Expert Opinion

Nucleoside analogue delivery systems in cancer therapy

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Nucleoside analogues (NAs) are important agents in the treatment of hematological malignancies. They are prodrugs that require activation by phosphorylation. Their rapid catabolism, cell resistance and overdistribution in the body jeopardize nucleoside analogue chemotherapy. Accordingly, therapeutic doses of NAs are particularly high and regularly have to be increased, resulting in severe toxicity and narrow therapeutic index. The major challenge is to concentrate the drug at the tumour site, avoiding its distribution to normal tissues. New drug carriers and biomaterials are being developed to overcome some of these obstacles. This review highlights novel NA delivery systems and discusses new technologies that could improve NA cancer therapy.

Keywords: DepoFoam™, dendrimers, inclusion complexes, liposomes, microparticles, nanoparticles, polymeric micelles, polyplex nanogels, Stealth® particles, vesicular phospholipid gel

Expert Opin. Drug Deliv. (2007) 4(5):513-531

1. Introduction

Nucleoside analogues (NAs) are critical components of anticancer, antiviral and immunosuppressive therapy. They are antimetabolites, a class of drugs that inhibit DNA synthesis either directly or through inhibition of DNA precursor synthesis on the *de novo* or salvage pathways [1,2]. The anticancer nucleosides include several analogues of physiological pyrimidine and purine nucleosides and nucleobases [1]. Among the presently available analogues of purine are cladribine, fludarabine and clofarabine, and those of pyrimidine are cytarabine and gemcitabine.

A major obstacle associated with the use of NA chemotherapeutic agents is the lack of selectivity toward cancerous cells. Consequently, the effective doses of NA anticancer agents are particularly high and have regularly to be increased. In this context, toxicity becomes the main limiting factor to the treatment. NA intracellular delivery is another major challenge, as these compounds are hydrophilic and therefore require facilitated transport to cross cellular membranes [3].

To exploit the specificity and potency of these drugs, several carrier systems, such as liposomes, nanoparticles, polyplex nanogels and polymeric micelles, have been developed as potential tools of tumour targeting, facilitating drug uptake by cancerous cells, and therefore increasing both safety and efficiency of NAs in cancer therapy.

In this paper, the authors overview the principal limitations of NAs and then present the presently available NA carriers and their role in NA tumour targeting.

2. The mechanism of action of nucleoside analogues

NAs mimic natural nucleosides. They are administered in forms that enter into the cells through nucleosides transporters before being phosphorylated to their active triphosphate form inside the cell by the kinases [4]. These agents can exert their

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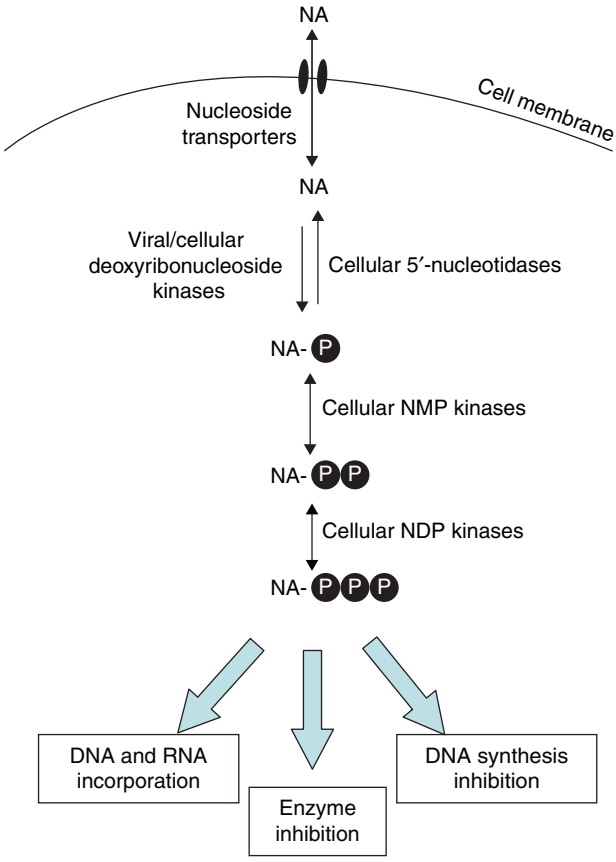

