1. Introduction

Improving the therapeutic index of drugs is a major impetus for innovation in many therapeutic areas such as cancer, inflammatory, and infective diseases. The search for new drug-delivery concepts and new modes of action are the major driving force in polymer therapeutics [1–3].

Today, the vast majority of clinically used drugs are low molecular weight compounds (typically under 500 g/mol$^{-1}$) that exhibit a short half-life in the bloodstream and a high overall clearance rate. These small-molecule drugs typically interact through a multiple but monovalent binding with a given receptor. Furthermore, they diffuse rapidly into healthy tissues and are distributed evenly within the body. As a consequence, relatively small amounts of the drug reach the target site, and therapy is associated with side effects. These disadvantages are especially pronounced with drugs that exhibit a narrow therapeutic index such as anticancer, antirheumatic, and immunosuppressive agents. Frequent side effects associated with these drugs are nephrotoxicity, bone marrow toxicity, neurotoxicity, cardiotoxicity, mucositis, and gastrointestinal toxicity, which are dose-limiting and thus prevent effective treatment.

A number of macromolecular delivery systems are under investigation to circumvent these limitations and improve the potential of the respective drug. Generally, these can be classified as nanoparticulate drug-delivery systems or as drug–polymer conjugates. Particulate delivery systems in which the drugs are physically incorporated into nanoparticles include emulsions, liposomes, and noncovalent polymeric carrier systems. In drug–polymer conjugates, however, a drug is covalently linked to polymers such as proteins, polysaccharides, or synthetic polymers. The coupling of drugs to macromolecular carriers received an important impetus from 1975 onwards with the development of monoclonal antibodies by Milstein and Kohler [4] and from Ringsdorf’s notion of a general drug-delivery system based on synthetic research work has focused on realizing drug conjugates with antibodies to selectively target cell-specific antigens or
receptors. This propagated the therapeutic concept of drug targeting that was founded on Paul Ehrlich’s vision of “the magic bullet” which he proclaimed at the beginning of the last century. However, it took many years for the dawning era of “polymer therapeutics” to “kick-off”.

In Ringsdorf’s original model (Figure 1) [5], a number of drug molecules are bound to a macromolecule through a spacer molecule, which can incorporate a predetermined breaking point to ensure release of the drug at the site of interest. The polymer conjugate can additionally contain moieties, for example, antibodies or sugar moieties, which target disease related antigens or receptors. In addition, solubilizing groups can be attached to the polymer backbone to modify the bioavailability of the drug–polymer conjugate.

![Figure 1. Ringsdorf’s model for drug-delivery systems based on synthetic polymers](image)

Macromolecules chosen for the preparation of drug–polymer conjugates should ideally be water-soluble, nontoxic, and nonimmunogenic, as well as degraded and/or eliminated from the organism [6]. Finally, the macromolecular carrier should exhibit suitable functional groups for attaching the respective drug or spacer. Initially, N-(2-hydroxypropyl) methacrylamide (HPMA) copolymers were intensively studied as linear polymers for therapeutic applications according to the Ringsdorf model [7–9]. However, a spectrum of other synthetic polymers with structural and architectural variations including, monofunctional linear (A),
polyfunctional linear (B), starlike (C), and dendritic architectures (D) are being investigated today (Figure 2).

**Figure 2. Selected structural and architectural types of drug–polymer conjugates**

Conjugates of drugs and polymers as well as other polymeric carrier systems have collectively been termed polymer therapeutics, which primarily encompass polymer–protein conjugates, drug–polymer conjugates, and more recently supramolecular drug-delivery systems as well as other defined nanosized systems [10–12]. Anchoring of enzymes or biological response modifiers to polyethylene glycol components (PEGylation) has led to polymer–protein conjugates with improved stability and pharmacokinetic properties. Several polymer–protein conjugates have received market approval (Table 1). The coupling of low molecular-weight anticancer drugs to polymers through a cleavable linker has been an effective method for improving the therapeutic index of clinically established agents, and the first candidates of anticancer drug polymer conjugates are being evaluated in clinical trials.

The advance of well-defined polyvalent and dendritic polymers [13] has paved the way for designing tailor-made systems with self-assembling properties which are also classified as polymer therapeutics. These include polyanionic polymers
for the inhibition of virus attachment and as heparin analogues, polycationic complexes with DNA or RNA (polyplexes) and polymer micelles with covalently bound drugs as well as dendritic core–shell architectures for the encapsulation of drugs.

Table 1: Polymer–protein conjugates with market approval

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Protein</th>
<th>Polymer</th>
<th>Indication</th>
<th>Marketed</th>
</tr>
</thead>
<tbody>
<tr>
<td>adagen</td>
<td>adenosine deaminase</td>
<td>5 kDa PEG</td>
<td>severe combined immunodeficiency disease</td>
<td>Enzon</td>
</tr>
<tr>
<td>oncaspar</td>
<td>asparaginase</td>
<td>5 kDa PEG</td>
<td>acute lymphatic leukemia</td>
<td>Enzon</td>
</tr>
<tr>
<td>pegvisomant</td>
<td>GH antagonist</td>
<td>5 kDa PEG</td>
<td>excessive growth (acromegaly)</td>
<td>Pfizer</td>
</tr>
<tr>
<td>PEG-intron</td>
<td>interferon a2b</td>
<td>12 kDa PEG</td>
<td>hepatitis C</td>
<td>Schering-Plough</td>
</tr>
<tr>
<td>pegasys</td>
<td>interferon a2a</td>
<td>40 kDa PEG</td>
<td>hepatitis C</td>
<td>Roche</td>
</tr>
<tr>
<td>neulasta</td>
<td>granulocyte colony stimulating factor</td>
<td>20 kDa PEG</td>
<td>neutropenia</td>
<td>Amgen</td>
</tr>
<tr>
<td>SMANCS/lipiodol</td>
<td>neocarzinostatin</td>
<td>copolymer of styrene maleic acid</td>
<td>hepatocellular cancer</td>
<td>Yamanouchi Pharmaceutical</td>
</tr>
</tbody>
</table>

1.1. Macromolecules as Drug-Delivery Systems: Biological Rationale

*Passive Drug and Specific Tissue Targeting: The EPR Effect*

It has long been known that biopolymers play an essential role as free and membrane-bound “therapeutics”. Therefore, it is surprising that synthetic polymers were originally only discussed as plasma expanders, for example, pervirlon or poly (vinyl pyrrolidone) during the Second World War [14]. Passive accumulation of macromolecules and other nanoparticles in solid tumors is a phenomenon which was probably overlooked for several years as a potential biological target for tumor-selective drug delivery.

The rationale for using macromolecules as efficient carriers for the delivery of antitumor agents, even if they are not targeted towards an antigen or receptor on the surface of the tumor cell, is based on the pioneering work of Maeda and
coworkers\[15, 16\] as well as Jain et al\[17, 18\]. The results of these studies gave
detailed insight into the pathophysiology of tumor tissue that is characteristic of
angiogenesis, hyper vasculature, a defective vascular architecture and an
impaired lymphatic drainage.

Differences in the biochemical and physiological characteristics of healthy and
malignant tissue are responsible for the passive accumulation of macromolecules
in tumors. This feature has been termed “Enhanced Permeability and Retention”
(EPR effect) \[19\]. In general, low-molecular-weight compounds diffuse into
normal and tumor tissue through the endothelia cell layer of blood capillaries.
Macromolecules, however, cannot pass through the capillary walls of normal
tissue. The entry of macromolecules into tumor tissue takes place in the
capillaries where blood flow is diminished and nutrients transfer into the tissue. In
contrast to the blood capillaries in most normal tissues, the endothelial layer of
the capillaries in the tumor tissue is fenestrated and leaky so that
macromolecules and other nanoparticles reach the malignant tissue. Tumor
tissue generally has a defective lymphatic drainage system with the result that
macromolecules are retained and can subsequently accumulate in solid tumors.

The size of the macromolecule is a crucial factor with respect to uptake by the
tumor. The EPR effect is observed for macromolecules with molecular weights
greater than 20 kDa. Therefore, there is a correlation between the half-life in
plasma, the renal clearance, and the accumulation in the tumor of the respective
macromolecule. In recent years, research groups involved in the development of
drug–polymer conjugates, selected macromolecular carriers with molecular
weights in the range of 20 to 200 kDa. It is generally assumed that in a healthy
organism, the renal threshold is in the range of 30–50 kDa to avoid leakage of
body proteins into the bladder \[20\]. A number of preclinical studies have
demonstrated that the physiochemical nature of the biopolymer or synthetic
polymer has a strong influence on its pharmacokinetic profile and degree of
accumulation in the tumor \[21, 22\]. The biodistribution and uptake by the tumor of
the polymer in question is essentially dictated by its molecular weight, charge, conformation, hydrophobicity, and immunogenicity. Preclinical studies have shown that the size of the tumor influences the uptake of the polymer in solid tumors. Smaller tumor nodules accumulate larger amounts of the polymer than larger nodules [23]. This observation points to the possibility that polymeric imaging agents could help to detect small tumor nodules.

