Course Weekly Outline

5th Class, 1st Semester

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Course Instructor	Dr. Basma M. Abd Razik
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Title	***Organic Pharmaceutical Chemistry* IV**
Course Coordinator	_____
Course Objective	To give the students knowledge and experience in pro-drug as part of their medicinal and pharmaceutical field. As well as combinatorial chemistry.
Course Description	Course describes how combinatorial chemistry and HTS are being used in drug design and discovery to find new lead structures in a shorter time.
Text book	***Wilson and Gisvold Textbook of Organic Medicinal and Pharmaceutical Chemistry; Delgado JN, Remers WA, (Eds.); 10th ed., 2004.***
References	***John McCurry; Organic Chemistry.; Thomason learningInc. ; CA,USA 7th ed 2008.***
Course Assessments	Midterm test: As (20%); Quizzes: As (5%); Project: As (5%); Final exam: As (70%)

Introduction

- Polymer-drug conjugates are a novel class of nanocairrers for drug delivery, which can protect the drug from premature degradation, prevent drug from prematurely interaction with the biological environment and enhance the absorption of the drugs into tissues (by enhanced permeability and retention effect or active targeting).
- Polymer-drug conjugates are often considered as new chemical entities (NCEs) owing to a distinct pharmacokinetic profile from that of the parent drug.
- A conjugation of a drug with a polymer forms so-called ‘polymeric prodrug’.

 Synthetic polymers may be conjugated covalently to a variety of natural or synthetic bio-molecules for many diverse uses.

 In 1975, Helmut Ringsdorf published his famous cartoon suggesting the use of a synthetic polymer backbone as a carrier for drug molecules.

 In 1977, Abuchowski et. al. published the first paper on the conjugation of poly(ethylene glycol) to protein drugs.

 Polymeric conjugates of conventional drugs (polymeric prodrugs) have several advantages over their low molecular weight precursors.

In general, the conjugation of hydrophilic polymers deeply changes the behavior of the parent (free) compound both in vitro and in vivo.

 This change happens with both proteins and low molecular weight agents.

Advantages of Polymer-drug conjugates

The main advantages include…

1. Increased water solubility; enhancement of drug bioavailability.

2. Protection of drug from deactivation and preservation of its activity during circulation, transport to targeted organ or tissue and intracellular trafficking.

3. An improvement in pharmacokinetics.

4. A reduction in antigenic activity of the drug leading to a less pronounced immunological body response.

5. The ability to provide passive or active targeting of the drug specifically to the site of its action.

6. In addition to drug and polymer carrier, may include several other active components that enhance the specific activity of the main drug.

7. Specific accumulation in organs, tissues or cells, by actively targeted polymers or exploiting the known “enhanced permeability and retention(EPR) effect”.

Characteristics of Ideal drug for conjugation

The following properties of the drug molecules make it suitable as an ideal candidate to form the polymeric conjugate…

a. lower aqueous solubility,
b. instability at varied physiological pH,
c. higher systemic toxicity, and
d. reduced cellular entry.

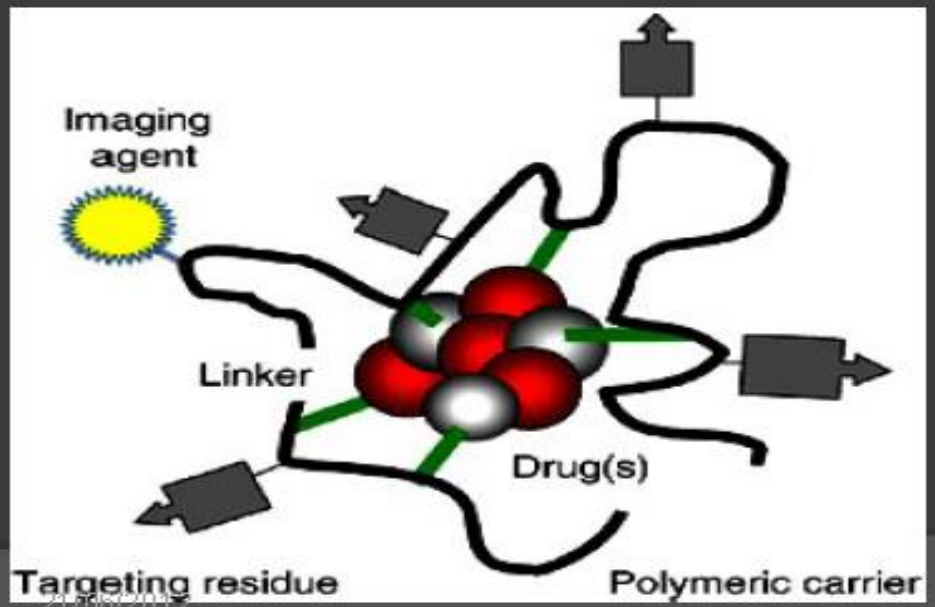
Clinical status of polymer–drug conjugates