Figure 1. Nucleoside analogues require intracellular phosphorylation for pharmacological activity. The nucleoside analogues are transported across the cell membrane and phosphorylated by cellular kinases to their triphosphate form.
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NDP: Ribonuclotide diphosphate; NMP: Ribonuclotide monophosphate.

cytotoxic activity by being incorporated into and altering the DNA and RNA macromolecules themselves and/or by interfering with various enzymes involved in the nucleic acid synthesis, such as DNA polymerases and ribonucleotides reductases [1]. These actions result in the inhibition of DNA synthesis and the induction of apoptic cell death (**Figure 1**).

3. Physiological deoxyribonucleotide and ribonucleotide metabolism

Deoxyribonucleotides and ribonucleotides have to be synthesized within the cells because there is no carrier protein for them in the cell membrane. There are two synthesis pathways, called the *de novo* pathway and the salvage pathway [2,4].

Via the *de novo* pathway, ribonucleotides are synthesized from small molecules (amino acids, ribose-5′-phosphate and CO_2) to mononucleotides and then they undergo further phosphorylation (**Figure 2**). The 2′-OH group of the ribonuclotide diphosphate can be reduced to the corresponding 2′-deoxyribonucleotide diphosphate by ribonucleotide reductases.

In the salvage pathway, deoxyribonucleotides are synthesized from deoxyribonucleosides, catalyzed by the deoxyribonucleoside kinases, nucleoside monophosphate and diphosphate kinases. In addition, there are two salvage pathways for ribonucleotides. The first is from free bases, which undergo further phosphorylation through direct sugar phosphate transfer. The second salvage ribonuclotide pathway is from ribonucleosides, which are transformed by further phosphorylation to their triphosphate form [2,4]. Ribonucleosides and deoxyribonucleosides are imported into cells by nucleoside transport proteins that facilitate diffusion or actively transport nucleosides across the membrane.

In proliferating cells, the de novo deoxyribonucleotides synthesis is the main source for nuclear DNA replication [2,4] and takes place only in S phase of the cell cycle. Deoxyribonucleotides synthesized in the salvage pathway are believed to be important for DNA repair and this pathway is active throughout the cell cycle. The deoxyribonucleosides salvage pathway is of a particular interest to pharmacologists because NAs used to treat cancer are administered as prodrugs that are activated inside the cells by the salvage enzymes. Consequently, the rate of NA phosphorylation to the active triphosphate form may directly determine the therapeutic efficacy of these agents.

4. Limitations to nucleosides analogues

The chemotherapeutic treatment of tumours with NAs is potentially limited by their narrow therapeutic index, due to low anticancer activity and/or severe side effects. NAs are not naturally specific to tumour cells; hence they accumulate not only in tumours but also in healthy tissues. Furthermore, most NAs have only short half-lifes in the systemic circulation due their rapid enzymatic degradation; as a consequence, very high doses have to be given for efficient tumour treatment, leading to severe side effects.

4.1 Cytotoxicity and pronounced side effects

NAs are cytotoxic agents that can disturb the cellular metabolism, deregulating the physiological nucleoside/nucleotide pools in both normal and cancerous cells, due to the lack of their selectivity towards malignant cells. At conventional doses, NAs induce myelosuppression, hepatotoxicity (gemcitabine, fludarabine and cladribine) [1,5], renal toxicity (gemcitabine), leucopoenia, thrombocytopenia, mucositis and hair loss (cytarabine). Furthermore, NA high doses have commonly been associated with neurotoxicity and pericarditis (cytarabine and fluorouracil).