The influence of the different factors on the EPR mediated uptake of the polymer in solid tumors is not yet completely understood. As a general rule, a polymer with a molecular weight above the renal threshold (ca. 30 kDa) as well as a neutral charge ensures a long half-life in plasma. This prolonged plasma residence time is an important prerequisite for a significant accumulation of the circulating polymer in the tumor. A similar uptake mechanism is also apparent in other leaky tissues, such as inflamed or infected tissue, and can result in an enhanced uptake of macromolecules at the respective sites. In contrast to this simple passive targeting by size, cell specific targeting using antibodies, oligosaccharides, and peptides has also been addressed by many research groups [24].

**Cellular uptake of polymers, site-specific drug release and implications for drug design**

In general, macromolecules are taken up by the cell through receptor-mediated endocytosis, adsorptive endocytosis, or fluid-phase endocytosis (Figure3) [25]. During endocytosis a significant drop in the pH value takes place from the physiological value (7.2–7.4) in the extracellular space to pH 6.5–5.0 in the endosomes and to around pH 4.0 in primary and secondary lysosomes. A great number of lysosomal enzymes become active in the acidic environment of these vesicles, for example, phosphatases, nucleases, proteases, esterases, and lipases.
Drug–polymer conjugates or complexes should be sufficiently stable in the bloodstream prior to the drug being liberated at the site of action. In principle, the polymer-bound drug can be released in the body by unspecific hydrolysis by enzymes, by reduction, or in a pH-dependent manner. In an ideal case, cleavage of the drug–polymer conjugate at the tumor site is triggered by a biochemical or physiological property unique for the individual tumor. Although such truly tumor specific features are rarely encountered, the over expression of certain enzymes, an acidic and hypoxic environment in solid tumors, as well as the endocytotic pathway of macromolecules offer several options for designing drug–polymer conjugates that are preferentially cleaved within the tumor.

The design of drug–polymer conjugates initially focused on incorporating enzymatically cleavable bonds that allow the prodrug to be cleaved intracellularly after cellular uptake. More recently, cleavage mechanisms involving triggering events that lead to a release cascade have been represented. The advantage of this approach is a high local drug concentration with a potential increase in efficacy [26, 27]. The low pH values in endosomes and lysosomes as well as the presence of lysosomal enzymes are therefore have intracellular properties which have been exploited for releasing the polymer-bound drug specifically in tumor cells. Furthermore, the microenvironment of tumors has been reported to be
slightly acidic in animal models and human patients. Non-invasive techniques have demonstrated that the pH value in tumor tissue is often 0.5–1.0 units lower than in normal tissue [28]. This difference could contribute to the extracellular release of drugs bound to polymers through acid-sensitive linkers, especially if the drug is trapped by the tumor for longer periods of time. Finally, drug–polymer conjugates can also be designed to slowly release the polymer-bound drug through hydrolysis under physiological conditions, as exemplified by conjugates of drugs and polyethylene glycol [29].

**Polymer Conjugates for Protein Stabilization**

Coupling polymers to therapeutically relevant proteins imparts several potential advantages. Conjugation can reduce the immunogenicity of the native protein, increase its stability, and prolong its biological half-life, thus resulting in less frequent administration to the patient. Poly (ethylene glycol) (PEG) has been the polymer of choice for preparing polymer–protein conjugates. In this “PEGylation” technology, linear or branched PEG derivatives are coupled to the surface of the protein [21, 30]. Companies like, Shearwater Polymers and Enzon initiated and refined this technology, which has resulted in the development of clinically as well as commercially successful products such as PEGylated asparaginase, PEGylated adenosine deaminase, PEGylated interferons, and PEGylated granulocyte colony stimulating factor [31–33].

**Multivalent Interactions**

In recent years, the development of multivalent drugs which are bridged by polymeric spacers has advanced dramatically [34, 35]. The great potential of these systems is the high entropic gain in the formation of the multivalent complex. For example, the binding constants of bivalent interactions can be a factor of 1000 higher than monovalent binding, and for tri- and pentavalent interactions values up to $10^8$ have been reported. This possibility allows for completely new ways to develop drugs. However, only a few efforts have been made so far to develop the first candidates for clinical trials.
A challenging approach to the application of multivalent interactions is the mimicry of functional biomacromolecules with therapeutic relevance. Several attempts have been made to mimic specific proteins (e.g., histones) or polysaccharides (e.g., heparin). In these cases, mimicry is mostly based on the surface charge of the polymer molecules. Applications range from DNA transfection agents (polycationic systems) to anticoagulating, anti-inflammatory, and anti-HIV drugs (polyanionic systems).

*Drug–Polymer Conjugates with Cleavable Linkers*

The development of drug–polymer conjugates is a promising strategy to improve the therapeutic index of toxic drugs, especially in the field of cancer chemotherapy. Several drug–polymer conjugates are being investigated in the Phase I–III studies at present.

Although great efforts are being made to develop novel polymeric carriers, synthetic polymers that have been used in clinically evaluated drug conjugates have been mainly restricted to HPMA, PEG, and poly (glutamic acid) (PG). In addition, albumin, a biopolymer carrier, is being evaluated as a drug-delivery system in anticancer therapy. The cytostatic agents that have been primarily selected for preparing drug–polymer conjugates are doxorubicin, camptothecin, taxol, methotrexate, and platinum complexes.

Several drug–polymer conjugates with HPMA copolymers have been studied clinically. A doxorubicin–(HPMA copolymer) conjugate, PK1 was the first drug–polymer conjugate to enter clinical trials [36]. PK1 has a molecular weight of approximately 28 kDa and contain doxorubicin (about 8.5 wt %) linked through its amino sugar to the HPMA copolymer by a tetrapeptide spacer, Gly-Phe-Leu-Gly. This peptide sequence is cleaved by lysosomal enzymes of tumor cells. Preclinical studies showed that the level of lysosomal enzyme expression in solid tumors, as well as their vascular permeability for macromolecules, correlated with the activity of this conjugate *in vivo* [37].
A Phase I study revealed that the maximum tolerated dose (MTD) of doxorubicin was 320 mg m\(^{-2}\) equivalents, which is a fivefold increase relative to the standard dose for doxorubicin. The dose-limiting factors observed in this study were mucositis and bone-marrow toxicity. Other side effects, for example, nausea and diarrhoea, were moderate. A noteworthy finding of this study was that no acute cardiotoxicity was observed even at these high doses. Two partial remissions and two minor responses were seen in four patients with lung, breast, and colorectal cancer. The recommended dose for phase II studies was 280 mg m\(^{-2}\) every three weeks. Phase II trials in breast, non-small-cell lung and colon cancer were initiated at the end of 1999 and an interim report indicated positive responses in a few cases [38].

PK2 is a related compound to PK1, but incorporates an additional targeting ligand, namely, a galatosamine group that was designed to be taken up by the asialoglycoprotein receptor of liver tumor cells. In a Phase I study, 31 patients with primary or metastatic liver cancer were evaluated [39]. The MTD of PK2 was 160 mg m\(^{-2}\) doxorubicin equivalents which is approximately half the MTD value of PK1, although the molecular weight and the loading ratio are very similar in both conjugates. Dose-limiting toxicity was associated with severe fatigue, neutropenia, and mucositis; a dose of 120 mg m\(^{-2}\) doxorubicin equivalents was recommended for Phase II studies. Two partial remissions and one minor response were achieved in this study.

Two other HPMA conjugates with either taxol or camptothecin, respectively entered Phase I trials (Table 2). PNU-166945 is a water-soluble conjugate in which taxol at its 2-OH position is bound through a Gly-Phe-Leu-Gly linker to the polymer backbone. The camptothecin–(HPMA copolymer) conjugate consists of camptothecin linked at its 20-OH group to the HPMA copolymer through a spacer (Gly-6-aminohexanoyl-Gly). Although preclinical results in tumor bearing mice have been promising, both conjugates have had limited success in early clinical trails because of their toxicity profile [40, 41].
Two drug–HPMA conjugates that have only recently entered Phase I studies are AP5280 and AP5286, in which a diamine- or a diaminocyclohexaneplatinum (ii) moiety is bound to a dicarboxylate ligand that is coupled to the polymer through the tetrapeptide spacer Gly-Phe-Leu-Gly. This cathepsin B sensitive linker is also present in PK1, PK2, and PNU-166945[42, 43]. Preclinical assessment showed a high antitumor efficacy and a significantly increased MTD value for AP5280 compared to the clinical standards (cis- and carboplatin). Another approach to doxorubicin–polylactide conjugates was reported by Sengupta et al [44]. These conjugates have been embedded into a biodegradable polylactide nanoparticle (ca.150 nm) to achieve better tumor selectivity through the EPR effect.

Prothecan, a camptothecin conjugate, is the first drug conjugate with polyethylene glycol that has been clinically assessed. Conjugating the 20-OH position of camptothecin with PEG through a glycine spacer [45–47] proved to have several advantages: a) the EPR effect results in a drug-targeting effect, b) esterifying the 20-hydroxy group of CPT stabilizes the drug in its active lactone form which otherwise tends to hydrolyze under physiological conditions and lead to the inactive hydroxycarboxylic acid form, c) incorporation of a glycine spacer ensured a controlled release of the drug and d) use of hydrophilic PEG leads to a highly water-soluble formulation of camptothecin.