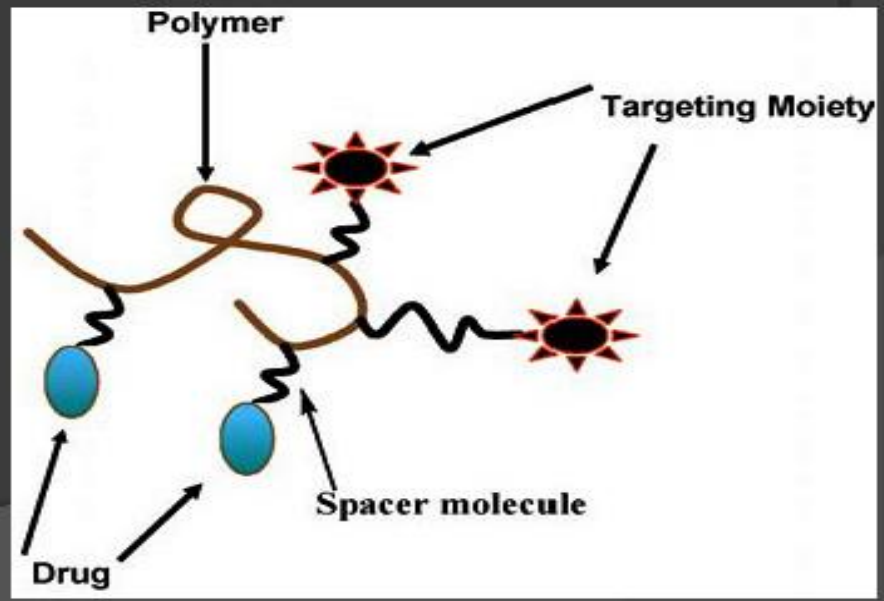
Conjugate	Indication	Year of market status	Company
A)High molecular drug			
SMANCS(ZINOSTIDIN,STIMILAMAN)	Hepatocelluler carcinoma	1993	Yamanochi pharmaceutical
PEG adenosine deamainase	SCID syndrome	1990	Enzon
PEG-anti-TNF Fab (CDP870)	RA, CROHN'S DISEASE	2008	UCB
B)Low molecular Wight drug			
HPMA co polymer-doxorubicin(PK1;FCE28068)	Breast cancer ,Lung cancer	Phase II	Pfizer
PEG-paclitaxcel	Solid cancer	Phase I	Enzon