4.2 Emergence of drug resistance and low anticancer activity

There are three general mechanisms of resistance to NAs that have been described in cell lines and clinical samples:

- Insufficient intracellular concentrations of NA triphosphate, which might be due to inefficient cellular uptake caused by

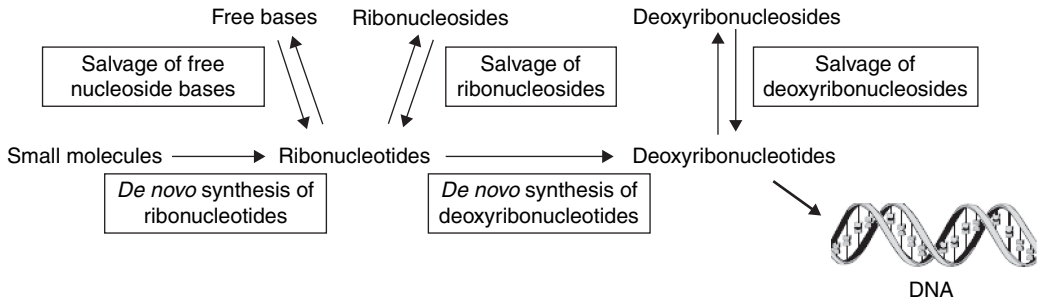

Figure 2. *De novo* and salvage synthesis of ribonucleotides and deoxyribonucleotides.
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deficiency in cellular membrane nucleoside transporters [3] or elevated levels of 5′-nucleotidases that remove the phosphate group from mononucleotides. The resulting nucleosides can be exported by nucleoside transporters outside of the cell [2], the levels of activating enzymes, such as nucleosides and deoxynucleoside kinases decrease [6], and catabolism caused by cellular over expression of 5′-nucleotidases [7] or deaminases increases [5,8].

- Inability to achieve sufficient alterations in DNA strands or deoxynucleotide triphosphate pools, which might result from altered interactions with DNA polymerases. This would reduce the affinity of these enzymes for NAs, reduce the inhibition of ribonucleotide reductases and cause expansion of deoxynucleotide triphosphate pools that may compete with NAs for incorporation into DNA [6].
- Defective induction of apoptosis. The key event of apoptosis is caspase activation, which may be a consequence of death triggering mitochondrial activation [9].

4.3 Hydrophilicity and low membrane permeability

NAs are hydrophilic polar molecules with low membrane permeability [5], and, thus, require specialised nucleoside transporter proteins to enter cells. Therefore, the abundance and tissue distribution of these transporters contributes to cellular specificity and sensitivity to nucleoside analogues [1]. On the other hand, there are no transporter proteins for ribonucleotides and deoxyribonucleotides in the cell membrane, and their negatively charged phosphate groups prevent their diffusion across the membrane [4].

4.4 Bioconversion *in vivo* drug catabolism and rapid clearance

The cytidine analogues, such as gemcitabine and cytarabine, undergo extensive degradation by cytidine deaminase to inactive metabolites (uracil arabinoside) in the liver and kidneys, which adversely affects their activities [5,8,10]. Consequently, these analogues have short circulation half-lifes of 10 – 20 min, and so they fail to maximize the intracellular accumulation of their active metabolites. After oral administration, although these drugs have shown to be stable at pH 1, and well-absorbed via the gastrointestinal tract, they are metabolized extensively in the first pass through the liver, as well as by the bacterial gut flora; as a consequence, they have poor oral bioavailability due to rapid hepatic deamination [11].

Purine analogues, such as cladribine, are unstable at low pH and are deglycosylated by bacterial gut flora purine nucleoside phosphorylases to chloroadenine, which has a lower cytotoxic effect [12]. Furthermore, cladribine can be cleaved in an enzymatic reaction to chloroadenine in the presence of the hepatic enzyme methylthioadenosine phosphorylase [12].

Given the previous limitations, a variety of novel drug delivery systems are presently being developed in an attempt to address some of the problems associated with the lack of NA stability and selectivity towards tumour tissues.