Prothecan is currently being assessed in Phase II studies for the treatment of gastric and gastroesophageal tumors after a Phase I study showed moderate nonhematologic toxicities at its MTD of 200 mg m$^{-2}$ camptothecin equivalents [48].
Table 2- Drug–polymer conjugates in clinical trials

<table>
<thead>
<tr>
<th>Compound</th>
<th>Spacer</th>
<th>Molecular weight (kDa)</th>
<th>Status of development</th>
</tr>
</thead>
<tbody>
<tr>
<td>PK1, doxorubicin–(HPMA copolymer)</td>
<td>Gly-Phe-Leu-Gly</td>
<td>30</td>
<td>phase II</td>
</tr>
<tr>
<td>PK2, galactosaminated doxorubicin–(HPMA-copolymer)</td>
<td>Gly-Phe-Leu-Gly</td>
<td>30</td>
<td>Phase I discontinued</td>
</tr>
<tr>
<td>PNU-166945, taxol–((HPMA copolymer)</td>
<td>Ester</td>
<td>40</td>
<td>Phase I completed</td>
</tr>
<tr>
<td>MAG-CPT, camptothecin–(HPMA copolymer)</td>
<td>Gly-6-aminohexanoyl-Gly</td>
<td>30</td>
<td>Phase I completed</td>
</tr>
<tr>
<td>AP5280, diammineplatinum(II)- (HPMA copolymer)</td>
<td>Gly-Phe-Leu-Gly</td>
<td>25</td>
<td>Phase I completed</td>
</tr>
<tr>
<td>AP5286, diaminocyclohexaneplatinum (II)-(HPMA copolymer)</td>
<td>Gly-Phe-Leu-Gly</td>
<td>25</td>
<td>Phase I</td>
</tr>
<tr>
<td>Prothecan, camptothecin-PEG conjugate</td>
<td>Alanine ester</td>
<td>40</td>
<td>Phase II</td>
</tr>
<tr>
<td>CT-2103, taxol-polyglutamate conjugate</td>
<td>Ester</td>
<td>40</td>
<td>Phase II / III</td>
</tr>
<tr>
<td>CT-2106, camptothecin-polyglutamate conjugate</td>
<td>Gly-ester</td>
<td>50</td>
<td>Phase I</td>
</tr>
<tr>
<td>MTX-HSA, methotrexate-albumin conjugate</td>
<td>--</td>
<td>67</td>
<td>Phase II</td>
</tr>
<tr>
<td>DOXO-EMCH, 6-maleimidocaproyl hydrazone derivative of doxorubicin</td>
<td>Acid-sensitive hydrazone (albumin-bound prodrug)</td>
<td>67</td>
<td>Phase I completed</td>
</tr>
</tbody>
</table>

PG-TXL (CT-2103), a poly(l-glutamic acid) conjugate of taxol is probably the most successful drug–polymer conjugate to date and is currently undergoing Phase III trials in combination with standard chemotherapy against ovarian cancer and non-small-cell lung cancer [49]. PG-TXL has a higher loading ratio (ca. 37 wt% taxol) than other drug–polymer conjugates, and the taxol is linked through its 2'-OH group to the poly (glutamic) acid backbone. Phase I and II
studies of various cancers showed promising response rates, even for patients who were resistant to taxane therapy [50, 51]. A noteworthy feature of PG-TXL is the biodegradability of the polyglutamic acid backbone and the liberation of taxol and taxol glutamic acid derivatives \textit{in vitro} and \textit{in vivo}, which, in part, appear to be mediated by cathepsin B [52]. A Phase I study with an analogously constructed PEG conjugate with camptothecin has recently been completed successfully [53].

Besides synthetic polymers, albumin is also being investigated as a drug carrier in clinical trials. A methotrexate–albumin conjugate (MTX–HSA) was synthesized by directly coupling methotrexate to human serum albumin (HSA). This conjugate showed significant accumulation in rat tumors and high antitumor activity in selected nude mice models [54, 55].

New approaches have concentrated on forming a drug–albumin conjugate \textit{in vivo} by binding prodrugs selectively to circulating albumin after intravenous administration [56–59]. This prodrug concept is based on two features, a) rapid and selective binding of a maleimide prodrug to the cysteine 34 position of endogenous albumin after intravenous administration, and release of the albumin-bound drug at the target site as a result of the incorporation of an acid sensitive or an enzymatically cleavable bond between the drug and the carrier.

Although the clinical data for drug–polymer conjugates is limited to a few hundred patients, some general trends are apparent. The increase in the maximum tolerated dose (MTD) of the drug–polymer conjugates compared to the parent drug noted in preclinical studies is also manifested in clinical trials. Furthermore, no particular toxicity can be attributed to the polymer, and dose-limiting toxicities are comparable to the free drug. The significance of the molecular weight and of the cleavable linker of the drug–polymer conjugate remains unclear. Although the majority of nonbiodegradable polymers have molecular weights close to the renal threshold (30–50 kDa.), which allows enhanced permeation and retention in solid tumors, as well as a certain degree of renal clearance, a few recent examples of
conjugates with albumin, polyglutamic acid, and PEG have molecular weights of 40–80 kDa. Whether the differences in the pharmacokinetic profile as a result of the different molecular weights influence the toxicity and tumor response needs to be evaluated in a larger population of patients.

The effectiveness of predetermined breaking point incorporated in drug-polymer conjugate also remains a matter of debate. The majority of drug–HPMA conjugates have made use of the tetrapeptide Gly-Phe-Leu-Gly, which is cleaved by lysosomal enzymes such as cathepsin B. However, preclinical data indicate that antitumor efficacy of such designed conjugates correlates with the expression of cathepsin B in the tumor a fact that has not been adequately addressed in clinical trials. Detailed knowledge of the expression of tumor-related proteases in individual tumor entities would certainly be helpful for the future development of cleavable drug–polymer conjugates. Whether drug–polymer conjugates that are cleaved by unspecific hydrolysis or at acidic pH values are more universally applicable needs to be addressed in clinical studies. Preliminary preclinical studies with doxorubicin–HPMA conjugates have indicated that an acid-sensitive linker is more effective than a cathepsin B sensitive one [60].

_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 [61]. Steric hindrance describes how molecular groups interfere with other groups in the structure or other molecules during chemical conjugation. This effect is due to the interaction of molecules as dictated by their shape and/or spatial relationships. For example, molecular atoms that have affinity for one another may not be at an appropriate distance to attract each other due to their shape or they may have other atoms blocking them. The macroscale architecture of polymers causes steric hindrance for covalent conjugation with drugs in general and large peptides molecules in particular. Steric hindrance drives chemical transformations and may affect the chemical conjugation with bulkier and unstable molecules. A
conjugation reaction involving polymers, peptides and unstable molecules, therefore, requires methodologies to reduce this effect. The most preferred method to decrease steric hindrance has been to alter the synthetic approach either by incorporating a spacer arm or by increasing the reactivity of the polymer or biomolecules [62].

The incorporation of a spacer arm can enhance ligand–protein binding and has application in prodrug conjugates and in biotechnology [62]. Ideal linkers should possess the characteristics like stability in the physiological pH if the drug is to be delivered to the tumor vasculature and release the bioactive agent at an appropriate site of action. Amino acid spacers such as glycine, alanine, and small peptides are preferred due to their chemical versatility for covalent conjugation and biodegradability. Heterobifunctional coupling agents containing succinimidyl have also been used extensively as spacers. Therapeutic potential of a carboxypeptidase monoclonal antibody conjugate were reported using Nsuccinimidyl anhydrides [63–67]. The higher conjugation ratio of an antibody with a drug can result in a decrease in the ability of the antibody to bind to its specific receptor. This could be overcome by introducing a spacer between the targeting antibody and the drug. The use of an intermediate polymer with drug molecules carried in its side chains increases the potential number of drug molecules able to attach to that antibody by modification of only a minimum amount of existing amino acid residues. In most of the bioconjugates, the NHS ester anhydride is reacted with primary –NH₂ of the peptide at higher pH (7.5) to form an amide bond which links the maleimide group to the protein and releases NHS. N-hydroxysuccinimide released from the protein can be easily removed either by dialysis or gel filtration using Sephadex columns such as G10 or G25. Thereafter, the maleimide group can be further reacted with the thiol containing moieties or proteins to form a thioether bond in the presence of a slightly acidic or neutral pH [67].

The higher conjugation ratio of an antibody with a drug can result in a decrease in the ability of the antibody to bind to its specific receptor. This could be
overcome by introducing a polymer spacer between the targeting antibody and the drug. The use of an intermediate polymer with drug molecules carried in its side chains increases the potential number of drug molecules that are able to attach to the antibody by modification of only a minimum amount of existing amino acid residues [67].