Designing the polymer drug conjugates

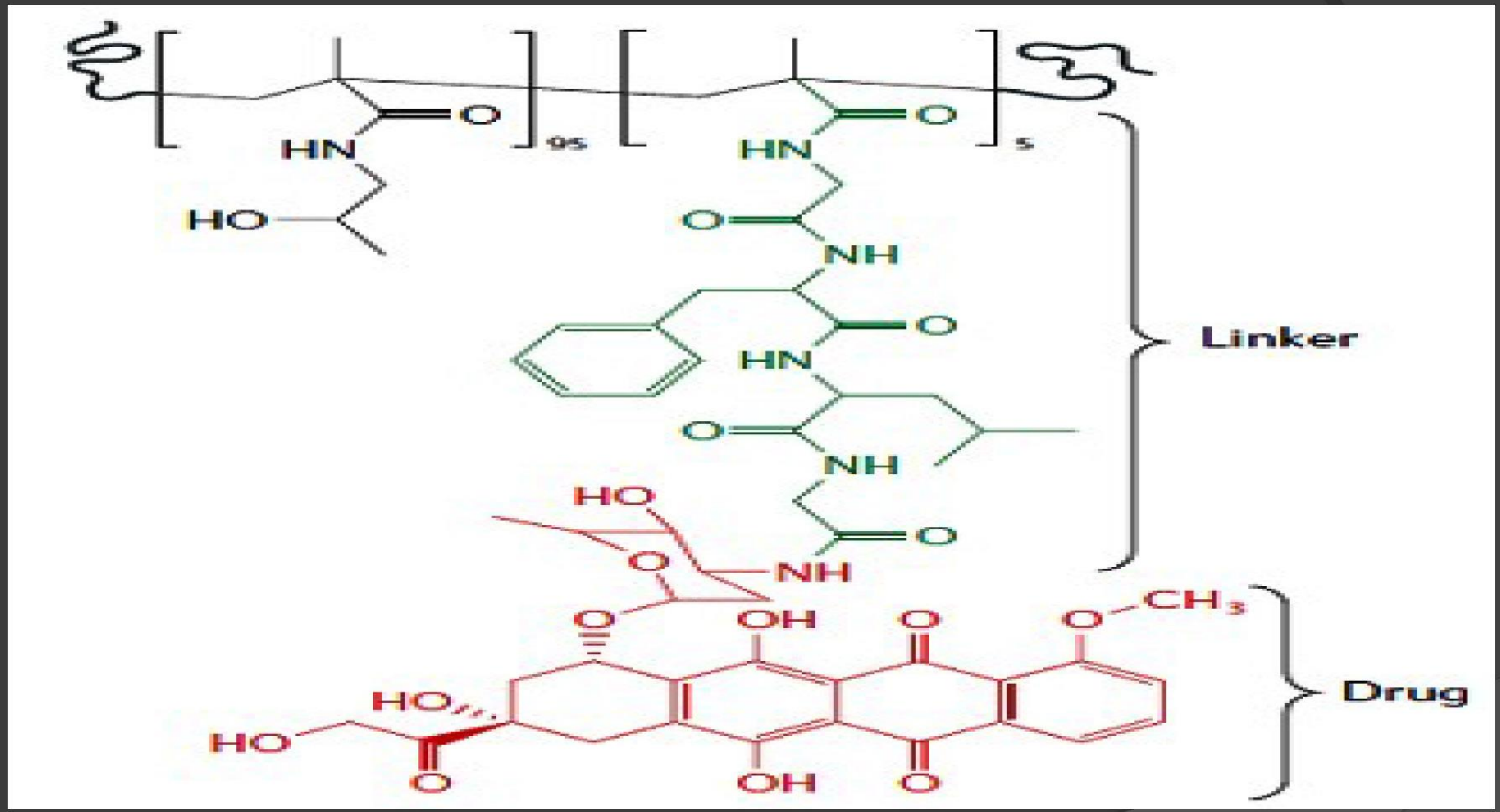
In a polymer-drug conjugate, there are at least three major components.....

1. a soluble polymer backbone
2. a biodegradable linker, and
3. a covalently linked drug which is deactivated as a conjugate.





Designing the polymer drug conjugates



HPMA-doxorubin-galactosamine

Designing the polymer drug conjugates

- Three major types of polymeric prodrug are currently being used….

1. Prodrug of the first type are broken-down inside cells to form active substance or substances.
2. The second type of prodrug is usually the combination of two or more substances. Under specific intracellular conditions, these substances react forming an active drug.
3. The third type of prodrug, targeted drug delivery systems, usually includes three components: a targeting moiety, a carrier, and one or more active component(s).