5. Nucleoside analogue delivery systems in cancer therapy

As previously mentioned, the efficacy of cancer chemotherapy is limited by the non-specificity of anticancer drugs, leading to severe systemic toxicity. The ideal scenario would be to sequester the drug in a package that would have minimal interactions with healthy cells, and to release drug at the appropriate time from the sequestering carrier at the tumour site.

Several drug delivery systems, namely liposomes, microparticles, nanoparticles, polymeric micelles, dendrimers, hydrogels, polyplex nanogels and cyclodextrin inclusion complexes, have been introduced in order to facilitate effective chemotherapy and to overcome some of the above limitations of NAs. The extended release of NA molecules has been made possible using microsized system, such as microparticles (e.g., DepoFoam™; SkyePharma), resulting in reduced toxicity and improved efficacy, especially for cell cycle-specific NAs. However, nano-sized carriers can either allow NAs to be passively targeted, as with long-circulating liposomes, or actively targeted, in the case of magnetic or pH-sensitive nanoparticles. Furthermore, polyplex nanogels seem to be an ideal carrier for NAs, by administering them in their active tri-phosphate form, avoiding the emergence of cellular

resistance related to the decreased activity of deoxycytidine kinase. Finally, their small size and the option to bind ligands to their surface resolve the relevant problems of NA poor cellular uptake and poor tumour cell selectivity, respectively.

The following sections cover the use of all these systems as carriers for NAs in detail.

5.1 Liposomal formulations

Liposomes are self-assembling vesicles with an inner aqueous compartment surrounded by a lipid bilayer, which consists of naturally occurring phospholipids as a main component [13]. Lipophilic and amphiphilic drugs can be incorporated into the liposomal bilayers, whereas hydrophilic drugs can be incorporated into the inner aqueous compartment. Thus, the systemic environment does not recognize the free drug. It recognizes only the liposomes and the drug pharmacokinetics become replaced by the pharmacokinetic behaviour of the liposomes..

Liposomes can be classified in two ways [14]:

- Classification according to structure and size (**Figure 3**):
  - Unilamellar liposomes, comprising one lipid bilayer and having diameters of 50 – 250 nm. They contain a large aqueous core and are used for the encapsulation of water-soluble drugs.
  - Multilamellar liposomes composed of several concentric lipid bilayers in an onion-skin arrangement and have diameters of 1 – 5 µm.
  - Multivesicular liposomes (MVLs, e.g., DepoFoam), consisting of numerous non-concentric lipid bilayers in a honey comb arrangement and have diameters of 1 – 100 µm.
- Classification according to a phylogenetic scheme:
  - Classical or conventional liposomes (simple mixtures of phospholipids and cholesterol) target the reticulo-endothelial system (RES) and are called RES-targeted liposomes. Vesicle size is inversely correlated with the amount of RES uptake.
  - Sterically stabilized liposomes, or surface-modified liposomes (by coating liposomes with polyethylene glycol; STEALTH® technology [ALZA]) escape from the RES uptake, allowing drug to target malignant tissues.

Liposomal formulations aim to reduce the toxic side effects of cytotoxic drugs without hampering their efficacy. This can be achieved by a selective drug accumulation in tumour tissues by means of active targeting (coating liposomes with antibodies or ligands in order to specifically recognize epitopes or receptors of malignant cells) [15,16], or by passive targeting due to the enhanced permeability and retention effect of sterically stabilized liposomes, due to differences in the vasculature between tumours and healthy organs or tissues [16].

5.1.1 *Conventional liposomes*

As most of NAs are polar (amphipathic at minimum) and water soluble, most early efforts in liposomal anticancer drug development focused on entrapping agents such as cytarabine in an attempt to improve its therapeutic index. However, significant protection from deamination and improvement of antitumour activity could not be achieved, mainly due to the drug leakage (initial burst) from liposomes, which is caused by their well-characterised instability and short half-life *in vivo*. The half-life of liposomes is generally influenced by their stability in serum [17] and their uptake by RES cells [18]. The uptake of liposomes by the RES is triggered by the binding of serum proteins to their phospholipid bilayer (opsonization). Once opsonized, liposomes can be rapidly recognized and phagocytosed. On the other hand, it is very difficult using presently available technologies to stably encapsulate water-soluble low molecular weight drugs such as NAs into conventional liposomes: these molecules diffuse rapidly through liposome bilayers. Thus, shortly after their preparation, rapid diffusion of the drug out of the liposomes occurs, limiting the shelf life and, therefore, the clinical use of conventional liposomes.