The reactivity of functional polymers to couple with other biomolecules, which may be low, could be enhanced by first conjugating the polymer with reactive bis functional molecules. The resulting polymer–spacer conjugate moiety often enhances the reactivity and decreases steric hindrance for further coupling with drugs or biomolecules.

1.2. A Combined Approach: The PDEPT Concept

Polymer-directed enzyme–prodrug therapy (PDEPT) is a novel two-step antitumor approach that combines a polymeric prodrug and a polymer–enzyme conjugate to generate a cytotoxic drug at the tumor site [68]. PDEPT involves initial administration of the polymeric drug to promote tumor targeting before the activating polymer–enzyme conjugate is administrated. PDEPT has certain advantages compared to antibody-directed enzyme–prodrug therapy (ADEPT): the relatively short residence time of the polymeric prodrug in the plasma allows subsequent administration of the polymer–enzyme conjugate without fear of activation of the prodrug in the blood stream, and also the polymer–enzyme conjugates could have reduced immunogenicity.

Two PDEPT approaches have been investigated with doxorubicin. In the first case, the polymeric prodrug PK1 (FCE 28068), which is currently under Phase II clinical evaluation, was selected as a model prodrug in combination with an (HPMA copolymer)–(cathepsin B) conjugate. In the polymer-bound form, the (HPMA copolymer)–(cathepsin B) conjugate retained approximately 20–25% of the cathepsin B activity in vitro. After intravenous administration of the conjugate to tumor bearing B16F10 mice there was a 4.2-fold increase in its accumulation in tumors relative to the free enzyme. When PK1 and the PDEPT combination
were used to treat established B16F10 melanoma tumors, the antitumor activity (%T/C, the survival time of treated versus control animals) for the PDEPT combination was 168% compared to 152% for PK1 alone, and 144% for free doxorubicin [69].

Another more successful PDEPT combination consisting of (HPMA-copolymer)– (methacryloyl-gly-gly-cephalosporin) – doxorubicin (HPMA-co-MA-GG-C-Dox) as the macromolecular prodrug and an HPMA copolymer conjugate containing the nonmammalian enzyme b-lactamase (HPMA-co-MA-GG-b-l) as the activating component has been reported. HPMA-co-MA-GG-C-Dox had a molecular weight of about 31600 Da and a doxorubicin–cephalosporin content of 5.85 wt%. Free b-lactamase has a molecular weight of 45 kDa, whereas the HPMA-co-MA-GG-b-L conjugate had a molecular weight in the range of 75–150 kDa. The HPMA-co-MA-GG-b-L conjugate retained 70% and 80% of its activity against the cephalosporin C and HPMA-co-MA-GG-C-Dox substrates, respectively. Intravenous administration of HPMA-co-MA-GG-C-Dox to mice bearing subcutaneously implanted B16F10 melanoma, followed after five hours by HPMA-co-MA-GG-b-L induced the release of free doxorubicin in the tumor. When the PDEPT combination caused a significant decrease in the size of the tumor (T/C=132%), neither free doxorubicin nor HPMA-co-MAGG- C-Dox alone displayed activity. Furthermore the PDEPT combination showed no toxicity at the doses used [70].

**Multivalent Therapeutics**

A fundamentally different approach to polymer therapeutics is based on the multiple interactions of ligands conjugated with a polymer which interact simultaneously with multiple receptor sites in protein complexes or multiple receptors on the cell surface. This concept is a close mimicry of biological interactions such as cellular recognition and signal transduction where multivalent processes play an important role. Although many interesting approaches have been reported, only a few clinical developments have so far been pursued.
**Multivalent Drug Concepts (Toxins and Bacteria)**

A number of multivalent inhibitors have been designed that are based on low molecular-weight drugs and target dimeric or multimeric proteins that contain multiple identical receptor sites. For example, a pentavalent starlike carbohydrate ligand has been reported that fitted precisely into the binding pocket of the five subunits of the Shiga-like bacteria toxin, a close analogue of the cholera toxin [71]. An increase in the binding affinity by a factor of $10^7$ was observed for this pentavalent interaction relative to the monovalent ligand. This example clearly demonstrates that dendritic and starlike molecules are perfect scaffolds for presenting ligands for multivalent interactions.

**Multivalent Interactions at Surfaces—Inhibition of Virus Attachment**

The inhibition of virus attachment to cell surfaces is a fundamental problem for the prevention of viral infections, such as influenza and HIV. Traditional monovalent drugs cannot prevent the multiple adhesion of the virus to the cell surface. Therefore, the development of multivalent ligands that bind to membrane proteins of viruses is an important goal.

Several polymer architectures, including linear, starlike, and dendritic structures, have been considered as scaffolds for multivalent drugs [72-74]. Besides linear glycopolymers, various dendrimer structures have been investigated as multivalent ligands for sugar-binding proteins (for example, lectins), with multiple carbohydrate moieties attached at the exterior to form a so-called “sugar-coating”. For example, L-lysine dendrimers with 2 to 16 sialic acid units show enhanced binding affinities in the Limax flavus lectin precipitation assay and the hemaglutination assay of erythrocytes [75]. In these systems, four to six sialic acid residues appeared to be an optimal number of functional groups for antiviral activity against the influenza A virus. An approximately 200 fold increase in the binding affinity to the trivalent hemaglutinin as compared to the monovalent ligand was observed. The small size of dendrimers (3–5 nm) relative to the spacing of receptor sites on the virus surface is a major limitation of this
approach. In comparison, a high-molecular weight (106 Da) linear acrylamide polymer has shown *in vitro* an up to 108-fold increase in binding affinity, and hence is much more effective in blocking the attack of the influenza virus at the cell surface [76, 77]. However, the molecular weight of the polymer is too high to be cleared from the body by the kidneys, and rapid biodegradation is unlikely. In addition to its extremely high binding constant, the polymer can also sterically shield the virus particle when applied in combination with other monovalent ligands [78].

1.3. Polyanionic Polymers: Heparin Analogues

Heparin, a glycosaminoglycan, has been the drug of choice in the prevention and treatment of thromboembolic disorders for nearly 70 years. There is great interest in finding alternatives to both unfractionated heparin (UFH) and low-molecular-weight heparins (LMWH) because heparin has several disadvantages. First, it has to be isolated from mammalian organs, which implies a potential risk of contamination with pathogens such as viruses or prions, second, the increased use of heparin, especially of LMWH, means there is a growing shortage of the raw material, and third, heparin is a polydisperse mixture of molecules with different chain lengths and chemical structures. Numerous parameters, such as the animal species used for providing heparin, the method of isolation, and the purification step of the product, influence its respective composition and results in wide chemical and subsequent pharmacological variations between different heparin preparations.

In addition to their antithrombic activity, the characteristic feature of heparin and other natural sulfated polysaccharides are complement inhibition, anti-inflammatory, antiangiogenic, antimetastatic, antiatherosclerotic, antiproliferative, antiadhesive, and antiviral effects. These additional modes of action can contribute to the overall therapeutic benefit of heparin in some cases. Consequently, heparin analogues with a similar or even improved pharmacological profile, but lacking the disadvantages of this animal product, are
of interest. Besides partially synthetic sulfated linear polysaccharides, [79] fully synthetic sulfated linear polymers, [80] which are produced without a starting carbohydrate, may represent promising heparin mimetics [81].

Recently, a new type of polysulfated heparin analogue based on branched polysaccharides was described that possesses a much higher anticoagulant activity than its linear counterparts [82]. However, the accessibility of branched polysaccharides is problematic because of limited natural sources. Thus, a simple and efficient approach to highly branched polysulfated heparin analogues based on dendritic polyglycerols has been developed [83]. These polyglycerol sulfates prolong the time of activated partial thromboplastin as well as thrombin and inhibit both the classical and alternative complement activation more effectively than heparin itself. In contrast to sulfated polysaccharides, their activities are not directly dependent on the molecular weight, which might be a result of the globular 3D structure of the dendritic polyglycerol sulfates. Since coagulation, complement activation, and inflammation are often present in the pathophysiology of numerous diseases, polyglycerol sulfates with both anticoagulant and anticomplementary activities represent promising candidates for the development of future drugs.

Recently, immunomodulatory and antiangiogenic properties of glucoseamine-modified polyamidoamine (PAMAM) dendrimers have been described. The use of dendrimeric glucosamine and dendrimeric glucosamine 6-sulfate together in a validated and clinically relevant rabbit model of scar tissue formation after glaucoma filtration surgery resulted in the long-term success of the surgery increasing from 30% to 80% [84].

Polycationic Polymers as DNA/RNA Transfection Agents
The search for nonviral alternatives remains a challenge because of problems associated with viral gene transfection, such as immune response and limited selectivity [85]. In the past decade several approaches were pursued in which cationic amphiphiles, polymers, or block copolymers and other pH-responsive
polymers were used [86]. The colloidal surface and chemical properties of DNA and RNA complexes with polycations are responsible for controlling the extent and rate of delivery of genes to cells. However, additional hurdles on the cellular level have to be overcome on the surface of the polyplexes, such as size, charge, hydrophobicity, and buffering capacity, play a major role in the efficient transport and biological activity of the gene-based drugs [87].