Designing the polymer drug conjugates

- The targeting ability of the delivery system depends on the several variables including: receptor expression; ligands internalization; choice of antibody, antibody fragments or non-antibody ligands; and binding affinity of the ligand.
- Therefore, the selection of a suitable polymer and a targeting moiety is vital to the effectiveness of prodrugs.
- It is essential that the polymer used is neither inherently toxic nor immunogenic. If the polymer is non-degradable in main chain the carrier must have a molecular weight sufficiently low to allow renal elimination (i.e. less than 30–40 kDa) and thus prevent progressive accumulation in the body.

Designing the polymer drug conjugates

- Unless the drug bound to the polymeric chain is membrane active, a non biodégradable polymère drug linker will yield an inactive conjugate. Preferably the linker chosen should be stable in the circulation but amenable to specific enzymatic or hydrolytic cleavage intra-tumourally.
- The cleavage of the polymer drug linker results in the release and re-activation of the attached drug molecules.
- Despite the variety of novel drug targets and sophisticated chemistries available, only four drugs (doxorubicin, camptothecin, paclitaxel, and platinate) and four polymers {N-(2-hydroxylpropyl)methacrylamide (HPMA) copolymer, poly-L-glutamic acid, poly(ethylene glycol) (PEG), and Dextran} have been repeatedly used to develop polymer–drug conjugates.

Designing the polymer drug conjugates

- To date, at least 11 polymer–drug conjugates have entered Phase I and II clinical trials and are especially useful for targeting blood vessels in tumors.

Polymers for drug conjugation

Many polymers have been investigated as candidates for the delivery of natural or synthetic drugs. In general, an ideal polymer for drug delivery should be characterized by.....

1. biodegradability or adequate molecular weight that allows elimination from the body to avoid progressive accumulation in vivo;
2. low poly dispersity, to ensure an acceptable homogeneity of the final conjugates;
3. Longer body residence time either to prolong the conjugate action or to allow distribution and accumulation in the desired body compartments; and
4. for protein conjugation, only one reactive group to avoid cross-linking, whereas for small drug conjugation, many reactive groups to achieve a satisfactory drug loading.

Polymers for drug conjugation

- ***Synthetic polymers:*** PEG, N-(2-hydroxypropyl)-methacrylamide copolymers (HPMA), poly(ethy-leneimine) (PEI), poly(acroloylmorpholine) (PAcM), poly(vinylpyrrolidone) (PVP), polyamidoamines, divinylethermaleic anhydride/acid co-polymer (DIVEMA), poly(styrene-co-maleic acid/anhydride) (SMA), polyvinylalcohol (PVA);

- ***Natural polymers:*** dextran, pullulan, mannan, dextrin, chitosans, hyaluronic acid, proteins;

- ***Pseudosynthetic polymers:*** PGA, poly(L-lysine), poly(malic acid), poly(aspartamides), poly((N-hydroxyethyl)-L-glutamine) (PHEG).

Recent studies

1. Anti-diabetic γ-PGA–Phloridzin conjugates :

- Yusuke Ikumi *et. al.* studied Polymer (γ-PGA)–Phloridzin conjugates for anti-diabetic action that Inhibits glucose absorption through the Na+/glucose co-transporter (SGLT1).
- They used γ-PGA of weight-average molecular weight: 382,000 as the polymer for conjugation.
- The strong inhibitory effect of PGA-PRZ may be explained by considering that bulky γ-PGA chains prevent the Phloridzin moiety of PGA-PRZ from degrading in the small intestine.

Recent studies

2. Anti cancer MPEG-b-PCL-b-PLL Cisplatin conjugate:

- Haihua Xiao *et. al.* studied the conjugate of Cisplatin with MPEG-b-PCL-b-PLL.
- They assembled the conjugate into nano-micelles.
- In vitro release experiments showed that drug release from the polymer - Cisplatin micelles follows an acid responsive and oxidation-reduction sensitive kinetics.
- HPLC-ICP-MS analysis revealed that Cisplatin can be released from the conjugate under an acidic plus a reductive condition which is available inside a cancerous cell.