Much research work has tried to overcome the instability of liposomes in serum by optimizing their formulation. It was found that the addition of cholesterol in quantities of 35 – 50% mol of the cytarabine liposomal formulation stabilized membranes by reducing membrane fluidity and enhanced the encapsulation efficiency of cytarabine-loaded liposomes [19]. Furthermore, liposomal membranes consisting of glycerophospholipids with long hydrogenated fatty acid esters (e.g., synthetic phospholipids), such as distearoylphosphatidylcholine, have been shown to be more rigid than those consisting of glycerophospholipids made up of fatty acids of different lengths and saturation (e.g., egg lecithin, soya lecithin) [13,16]. As a consequence, these liposomal membranes are more stable against lipid exchange by serum proteins, as a rigid membrane decreases the efflux of drugs from liposomes and stabilizes the liposomes themselves.

5.1.2 *Stealth liposomes*

The modification of liposome composition with PEG-containing lipids greatly enhances their stability in the circulation. Components that sterically stabilize liposomes, such as PEG-phosphatidylethanolamine (Stealth components), lower the recognition and uptake by the RES by increasing the liposome hydrodynamic circumference (**Figure 4**). When gemcitabine was encapsulated in pegylated liposomes (Stealth liposomes), a significant antitumour effect was observed using lower drug concentrations and after an earlier exposure time [20]. Also, the cytotoxic activity of cytarabine encapsulated in long-circulating PEG liposomes (Stealth liposomes) was superior to that of other liposome formulations or of the free drug [21]. In fact, the increased permeability of the tumour endothelium allows liposomes to be extravasated, and the deficient lymphatic drainage lead to the drug being selectively accumulated in the site of action (enhanced permeability and retention effect) [22].