The “proton- sponge hypothesis” postulates enhanced transgene delivery by cationic polymer–DNA complexes (polyplexes) containing proton-buffering polyamines through enhanced endosomal accumulation of chloride, which leads to osmotic swelling and lysis of the endosome [88]. For therapeutic applications, however, an early endosomal escape mechanism, rather than lysosomal fusion, would be preferable to avoid the release of lysosomal enzymes into the cytosol [89].

The most frequently used cationic polymers for in vitro gene delivery are poly (ethylene imine) (PEI), poly (l-lysine), and chitosans. Another approach is the use of perfect polyamine-dendrimers [86, 90] to mimic the globular shape of the natural protein complex. However, the synthetic workload to obtain dendritic structures in the size range of the natural histone complex (ca. 8 nm) [91] is tremendous. Also, the observation that a partially destroyed (hydrolyzed) dendritic backbone showed even higher transfection efficiencies [90] underlines the significance of readily available alternatives.

1.4. Supramolecular Drug–Polymer Complexes

Block Copolymer Micelles
Polymeric micelles are generally more stable than micelles of small surfactant molecules and can retain the loaded drug for a longer period of time [92, 93]. The block copolymer micelles form spontaneously by self-assembly in water when the concentration of the amphiphilic block copolymer is above the critical micellar concentration (CMC) [94]. The driving force can be the hydrophobic
interactions of the inner block, for example, a nonpolar poly(caprolactone) block (PCL), or ionic interactions, for example, a poly(aspartate) block (PAsp), complexed to a negatively charged polymer such as DNA that forms a polyion micelle [95]. The outer block often consists of a polar poly (ethylene oxide) (PEO) block which forms the shell of the nanocarrier and protects its core. It has been demonstrated that PEO prevents the adsorption of proteins [96, 97] and hence forms a biocompatible polymeric nanocarrier shell. The size of these block-copolymer micelles is determined by thermodynamic parameters, but partial control over the size is possible by variation of the block length of the polymer [98]. Typically, these block-copolymer micelles are 20–50 nm in diameter with a relatively narrow distribution and are therefore similar in size to viruses, lipoproteins, and other naturally occurring transport systems [92].

A major obstacle for these nanocarrier systems is their nonspecific uptake by the reticuloendothelial systems. The size and the surface properties of the nanocarriers based on block copolymers require careful design to achieve long circulation times in the blood and site-specific drug delivery [99]. The polarity and functionality of each block allow control over the spontaneously formed core shell architecture. While terminal functionalities on the outer block (the shell) control biocompatibility and may incorporate potential targeting properties, the inner block of such nanocarriers can be used to complex or covalently couple active drug molecules. This core–shell concept is frequently used to dissolve nonpolar drugs. Examples of block copolymers that have poor solubility in water are the pluronics PEO-b-PPO or PEO-b-PPO-b-PEO.

Supramolecular constructs have also been generated by using block copolymers as shells for dendritic porphyrins [100]. These “blown up” micelles (ca. 100 nm) may have a much higher targeting specificity for tumor tissue as a result of an enhanced EPR effect. Kataoka and co-workers have recently reported a pH sensitive supramolecular nanocarrier for doxorubicin based on biocompatible block-copolymer micelles [101]. In contrast to drug–polymer conjugates, in which antitumor agents are covalently attached to a single macromolecule chain,
doxorubicin was coupled through an acid-labile hydrazone linker to PEO-b-PAsp copolymer. After spontaneous self-assembly of the drug-loaded supramolecular nanocarrier, kinetic analysis clearly demonstrated the effective cleavage of the hydrazone bonds at pH-5, with concomitant release of doxorubicin. Release of doxorubicin was negligible under physiological conditions in cell culture medium (pH-7). The doxorubicin nanocarrier demonstrated in vitro cytotoxicity against a human small-cell lung cancer cell line (SBC-3) in a time-dependent manner, thus suggesting cellular uptake by endocytosis. The first candidates of antitumor drugs based on polymer micelles have entered clinical trials in Japan [102].

1.5. Acyclovir

Acyclovir, (2-Amino-1, 9 dihydro-9-[(2 hydroxy ethoxy) methyl]-6H-purine-6-one), is a synthetic purine nucleoside analogue and a highly selective antiviral drug which is activated by viral thymidine kinase. Acyclovir triphosphate inhibits DNA synthesis by acting as a chain terminator and the inhibition activity of acyclovir is highly selective due to its special affinity for the thymidine kinase encoded by herpes simplex virus and vericella zoster virus [103], and its anti-hepatitis B virus activity has been reported [104].

![Acyclovir](image)

On oral administration it leads to side effects such as nausea, vomiting, diarrhoea, headache, dizziness, fatigue, skin rash, edema, inguinal adenopahy, anorexia, leg pain, accelerated hair loss, fever, palpitation, muscular cramps and menstrual abnormalities. On parental administration there are side effects like
renal toxicity, encephalopathy changes and transient elevation of serum creatinine rash, hematuria, hypotension, headache, nausea and thromocytosis.

Absorption, Bioavailability and Distribution
Acyclovir is well absorbed following oral administration. Bioavailability is between 10-20%. Peak plasma concentrations occur within 1-2 h after dosing. In healthy volunteers, at a therapeutic dose of 200-800 mg, mean steady-state $C_{\text{max}}$ of acyclovir in plasma is 1.6 µg/ml. The mean area under the curve (AUC) over a dosing interval of 12 h is 2.3 µg/ml. The estimated volume of distribution is high. Protein binding is 9-33%.

Metabolism / Elimination
The observed elimination half-life is 2.5-3.3 h. It is renally excreted, partly by glomerular filtration and partly by tubular secretion. Almost 62-91 % of acyclovir and its major metabolite, 9-carboxy-methoxymethylguanine (CMMG), are excreted in urine [105].

1.6. Lamivudine
Lamivudine, (−)-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl] cytosine, is an orally administered nucleoside reverse transcriptase inhibitor (NRTI). The active form is lamivudine triphosphate which is generated via an intracellular triple phosphorylation process. Lamivudine triphosphate competitively inhibits viral reverse transcriptase by causing termination of DNA replication [106, 107], thus, interrupting HIV replication. It used in combination with other antiretroviral agents to treat human immunodeficiency virus (HIV) Type1 infection in patients with acquired immunodeficiency syndrome (AIDS) and as monotherapy in the treatment of hepatitis virus (HBV) infection but has a significant depressant effect via short-term treatment.
However, relapse is easily triggered after drug withdrawal and drug resistance is most likely to be generated after long-term medication [108]. The adverse events of lamivudine reported includes headache, insomnia, nausea, vomiting, diarrhoea, abdominal pain, fever, muscle pain, hepatitis, pancreatitis, peripheral neuropathy, and red cell aplasia, most of which are reported to be mild to moderate. Lactic acidosis and severe hepatomegaly with steatosis, including fatal cases, have also been reported [109, 110]. Dosing regimen appears to have little influence on side effects.

**Absorption, Bioavailability and Distribution**

Lamivudine is well absorbed following oral administration. Bioavailability is between 80-85%. Peak plasma concentrations occur within half an hour after dosing. In healthy volunteers, at a therapeutic dose of 150 mg twice daily, mean steady-state $C_{\text{max}}$ of lamivudine in plasma is 1.2 µg/ml [111]. The mean area under the curve (AUC) over a dosing interval of 12 h is 3.4 µg/ml. The estimated volume of distribution is 1.3L/Kg. Protein binding is 36%.

**Metabolism / Elimination**

The observed elimination half-life is 5-7 h. It is eliminated by kidneys. Almost 70% of lamivudine and its major metabolite, trans-sulfoxide is excreted in urine [112].
1.7. Tenofovir

Tenofovir, \(((2R)-1-(6\text{-amino}-9H\text{-purin}-9\text{-yl}) \text{propan-2-yl}] \text{oxy}) \text{methyl}) \text{phosphonic acid}\) is a nucleotide analogue of adeno-sine monophosphate. Its active metabolite, tenofovir diphosphate, competes with natural deoxyadenosine triphosphate for the active binding site on the HIV-induced reverse transcriptase (HIV DNA polymerase) and reverse transcription, the key step in HIV proliferation, is inhibited [113]. It is used in combination with other anti-retroviral drugs [114] to form the backbone of HAART (highly active anti-retroviral therapy) [115]. The use of tenofovir in the first-line treatment of HIV is considered superior to treatments due to a lower incidence of toxicity and lower rate of resistance development [116]. It is in advanced phase III clinical trials for the treatment of hepatitis B virus infections.

![Tenofovir structure](image)

The most common side effects associated with tenofovir include nausea, vomiting, diarrhoea, and asthenia. Tenofovir has also been implicated in causing renal toxicity, particularly at elevated concentrations. It can cause acute renal failure, proteinuria or tubular necrosis. Bone disorders, including a vascular necrosis of the hip and compression fracture of the lumbar spine, in HIV-infected patients receiving antiretroviral therapy [117].