Recent studies

- In vitro MTT assay demonstrated that the polymer- Cisplatin micelles display higher cytotoxicity against SKOV-3 tumor cells than pure Cisplatin.
- This enhanced cytotoxicity is attributed to effective internalization of the micelles by the cells via endocytosis mechanism, which was observed by fluorescence imaging and by direct determination of the platinum uptake by the cells.
- This polymer- Cisplatin conjugate is a promising polymeric pro-drug of Cisplatin in micellar form. It can protect the drug against blood clearance. It can enter cancerous cells via endocytosis mechanism and then Cisplatin can be released.
- Therefore, this polymeric pro-drug of cisplatin is expected to find clinical applications in the future.

Recent studies

3. Rotem Erez *et. al.* have synthesized an N-(2 hydroxypropyl)-meth-acrylamide (HPMA) copolymer–paclitaxel conjugate with an AB3 self-immolative dendritic linker.

- The water-soluble polymer–drug conjugate was designed to release a triple payload of the hydrophobic drug paclitaxel as a result of cleavage by the endogenous enzyme cathepsin B.
- The polymer–drug conjugate exhibited enhanced cytotoxicity on murine prostate adeno-carcinoma (TRAMP C2) cells in comparison to a classic monomeric drug–polymer conjugate.

Recent studies

4. A novel folate-decorated maleilated pullulan–doxorubicin conjugate (abbreviated as FA–MP–DOX) for active tumor targeting was set up by Haitao Zhang *et. al.*

- Based on the IC_{50} values, the conjugate was found more effective with ovarian carcinoma A2780 cells than the parent drug.
- These results suggested that FA–MP–DOX conjugate could be a promising doxorubicin carrier for its targeted and intracellular delivery.

Polymeric prodrugs:

- Polymers, including **biopolymers**.sremonom dellac stinu evtitieper fo edam era ,
- **Biopolymers** ,snietorp ,ntiihc ,hcrats ,esolulleC .smsinagro gnivil yb decudorp sremylop era ,stinu ciremonom eht hcihw ni ,sremylopoib fo selpmaxe lla era ANR dna AND ,sedtipep .sedtioelcun dna ,sdica onima ,sragus era ,ylevticepser
- Polymers are used as carriers for the delivery of drugs, proteins, targeting moieties, and imaging agents.
- Several polymers have been successfully utilized in clinical research:
- 1. poly(ethylene glycol) (PEG),
- 2. N-(2-hydroxypropyl)methacrylamide (HPMA),
- 3. poly(lactide-co-glycolide) (PLGA) copolymers

polymeric prodrug

- A conjugation of a drug with a polymer forms so-called ‘polymeric prodrug’.
- Based on the site and the mode of action, polymer conjugates possess either ‘tuned’ degradable or non-degradable bonds.
- Polymeric prodrugs have several advantages over their low molecular weight precursors. The main advantages include:
- 1. An increase in water solubility of low soluble or insoluble drugs, and therefore, enhancement of drug bioavailability.

- 2. Protection of drug from deactivation and preservation of its activity during circulation, transport to targeted organ or tissue and intracellular trafficking.
- 3. An improvement in pharmacokinetics.
- 4. A reduction in antigenic activity of the drug leading to a less pronounced immunological body response.
- 5.The ability to provide passive or active targeting of the drug specifically to the site of its action.
- 6. The possibility to form an advanced complex drug delivery system, which, in addition to drug and polymer carrier, may include several other active components that enhance the specific activity of the main drug.

- Depending on the nature and site of action of a drug, either homopolymers, or graft or block polymers are being extensively used in bioconjugates.
- Due to their higher molecular weight, polymers are known to dominate the physical properties of the bioconjugated moiety.
- Along with the polymer, the physico-chemical properties of the drug or biomolecule to be conjugated are equally important.

- The following properties of the drug molecules make it suitable as an ideal candidate to form the polymeric conjugate:
- 1. Lower aqueous solubility.
- 2. Instability at varied physiological pHs.
- 3.Higher systemic toxicity, and
- 4. Reduced cellular entry

- Numerous polymeric prodrugs are in clinical phases and several others have been approved e.g. liposomal _ Amphotericin B & PEG_Adenosine deaminase.
- Covalent conjugation of biomolecules, e.g. protein drugs to synthetic polymers, particularly poly (ethylene glycol) (PEG) does:
- 1. Increase their plasma stability.
- 2. Reduces protein immunogenicity and
- 3. Can increase therapeutic index.