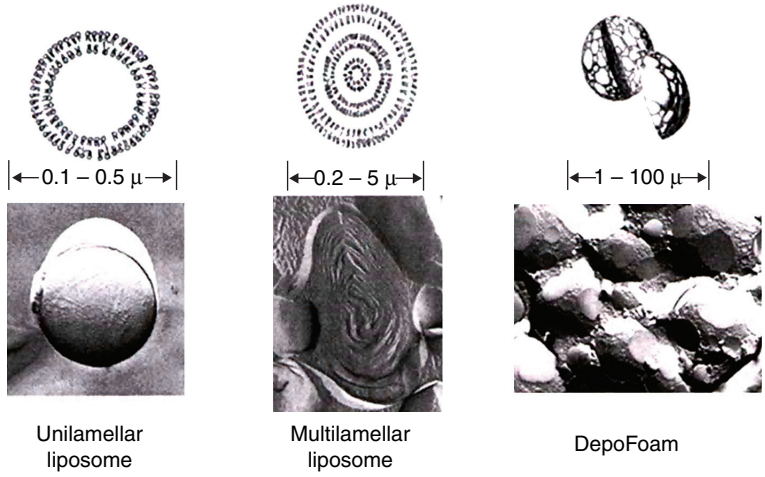

Figure 3. Stuctural differences between conventional liposomes and DepoFoam.
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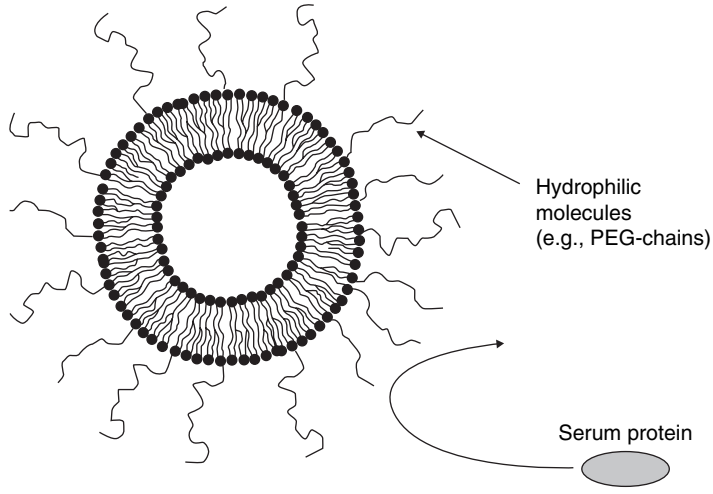

Figure 4. Sterically stabilized liposomes (Stealth liposomes): the interaction of serum proteins with the liposome bilayers is reduced by surrounding the liposomes with large hydrophilic molecules (e.g., PEG chains, pegylation of liposomes).
Reproduced with permission from [13].

More exciting results were obtained by sequestering the drug in multivesicular lipid-based particles using DepoFoam technology.

5.1.3 *Multivesicular liposomes (DepoFoam)*

The DepoFoam drug delivery system was developed to permit sustained release of water-soluble drugs, capable of delivering drugs for periods extending from a few days to a few weeks, from a depot after direct injection into a body compartment or a tissue [23].

DepoCyt® (cytarabine-loaded multivesicular liposomes; SkyePharma) is the first product based on the DepoFoam technology to be approved by the FDA. DepoCyt is an intrathecally injectable suspension of cytarabine encapsulated in DepoFoam particles (SkyePharma). Each particle has a diameter of ~ 3 – 30 µm and consists of numerous non-concentric vesicles in a honeycomb arrangement. The chambers are separated from each other by lipid bilayers consisting of dioleyl-phosphatidylcholine, dipalmitoyl-phosphatidylglycerol, cholesterol and triolein [21] (**Figure 5**).

The characteristic nonconcentric nature of a DepoFoam particle results in a higher aqueous/lipid ratio than for a concentric multilamellar liposomes, leading to greater encapsulation efficiencies for water-soluble drugs. Furthermore, the interconnection between the internal membranes gives rise to greater mechanical strength and stability than those known for traditional liposomes of equivalent size and aqueous content [14]. At a storage temperature of 2 – 8°C,