**Absorption, Bioavailability and Distribution**

Tenofovir is well absorbed following oral administration. Bioavailability is 25%. Peak plasma concentrations occur within 2 h after dosing. In healthy volunteers,
at a therapeutic dose of 300 mg once daily, mean steady-state $C_{\text{max}}$ of tenofovir in plasma is 317 ng/ml. The mean area under the curve (AUC) over a dosing interval of 24 h is 2999 ng.hr/ml. The estimated volume of distribution is 0.813L/Kg. Protein binding is 7.2%.

Metabolism / Elimination

The observed elimination half-life is 12-14 h. The mean systemic clearance of tenofovir is approximately 510ml/h/kg. Almost 70-80% of tenofovir and its active metabolite is excreted in urine [118].

1.8. Poly (styrene-co-maleic anhydride) (PSMA)

Poly (styrene-co-maleic anhydride) (PSMA) is a synthetic co-polymer with attractive chemical and biological properties [119]. PSMA or SMA_nh is built-up of styrene and maleic anhydride monomers. The monomers are almost perfectly alternating, making it an alternating copolymer. It is formed by radical polymerization using organic peroxides. The main characteristics of PSMA copolymer are its transparent appearance, high heat resistance, high dimensional stability, and the specific reactivity of the anhydride groups. The solubility of PSMA in alkaline (water-based) solutions makes it suitable for various applications. PSMA is available in a broad range of molecular weights and maleic anhydride (MA) contents.

Poly (styrene-co-maleic anhydride) (PSMA)
The potential of PSMA as a polymeric drug has already been exploited with the clinical success of SMANCS a conjugate of poly (styrene–co–maleic anhydride) with neocarzinostatin a protein with anticancer activity which is partially esterified with n butyl alcohol increases the hydrophobicity, lipid solubility and the affinity to albumin[120]. The maleic copolymer was chosen because anhydride groups react with the amodic groups of the protein while the styrene units bring certain hydrophobicity. SMANCS conjugate improves the *invitro* and *in vivo* stability and the capacity to concentrate in the tumor tissue is also increased. SMANCS conjugate produces the inhibition of DNA synthesis, like free NCS, but it is also capable of activating macrophages and inducing interferon-γ. The activation of macrophages is maximal when the MA-St copolymer is in n-butyl ester form. If the copolymer is esterified with longer alkyl chains, the water solubility would be decreased, so the n-butyl ester was chosen as the most proper one.

The free NCS and the SMANCS conjugate possess low immunogenicity. SMANCS conjugate attaches to the cells faster than NCS, so the action to inhibit the malign cell development is enhanced. The hydrophobic character of SMANCS leads to an increased interaction with the cell membrane by penetrating the lipid bilayer. The clinical success of SMANCS, a conjugate of poly styrene co-maleic anhydride with the potent yet toxic anti-tumor polypeptide neocarzinostatin (NCS), in liver and lung cancer [121], [122], [123] have also been reported. Recent revelation of the potential of PSMA and its derivatives in effectively inhibiting human immunodeficiency virus type 1 (HIV-1) by preventing virus adsorption on the surface of target cell membranes, has generated considerable interest [124].

It has already been reported that the hydrophobic regions of PSMA play an important role in the penetration of the lipid bilayer of cell membranes, and the anionic portion of the copolymer facilitates the internalization and binding of the drug-polymer conjugate with the hydrophobic milieu of the cell [125]. PSMA copolymers have also been reported to be strong inhibitors of spermatozoa
motility. The spermicidal activity of the copolymer has been attributed to the presence of carboxylic groups, which induces a low-pH environment responsible for killing spermatozoa. It is also known to induce surface charge imbalance in human spermatozoa membrane, which leads to the release of acrosomal enzymes hyaluronidases and acrosin. This ultimately leads to spermatozoa damage. It therefore becomes imperative to elucidate the influence of PSMA on lipid matrix models of cell membranes in order to improve the therapeutic potential of the copolymer by enhancing biological activity and diminishing side effects [126].
1.9 Literature Review

1.9.1. Poly (styrene-co-maleic anhydride) (PSMA)

*Nate Larson and coworkers* [127] synthesized and evaluated polymeric micelles carrying the heat shock protein 90 inhibitor tanespimycin (17-N-allylamino-17-demethoxygeldanamycin) using poly (styrene-co-maleic acid) (SMA) copolymers. Tanespimycin was released from the micelles in a controlled manner *in vitro* at pH 7.4 buffer containing bovine serum albumin and also showed potent activity against DU145 human prostate cancer cells *in vitro*. The micellar drug delivery systems for tanespimycin exhibited properties that allow for increased blood circulation and tumor accumulation *in vivo*. It showed significantly higher anticancer efficacy as measured by tumor regression when compared to free tanespimycin at an equivalent single dose of 10 mg/kg. It was found that the polymeric micelles can be used as promising agents in the treatment of prostate cancer.

*P. Najafi Moghadam* [128] synthesized poly (styrene-alt-maleic anhydride) (PSMA) copolymer for controlled drug delivery of ceftriaxone antibiotic which was covalently bonded onto the polymeric framework. The chemical grafting reaction of ceftriaxone antibiotic on PSMA was carried out in various ratios. In addition, PSMA was modified by isopropyl amine for preparation of PSMA-Isopropyl amide (PSMA-IPA) pH sensitive hydrogel. The physical loading of ceftriaxone antibiotic on PSMA-IPA in various ratios was also carried out. *In vitro* drug release was performed under specific conditions to investigate the influence of pH on the releasing rate. *In vitro* release profile showed that the amount of drug release in chemical loading was higher than the physical loading. Also the release rate showed a high dependence on the pH of the release medium. In addition at pH 2, the release in chemical loaded form was not shown, whereas in the physical loaded form, there was a slight release at pH 2. The release dependence of the ceftriaxone antibiotic on the pH from the obtained loaded co polymeric system has made it a favorite system for applying as targeting drugs to specific locations such as the small intestine, since the polymeric formulation is not highly affected by the low pH such as stomach pH.
Weijun Fang and coworkers [129] synthesized poly (styrene-alt-maleic anhydride) derivatives as potent anti-HIV microbicide candidates. A series of poly [styrene-alt-(maleic anhydride)] derivatives were prepared by amidation or hydrolysis of the anhydride moiety. The derivatives were shown to be of low cell toxicity and effectively inhibited HIV-1 infections in an in vitro cellular model. Poly [styrene-alt-(maleic anhydride, sodium salt)] was the most potent inhibitor, being 100-fold more potent than dextran sulfate suggesting its potential application as a new class of polyanionic microbicides.

Jun Fang and coworkers [130] synthesized styrene-maleic acid copolymer (SMA)–(AHPP) (4-amino-6-hydroxypyrazolo [3, 4-d] pyrimidine) conjugate and evaluated for its antihypertensive activity. However as AHPP is insoluble in water, it hampered its in vivo application. Therefore, a water soluble polymeric conjugate of AHPP was prepared by using a styrene maleic acid copolymer (SMA, SMA–AHPP). In vivo experiments were carried out to examine the antihypertensive effect of SMA–AHPP using the spontaneously hypertensive rats (SHR) by i.v. injection or by oral administration. The results showed significantly reduced blood pressures of SHR rats and this antihypertensive effect was continued for at least 24 h after administration.

Marcel Popa and coworkers [131] synthesized some new polyanionic polymer conjugates with antitumoral activity by chemically binding mustard type alkylating derivatives of di-(β-chloroethyl)-amine and tri-(β-chloroethyl)-amine, respectively, onto poly(maleic anhydride-alt-vinyl acetate. The antitumoral activity of these polymeric drugs was evaluated in vivo using carcinosarcoma Walker 256 solid tumors in Wister rats. The conjugates exhibited good antitumor regression (ATR).

Marijana Zovko and coworkers [132] synthesized fenoprofen and gemfibrozil styrene-maleic acid (SMA) copolymer conjugates. Fenoprofen and gemfibrozil were chosen as model drugs because of their short plasma half lives. Both drugs were first converted to their 2-aminoethylamides, which possessed free amino groups capable of reacting with SMA anhydride rings. By modifying the degree and type of substitution, lipophilic and hydrophilic conjugates were obtained. The
results showed that the prepared polymer-drug conjugates underwent hydrolysis and released the bound fenoprofen and gemfibrozil.

**Khaled Greish and coworkers** [133] synthesized (SMA)–doxorubicin polymeric micellar drug for effective targeting to solid tumours. Copolymer of styrene-maleic acid (SMA) was used to construct micelles containing doxorubicin by means of a hydrophobic interaction between the styrene moiety of (SMA) and doxorubicin (Dox). The micelles obtained (SMA–Dox) showed a high solubility in water and a constant doxorubicin release rate of about 3–4%/day *in vitro*. The (SMA–Dox) micelle preparation was less cytotoxic to the SW480 human colon cancer cell line *in vitro* when compared with free doxorubicin. *In vivo* assay of (SMA–Dox) in mice bearing S-180 tumor revealed a potent anticancer effect with no remarkable toxicity up to a dose of 100 mg/kg of free doxorubicin equivalent. The drug concentration in tumor after administration of (SMA–Dox) was 13 times higher than that after the free drug. This result was attributed to the enhanced permeability and retention (EPR) effect of the macromolecular drugs observed in solid tumors. Complete blood counts and cardiac histology showed no serious side effects for *i.v.* doses of the micellar formulation as high as 100 mg/kg doxorubicin equivalent in mice. It indicated that *i.v.* administration of (SMA–Dox) micellar formulation can enhance the therapeutic effect of doxorubicin while reducing greatly cardiac and bone marrow toxicity.

