic emulgent such as sorbitan trioleate. Emulsification is accomplished using an electrically driven stirrer, higher speeds giving a smaller and narrower size range(164).

2.7.2 Chemical Reaction:

Wittbecker and Morgan(165) were the first to publish details of an interfacial polycondensation process involving a Schotton - Baumann type of reaction between an acid dichloride and a compound containing reactive hydrogen atom. (–NH, –OH, –SH) The general reaction for polyamide formulation is –

\[
H_2N.R_1.NH_2 + C1OC.R_2.COCl \xrightarrow{\text{diamine}} \text{acid dichloride} \xrightarrow{\text{HN-R_1-}} \text{ENHOC.R_2.CONHR_1} \text{3-NHOC.R_2} \text{Polyamide}
\]

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

\[
H_2N.R.NH_2 + C1OC \xrightarrow{\text{diamine}} \text{terephthaloyl chloride} \xrightarrow{\text{HN.R.NH-CO}} \text{CO} \xrightarrow{\text{n}} + \text{HCl} \text{polyterephthalamide}
\]

(67)
2.7.3 Factors Affecting Preparation of Polyamide Microspheres:

A number of workers have reported the factors affecting preparation of microspheres by interfacial polymerization process.

Koishi et al (166) described how polythalamide microcapsules could be prepared by an interfacial polymerization reaction between a water soluble monomer 1,6 hexamethylene diamine or piperazine and a monomer soluble in an immiscible organic solvent such as phthaloyl chloride or terephthaloyl chloride.

They used sodium carbonate as aqueous phase and chloroform : cyclohexane (1:4 or 1:3 mixture) as non aqueous phase and Span 85 as an emulgent. The phthaloyl dichloride in the mixed solvent was then added to the emulsion, whose container was immersed in ice to absorb the heat liberated during polymerization reaction. Further mixed solvent was added to quench the reaction, and the newly formed microcapsules were separated by centrifugation and dispersed in 50% aqueous Tween 20 solution. Size of microcapsules obtained was between 1 and 10 microns.

Koishi et al (166) also reported the effect of Span 85 on the formation of microcapsules, probably by increasing the solubility of diamines in organic phase due to solubilization and also on the size distribution of microcapsules.
Shigeri et al (167) reported that other factors, such as decrease in temperature or monomer concentration lowered the rate and extent of interfacial polymerization and can increase the size of polyamide capsules obtained. Shigeri et al (168) also prepared a range of carboxylated polyphthalamide microcapsules by reacting amino acids like L-arginine, L-citrulline, L-cystine, L-histidine and L-lysine. The yield of microcapsules was high when a high concentration of Span and a low temperature was used.

Ogawa et al (169) studied the effect of pH of the aqueous phase containing piperazine and variable concentration of Span 85 on the formation of polyterephthalamide microcapsules. Span 85 aided the formation of microcapsules increasingly as the pH increased from 9 to 12. Span 85 had no significant effect on the formation of microcapsules outside this pH range.

Muramatsu et al (170) have recently reported a novel emulsification technique which employs microporous glass membranes to prepare polyamide microcapsules of narrow size distribution.

Shiba et al (171) micro encapsulated an aqueous solution of bovine serum albumin in various polyamides and found that the strength of polyamide membrane increased when prepared in the presence of albumin indicating that amino acid residues of this protein are chemically involved with the acid dichloride in the formation of wall material.
Takahashi et al (172) and Takamura et al (173) investigated the permeability of polyphthalamide microcapsules.

2.7.4 Applications of Polyamide Microspheres:

The applications of polyamide microcapsules as carrier systems have also been reported by a number of workers.

Chang et al (174) reported the use of semi permeable nylon microcapsules as protective carriers for erythrocyte haemolysate.

Kondo et al (175) microencapsulated haemoglobin solution from sheep in poly (terephthaloyl L-lysine) microcapsules in an attempt to produce artificial red blood corpuscles. The microcapsules obtained were similar in suspension to dispersions of red blood corpuscles in that they exhibited a mean diameter of about 10 micron, negative surface charge due to dissociation of carboxyl groups of lysine residues.

Arakawa et al (176) extensively examined the flow properties of poly (terephthaloyl L-lysine) microcapsules as a model for blood and concluded from rheological studies using a capillary viscometer that in this regard they were a reasonably good model.

Muramatsu et al (177) reported that polyphthalamide microcapsules loaded with sheep hemolysate adsorbed large amounts of fibrinogen and Y-globulin.

Arakawa and Kondo (178) attempted to prepare by interfacial polymerization much smaller poly...
(terephthaloyl L-lysine) capsules in the nanometer size range loaded with sheep haemolysate that could pass through fine blood capillaries. An electrocapillary emulsification technique was employed.

Several workers have used polyamides for microencapsulating various enzymes like carbonic anhydrase(179), L-asparaginase(180-182), urease(183-185), invertase(186), arginase(187), secretin(188) etc. with a view to increase their stability and activity.

Interfacial polymerization has been used for encapsulating various proteins like haemolysate(189) albumin and fibrinogen.(190)

McGinity et al(191) 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(192) used nylon microcapsules as a prolonged release carrier for sodium pentobarbital.

Desoize and Levy et al(193) reported that nylon microcapsules prepared by crosslinking various proteins like lysozyme, casein, human serum albumin, haemoglobin and pepsin exhibited a cytotoxic activity in vitro towards leukemic cells by inhibiting cell division.

Sawant et al(194) prepared various polyamide microcapsules containing 5-fluorouracil by interfacial polymerization technique using a series of amino acids cysteine, lysine, arginine, citrulline, ornithine etc.
They reported a different in vivo organ distribution pattern of 5-fluorouracil from these microcapsules as compared to free 5-fluorouracil solution (88).