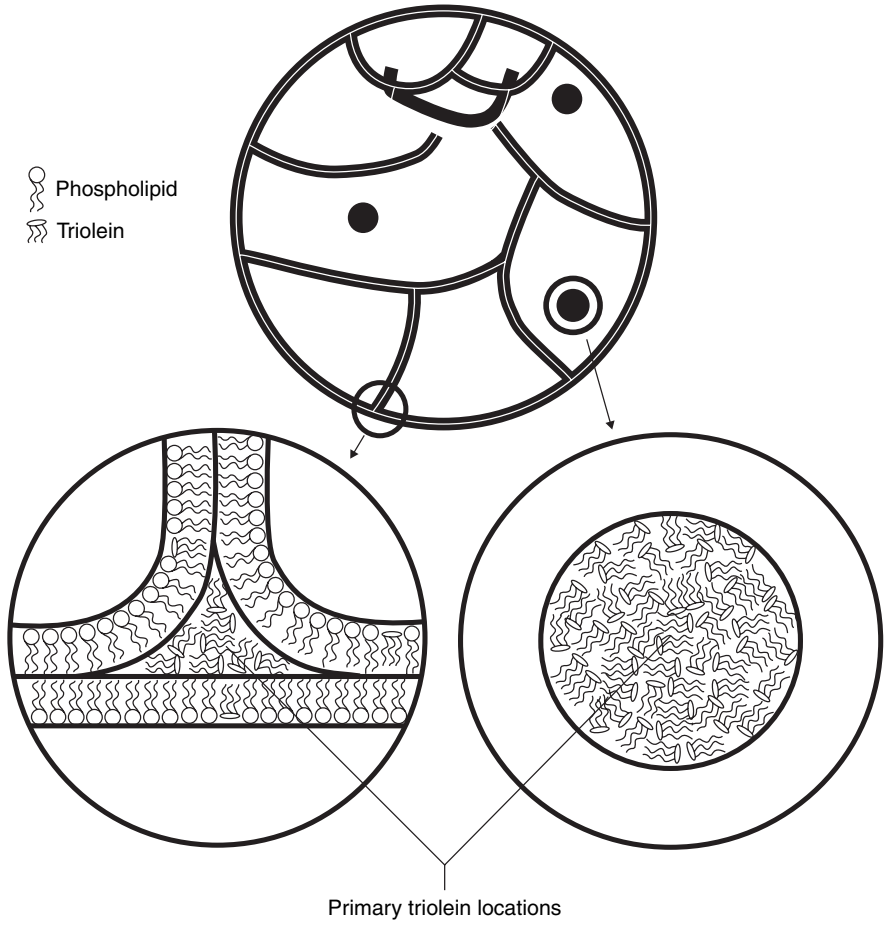

Figure 5. Schematic showing the location of triglycerides in DepoFoam.
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the particles are stable for 12 months. DepoCyt has a mean half-life of 130 – 277 h, compared with 3 – 4 h for free cytarabine [14].

Another NA that is an antiviral drug, acyclovir, was conditioned in MVLs in order to overcome the limitations of conventional liposomal therapies. The encapsulation efficiency in MVLs (45 – 82%) was found to be 3- to 6-times higher than that in conventional multilamellar vesicles. In addition, the *in vitro* release of acyclovir from MVLs was found to be in a sustained manner, and only 70% of drug was released in 96 h, whereas conventional multilamellar liposomes released 80% of drug in 16 h [24]. Moreover, formulations containing phosphatidylglycerol as negatively charged lipid showed better results because they increase the intralamellar distance between the successive bilayers of the MVL structure, which leads to a greater overall capture volume. Neutral oil is an integral structural component: it becomes a part of the corner or edges where membranes meet [24].

A more recent strategy to increase the shelf life of liposomal formulations is the encapsulation of small, hydrophilic molecules, such as NAs, into vesicular phospholipid gels (VPGs).

5.1.4 *Vesicular phospholipid gels*

VPGs are semisolid matrices of densely packed liposomes – mainly small unilamellar vesicles – which are prepared by high-pressure homogenization [25]. Due to their high lipid content, which leads to a considerably increased ratio of aqueous volume inside the vesicles compared with the surrounding aqueous volume, these formulations are suitable for entrapping water-soluble substances with high encapsulation efficiency compared with conventional liposomal formulations [26]. Moreover, due to their high lipid concentrations, these formulations have a semisolid or gel-like consistency. In contrast to conventional liposomal formulations, the non-encapsulated drug is not removed at the end of the preparation process, so that the drug is entrapped inside the liposomes and between them in the surrounding aqueous phase. This special characteristic leads to an increased shelf life and an increased encapsulation

efficiency of the drug-loaded VPG. This has been demonstrated for gemcitabine and 5-fluorouracil (5-FU)-loaded VPG formulations [27,28]. For gemcitabine, the drug still diffused through the liposomal bilayers, and thus its concentration was always in equilibrium between the inner and outer aqueous phases of the liposomes. As the volumes of the aqueous phases inside and outside the vesicles have the same magnitude, the concentration of the drug remained the same, resulting in a superior shelf life of gemcitabine-loaded VPG. Furthermore, the liposomal entrapment of gemcitabine in VPG was demonstrated to positively change its pharmacokinetics and pharmacodynamics and, hence, to enhance its antitumour activity [27] (**Figure 6**). This effect can be attributed to three reasons: i) prolonged circulation of the liposomally entrapped gemcitabine in blood and therefore prolonged drug exposure to the tumour; ii) protection of the drug against rapid metabolic inactivation; and iii) enhanced uptake and accumulation of the drug within the tumour by the enhanced permeability and retention effect [22].