**1.9.2. Acyclovir**

**Su-Ting Huang and coworkers** [134] synthesized acyclovir conjugated stearic acid -g- chitosan-oligosaccharide micelles via succinate linker and evaluated for anti-hepatitis B virus activity. Chitosan-g-stearate was synthesized by the reaction between the amino group of chitosan oligosaccharide and the carboxyl group of stearic acid. Both chitosan-g-stearate and acyclovir-chitosan-g-stearate could self aggregate to form micelles in aqueous solution. Acyclovir release from acyclovir-chitosan-g-stearate micelles could prolong to 24 h *in vitro*. For the free acyclovir and acyclovir-chitosan-g-stearate micelle with acyclovir concentration of 0.044 µM mL⁻¹, the inhibition of acyclovir on hepatitis B surface antigen was
increased from 12.7% to 22.3%, while the inhibition of acyclovir-chitosan-g-stearate was increased from 58.2% to 80.3% The cellular uptake and antiviral activity of acyclovir was successfully increased and improved through chemical conjugation of acyclovir to chitosan-g-stearate.

**Maria Grazia Sarpietro and coworkers** [135] conjugated squalene to acyclovir. Differential scanning calorimetry was used to study the interaction of acyclovir and its prodrug squalenoyl–acyclovir with biomembrane models to verify whether a stronger interaction of the prodrug with respect to the free drug can be obtained. To evaluate if acyclovir and its prodrug can be absorbed by the biomembrane model, an experiment was carried out in which the compounds were left in contact with the biomembrane model and their eventual uptake was evaluated analyzing the effect on the thermotropic behavior of the biomembrane model. A very small uptake was revealed for all the compounds. To check the potential use of liposomes as a delivery system for the prodrug, the biomembrane models were incubated with liposomes loaded compounds to deliver the squalenoyl-acyclovir. The results suggested that liposomes could be used to deliver the squalenoyl-acyclovir to the biomembrane models.

**Roberta Cavalli and coworkers** [136] prepared nanoparticles by the conjugation of acyclovir to β-cyclodextrin-poly (4-acryloylmorpholine) mono-conjugate (β-CD-PACM) and evaluated for its antiviral activity. Acyclovir-loaded nanoparticles were prepared from inclusion complexes of Acyclovir with that of β-CD-PACM. Both unloaded and drug-loaded nanoparticles were characterized in terms of particle size distribution, morphology, zeta potential, drug loading and in vitro drug release rate. The antiviral activity of Acyclovir loaded β-CD-PACM nanoparticles against two clinical isolates of HSV-1 was evaluated and found to be remarkably superior compared with that of both the free drug and the soluble β-CD-PACM complex. It was found that the nanoparticles were internalized in cells and located in the perinuclear compartment.

**Ravi S. Talluri and coworkers** [137] synthesized and evaluated enzymatically stable dipeptide prodrugs for improved absorption of acyclovir. L-Valine-L-valine-acyclovir (LLACV), L-valine-D-valine-acyclovir (LDACV), D-valine-L-valine-
acyclovir (DLACV) and D-valine-D-valine-acyclovir (DDACV) were successfully synthesized. The uptake and transport studies were conducted on a Caco-2 cell line. Buffer stability and metabolism of the prodrugs in Caco-2, rat intestine and liver homogenates were studied. The uptake and transport of $[^3]$H glycylsarcosine was inhibited by all prodrugs except DDACV. DLACV and DDACV exhibited no measurable degradation in Caco-2 homogenate. Except DDACV, other three prodrugs were hydrolyzed in rat intestine and liver homogenates. The order of permeability across Caco-2 was observed as LDACV>LLACV>DDACV >DLACV. A linear correlation between the amount of prodrug transported and overall permeability of acyclovir was established. This study showed that the incorporation of one D-valine in a dipeptide did not abolish its affinity towards peptide transporters (PEPT). Moreover, it enhanced enzymatic stability of prodrug to a certain extent depending on the position in a dipeptide conjugate.

_Padmavathy Tallury and coworkers_ [138] developed bio-compatible copolymer matrix of poly (ethylene-co- vinyl acetate) (EVA) to deliver acyclovir and chlorhexidine drugs at therapeutic levels over extended periods of time. The release rate of acyclovir and chlorhexidine diacetate (CDA) from EVA was investigated individually and as a mixture. The effect of drug combination, the composition of the copolymer and the coating of the matrix with the polymer on the rate of drug release were evaluated. It was found that the release rate of acyclovir was higher than that of CDA, both individually and in the mixture. Total release of acyclovir was higher than CDA in most compositions. The effect of increasing the vinyl acetate content of the EVA matrix increased the drug release rate while coating of films resulted in a decrease of the release rate of the drugs. _In vitro_ rate of drug release showed that there was a sustained release of drug at an almost constant concentration over extended period of time, thus providing a basis for oral treatment modality.

_M. Zacchigna and coworkers_ [139] synthesized chemically and enzymatically stable new poly (ethylene glycol)–acyclovir and poly (ethylene glycol)–valaciclovir prodrugs. _In vitro_ drug release studies demonstrated the conjugates to be stable in buffer solutions at pH 7.4 and 5.5, while only PEG–valaciclovir
was stable in a buffer solution at pH 1.2. The ability of the macromolecular conjugate to release the free drug was also evaluated in plasma, in which the most stable prodrug also proved to be PEG–valaciclovir. The derivatives were degraded in the presence of proteolytic enzyme. PEG–valaciclovir was found to be more suitable for therapeutic use since it was more stable in various buffers and released more of the free, active drug over a period of 24 h. As a result, the possibility of properly retaining this adduct in the bloodstream could represent an interesting way to improve the pharmacokinetics of acyclovir, particularly by increasing its half-life.

**Giammona and co-workers** [140] conjugated acyclovir by using succinic anhydride spacer with α, β-poly (N-2-hydroxy ethyl)-DL-aspartamide (PHEA) to give a PHEA-O-succinyl acyclovir conjugate. It was found that the drug-polymer conjugate was freely water soluble at room temperature. The stability, bioavailability of oral and intravenous administration of the conjugate was higher than that of free acyclovir.

1.9.3. Lamivudine

**Yan Zhong and coworkers** [141] synthesized a prodrug from lamivudine (LMV) and ursolic acid (UA) when coupled with ethylchloroacetate through an amide and ester linkage and evaluated for anti-HBV activity. The *in vitro* non-enzymatic and enzymatic hydrolysis and *in vivo* pharmacological activities of the prodrug were studied. The kinetics of hydrolysis were also studied in aqueous solution of pH 1 to 10, 80% buffered human plasma and in the presence of lipase from Porcine pancreas at 37 °C. It was found that the hydrolysis rate of the prodrug was significantly faster in lipase with half-life of 1.4 h compared to pH 7.4 phosphate buffer (t$_{1/2}$ 11.2 h) and buffered human plasma (t$_{1/2}$ 5.4 h). It was comparatively stable between pH 3 and 6 (half-life >40 h). *In vitro* studies indicated that the prodrug had the dual action of hepatoprotective effects and anti-hepatitis B virus activity against acute liver injury.

**Ashish Dev and coworkers** [142] developed Poly (lactic acid) (PLA)/chitosan (CS) nanoparticles for anti-HIV drug delivery applications. Lamivudine was
loaded into PLA/CS nanoparticles. The encapsulation efficiency and *in-vitro* drug release behaviour of drug loaded PLA/CS nanoparticles were evaluated. In addition, the cytotoxicity of the PLA/CS nanoparticles using MTT assay was also studied. The *in-vitro* drug release studies showed that the drug release rate was lower in the acidic pH when compared to alkaline pH. The drug release rate was found to be higher in the drug loaded formulation. These results indicated that the PLA/CS nanoparticles were a promising carrier system for controlled delivery of anti-HIV drugs.

**Qian Li and coworkers** [143] synthesized Stearic acid-g-chitosan oligosaccharide (CSO-SA) micelles and the prodrugs of Lamivudine (LA), Lamivudine stearate (LAS) via ester linkage between LA and stearic acid and evaluated them for anti-HBV activity. Stearic acid-g-chitosan oligosaccharide (CSO-SA) micelles demonstrated fast internalization and accumulation ability to tumor cells. The CSO-SA with 3.79% amino substitution degree (SD) was prepared for loading LAS. When LAS was incorporated, the micellar size decreased and the zeta potential increased. The LAS loaded CSO-SA micelles (CSO-SA/LAS) possessed high entrapment efficiency and drug loading. The release of LA from CSO-SA/LAS showed a pH-dependent behavior and increased significantly as the pH of release medium reduced from 7.4 to 6.2. CSO-SA/LAS presented a low cytotoxicity and high cellular uptake percentage of LAS against HBV transfected tumor cells. *In vitro* anti-HBV activities of CSO-SA/LAS presented more conspicuous inhibitory effects on antigen expression and DNA replication compared with LA and LAS.