Successful bioconjugation depends upon:

- The chemical structure.
- Molecular weight.
- Steric hindrance and
- The reactivity of the biomolecule as well as the polymer.

- In order to synthesize a bioconjugate, both chemical entities need to posses a reactive or functional groups such as –COOH, – OH, –SH or –NH2.
- However, the presence of multiple reactive groups makes the task a bit complex. Therefore, the synthetic methodology to form a conjugate involves either protection or deprotection of the groups.

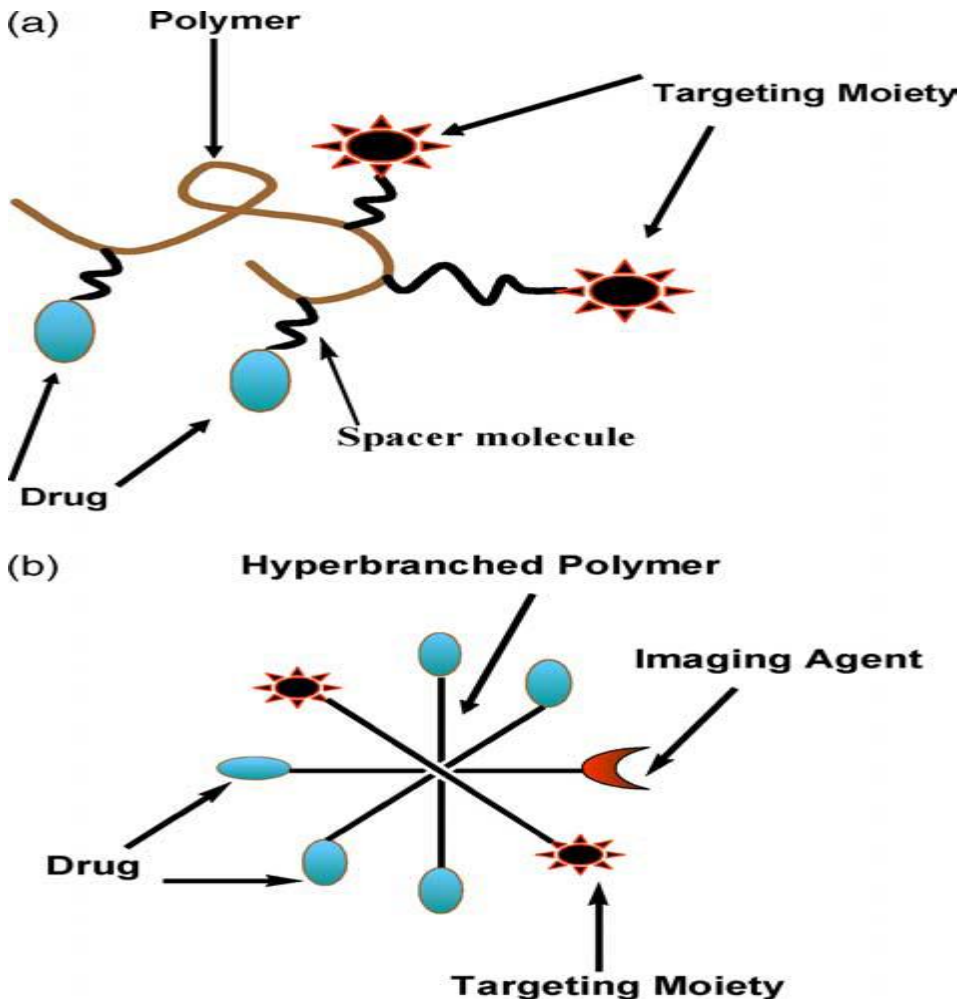
- Many of the most commonly used strategies involve use of coupling agents such as dicyclohexyl carbodiimide (DCC) or use of N-hydroxysuccinimide esters.
- Chemical conjugation of drugs or other biomolecules to polymers and its modifications can form stable bonds such as ester, amide, and disulphide.
- Covalent bonds (e.g. ester or amide) are stable and could deliver the drug at the targeted site, but such bonds may not easily release targeting agents and peptides under the influence of acceptable environmental changes.
- In the past, most of the polymeric prodrugs have been developed for the delivery of anticancer agents. High molecular weight prodrugs containing cytotoxic components have been developed to decrease peripheral side effects and to obtain a more specific administration of the drugs to the cancerous tissues.
- Polymer–drug conjugates can therefore be tailored for activation by extra- or intracellular enzymes releasing the parent drug in situ.