**Lin Yang and coworkers** [144] synthesized Chitosan-O-isopropyl-5′-O-d4T monophosphate conjugated nanoparticles with a phosphoramidate linkage and evaluated for anti-HIV activity. The anti-HIV activity and cytotoxicity of the polymeric conjugate were evaluated in MT4 cell line. *In vitro* drug release studies in pH 1.1 and 7.4 suggested that both chitosan-d4T conjugate and its nanoparticles prefer to release d4T 5′-(O-isopropyl) monophosphate than free d4T for prolonged periods, which resulted in the enhancement of anti-HIV selectivity of the polymeric conjugate relative to free d4T. Additionally, the cross
linked conjugated nanoparticles could prevent the coupled drug from leaking out of the nanoparticles before entering the target viral reservoirs and provided a mild sustained release of d4T 5'-O-(isopropyl) monophosphate without the burst release. The results suggested that this kind of chitosan-O-isopropyl-5'-O-d4T monophosphate conjugated nano-prodrugs could be used as a targeting and sustained polymeric prodrugs for improving therapy efficacy and reducing side effects in antiretroviral treatment.

**Soledad Ravetti and coworkers** [145] synthesized series of 5'-O-carbonates of 3TC (Lamivudine), using different aliphatic alcohols and N, N- carbonyldiimidazol and evaluated them for anti-HIV activity. The antiviral activity was determined in peripheral blood mononuclear cells (PBMCs) showing some carbonate derivatives with an activity similar to or better than 3TC. **In vitro** assays in PBMCs demonstrated that cytotoxicity increased as the carbon chain length of the alcohol moiety increased.

**Tathagata Dutta and coworkers** [146] investigated the targeting potential and anti HIV activity of lamivudine (3TC) loaded mannosylated Poly (propyleneimine) dendrimers (MPPI). The MPPI showed prolonged **in vitro** drug release profile. The sub toxic concentrations of free 3TC, blank MPPI, and drug loaded MPPI were determined on MT2 cells. A significant increase in cellular uptake of 3TC was observed when MPPI was used. Antiretroviral activity was determined using MT2 cell lines by estimating p24 antigen by ELISA. The 3TC loaded MPPI formulations were found to possess higher anti-HIV activity at low concentrations as compared to that of free drug. The results suggested that the proposed carrier held potential to increase the efficacy and reduce the toxicity of antiretroviral therapy.

**Yung-Chih Kuo and coworkers** [147] developed nanoparticles of polybutylcyanoacrylate (PBCA) and methylmethacrylate–sulfopropylmethacrylate (MMA-SPM) to investigate the permeability of zidovudine (AZT) and lamivudine (3TC) across the blood–brain barrier (BBB). Also, the influence of alcohol on the permeability of AZT and 3TC incorporated with the two polymeric nanoparticles (NPs) was examined. The loading efficiency and the permeability of AZT and
3TC decreased with an increase in the particle size of the two carriers. By employing PBCA NPs, the BBB permeability of AZT and that of 3TC became, respectively, 8–20 and 10–18 folds. The application of (MMA–SPM) NPs resulted in about 100% increase in the BBB permeability of the two drugs.

1.9.4. Tenofovir

Petr Jansa and coworkers [148] synthesized bis-amidate prodrugs of adefovir, tenofovir acyclic nucleoside phosphonates, starting from free phosphonic acids or phosphonates diesters and evaluated them for different biological activities. The bis-amidates of compounds exhibited anti-HIV activity at submicromolar concentrations with no observed cytotoxicity at the highest tested concentration. In addition, results of the antiproliferative activity assays demonstrated slightly more potent activity in human cancer cell lines of different origin. None of the prepared compounds displayed any immunomodulatory activities.

Dana Hocková and coworkers [149] synthesized series of prodrugs of adefovir, tenofovir acyclic nucleoside phosphonates (ANPs) with various nucleobases and 2-(2-phosphonoethoxy) ethyl (PEE) chain bearing substituents in β-position to the phosphonate moiety. The influences of structural alternations on antiviral activity were studied. The compounds exhibited antiviral activity against HIV and vaccinia virus (middle micromolar range), HSV-1 and HSV-2 (lower micromolar range) and VZV and CMV (nanomolar range), although the parent unbranched PEE–ANPs were inactive. Adenine as a nucleobase and the methyl group attached to the PEE chain proved to be a prerequisite to afford pronounced antiviral activity.

Tao Zhang and coworkers [150] designed tenofovir (TNF) or tenofovir disoproxil fumarate (TDF) loaded nanoparticles (NPs) prepared with a blend of poly(lactic-co-glycolic acid) (PLGA), metacrylic acid copolymer (S-100) and exhibited significant pH-responsive release of anti-HIV microbicides in the presence of human semen fluid simulant (SFS). Cellular uptake was elucidated by fluorescence spectroscopy and confocal microscopy. There was a 4 fold increase in the drug release rate in the presence of SFS. At a concentration upto
10 mg/ml, the PLGA/S-100 nanoparticles were noncytotoxic to vaginal endocervical/epithelial cells. The particle uptake by these vaginal cell lines mostly occurred through caveolin-mediated pathway. The PLGA/S-100 NPs were appeared as an alternative controlled drug delivery system for intravaginal delivery of an anti-HIV/AIDS microbicide.

**Jianing Meng and coworkers** [151] developed tenofovir loaded chitosan nanoparticles (NPs) and evaluated for anti-HIV activity and also to assess the influence of formulation variables on the size of NPs and drug encapsulation efficiency (EE %). The effect of the NPs on vaginal epithelial cells and *Lactobacillus crispatus* viability and their mucoadhesion to porcine vaginal tissue were assessed by cytotoxicity assays and fluorimetry, respectively. The small sized NPs exhibited burst drug release from medium and large sized NPs fitted the Higuchi and first-order release models, respectively. These NPs were not cytotoxic to both the vaginal epithelial cell line and *L. crispatus*. When the diameter of the NPs decreased the mucoadhesion increased. However, the combinatorial effect of EE% and percent mucoadhesion for larger size NPs was the highest. Overall, large-size, microbicide loaded chitosan NPs appeared to be promising nanomedicines for the prevention of HIV transmission.

**Todd J. Johnson and coworkers** [152] fabricated dual segmented polyurethane Intravaginal rings (IVRs) to enable sustained release of antiretroviral agents dapivirine and tenofovir to prevent the male to female sexual transmission of the human immunodeficiency virus. Due to the contrasting hydrophilicity of the two drugs, dapivirine and tenofovir were separately formulated into polymers with matching hydrophilicity via solvent casting and hot melt extrusion. The resultant drug loaded rods were then joined together to form dual segment IVRs. Compression testing of the IVRs revealed that they were mechanically comparable to the widely accepted NuvaRing® IVR. *In vitro* release of tenofovir from the dual segment IVR was sustained over 30 days while dapivirine exhibited linear release over the time period. A 90 day accelerated stability study confirmed that dapivirine and tenofovir were stable in the IVR formulation.
Altogether, they were an attractive formulation for the sustained vaginal delivery of drugs with contrasting hydrophilicity such as dapivirine and tenofovir.

Richard L. Mackman and coworkers [153] synthesized amidate prodrugs of tenofovir and evaluated for its anti-HIV activity. To effectively deliver its active phosphorylated metabolite into target cells, a series of amidate prodrugs were designed as substrates of cathepsin A, an intracellular lysosomal carboxypeptidase highly expressed in peripheral blood mononuclear cells (PBMCs). The prodrug demonstrated the favorable cathepsin A substrate properties, in addition to favorable in vitro intestinal and hepatic stabilities. Following oral dosing (3 mg/kg) in Beagle dogs, high levels (>9.0 µM) of active metabolite were observed in PBMCs, validated the prodrug as a clinical candidate.

Constantine G and coworkers [154] synthesized prodrugs of tenofovir and evaluated for its anti-HIV activity. In cell culture the parent compound demonstrated antiviral activity (EC$_{50}$ = 16 µM) within two-fold of a prodrug which is currently under clinical investigation, and within 5-fold of tenofovir (PMPA). The in vitro cellular metabolism studies confirmed that the active metabolite produced albeit at a lower efficiency relative to the prodrug.

Carlo Ballatore and coworkers [155] synthesized amidate prodrugs of PMEA (adefovir) and PMPA (tenofovir) and tested for their in vitro antiviral activity. The compounds showed greatly enhanced antiviral potency compared with the parent nucleotide analogue. In vitro enzymatic studies and structure–activity relationships indicated that the degradation mechanism of such prodrugs may be the same as that of the phosphoramidate triesters of nucleotide analogues.