Design and synthesis of polymeric prodrugs:

- The most complete realization of the prodrug approach is possible by the use of an advanced type of prodrug—the drug delivery system (DDS).
- Such a system can be constructed not only to target a desired organ as a whole, its cells or specific organelles inside certain cells but also to release a specified amount of the drug at specific times.

- Three major types of polymeric prodrugs are currently being used:
- 1. Prodrugs that are broken down inside cells to form active substance or substances.
- 2. Prodrugs that are usually the combination of two or more substances. Under specific intracellular conditions, these substances react forming an active drug.
- 3. Prodrugs that are include three components: a targeting moiety, a carrier, and one or more active component(s).

- In general, an ideal polymeric prodrug model consists mainly of a combination of one or more components:
- (a) A polymeric backbone as a vehicle,
- (b) One or more drugs of the biological active components,
- (c) Spacer for hydrolysis of the biomolecule and versatility for conjugation,
- (d) An imaging agent and
- (e) Targeting moiety (Fig. 1a and b).



- Fig. 1. Schematic presentation for (a) polymeric prodrug with targeting agent and (b) hyperbranched polymer conjugate with targeting and imaging agent.

- The drug delivery carrier can be either biocompatible or an inert biodegradable polymer.
- The drug is coupled directly or via a spacer arm onto the polymer backbone.
- Selection of the spacer arm is critical as it opens the possibility of controlling the site and the rate of release of the active drug from the conjugates either by hydrolysis or by enzymatic degradation.
- The most challenging aspect of this protocol is the possibility of altering the body distribution and the cellular uptake by cell-specific or non-specific uptake enhancers.

- The polymers selected for preparing macromolecular prodrugs can be categorized according to:
- 1. Chemical nature (vinylic or acrylic polymers, polysaccharides, poly (a-amino acids), etc.,
- 2. Biodegradability,
- 3. Origin (either natural polymers or synthetic polymers) and
- 4. Molecular weight (oligomers, macromers and polymers).

Polymeric drug delivery system (PDDS)

- Modification of a polymer to form a conjugate with a biomolecule depends upon two interrelated chemical reactions:
- (1) Reactive functional groups present in the polymer and
- (2) Functional groups present on the biological component.
- In general, most of the biomolecules such as ligands, peptides, proteins, carbohydrates, lipids, polymers, nucleic acid and oligonucleotide possess combinations of these functional groups. Selection of a suitable method, process, and reagents are crucial for successful chemical conjugation.

- The following are common strategies adapted to obtain a polymeric drug delivery system as biologically active prodrug conjugates:
- 1. N-hydroxysuccinimide (NHS) ester and coupling methods, due to their higher reactivity at physiological pH makes NHS a choice for amine coupling reactions in bioconjugation synthesis.
- 2. Incorporation of spacers in prodrug conjugates; various spacers have been incorporated along with the polymers and copolymers to decrease the crowding effect and steric hindrance.
- The incorporation of a spacer arm can enhance ligand–protein binding and has application in prodrug conjugates and in biotechnology.

- For example, amino acid spacers such as glycine, alanine, and small peptides are preferred due to their chemical versatility for covalent conjugation and biodegradability.
- 3. Carbodiimide coupling reactions or zero lengths cross-linkers;
- Coupling and condensation reactions are unique to obtain chemical conjugates involving drugs or other biocomponents with polymers. The smallest possible reagents for bioconjugate synthesis are called zero length cross-linkers