The influence of the VPG lipid composition on the encapsulation efficiency was studied in 5-FU-loaded VPG [28], using mixtures of hydrogenated soy phosphatidylcholine and cholesterol with molar ratios ranging from 55/45 to 75/25. Interestingly, it was found that a decreasing amount of cholesterol correlated with an increasing encapsulation efficiency, which was probably due to a reduced amount of smaller vesicles and the number of lamellae.

5.2 Hydrogels

A hydrogel is a three-dimensional network of hydrophilic polymers swollen in water, being maintained in the form of an elastic solid. Hydrogels usually contain water accounting for at least 10% of the total weight [29]. They are divided into chemical and physical gels depending on the nature of the crosslinking. Chemical gels are those having covalently crosslinked networks. They can be prepared by two different approaches. First, they can be made by the polymerization of water-soluble monomers in the presence of bi- or multi-functional crosslinking agents (crosslinking polymerization). Second, chemical gels can be prepared by crosslinking water-soluble polymer molecules using typical organic chemical reactions that involve functional groups of the polymers. Physical gels are continuous disordered three-dimensional networks formed by noncovalent interactions such as hydrogen bonding, ionic association, hydrophobic interaction, stereocomplex formation and solvent complexation [29].

Hydrogel delivery systems can be administered by implantation into the tumour site, for topical, oral or rectal administrations. The drug release rate from hydrogel implants can be controlled by adjusting the crosslinking density and/or by adding water-soluble components. For example, the entrapment of 5-FU in poly(2-hydroxyethyl methacrylate-co-acrylamide) hydrogels allowed 5-FU to be included in the feed mixture of polymerization, up to 16 mg/disc, without any chemical drug alteration; it also made possible the control of its release over a wide range of times varying between 7 h and 9 days, just by modulating the crosslinking degree of the copolymer (**Figure 7**), as well as their comonomeric composition, maintaining sufficiently high hydrate degrees (66 ± 24 wt%) [30].

5.3 Polyplex nanogel formulations

The polyplex nanogels are hydrophilic nanosized particles consisting of crosslinked cationic polymers. Hydrophilic ionisable polymers are able to bind biomolecules of opposite charge, forming polyionic complexes or polyplexes, and deliver them into various biological environments [31]. This delivery system was studied to be a candidate for the vectorization of NA 5′-triphosphates [32]. In this study, a polycationic polymer polyethylenimine (PEI) was used to bind the active 5′-triphosphate form of fludarabine (FATP) to PEI's protonated amino groups. Subsequent compaction of the flexible nanogel network has resulted in the encapsulation of the FATP/PEI complex in a dense core surrounded by a hydrophilic PEG envelope. This structure has provided the sustained release of the drug, as well as an efficient protection of FATP against enzymatic degradation.

Cancer cell-targeting molecules, such as folate, can be easily attached to nanogels (**Figure 8**), and this modification has resulted in a strong 10-fold increase of the carrier's internalization in human breast carcinoma MCF-7 cells.

Tri-phosphorylated NAs (NTPs) are efficient terminators of nucleic acid synthesis in proliferating cancer cells, but they are considered to be unstable for direct use in cancer chemotherapy. The application of these delivery systems for NTP encapsulation and targeting offers hope to resolve many of the problems associated with this chemotherapy, especially the avoidance of the development of drug resistance due to decreased nucleoside kinase activity. In addition, they also protected the drug against the enzymatic degradation. These formulations allow lower doses of NAs to be administered, while maintaining strong anticancer efficacy.

This approach is very mild, efficient, and non-damaging to the NTP structure compared with their encapsulation into biodegradable nanoparticles or liposomes, for example. Drug-loaded nanogel formulations are easily dispersed in water, aggregationally stable, and of great importance from a pharmaceutical viewpoint, as they can be lyophilized and redissolved instantly at the moment of their administration. Lyophilized formulations maintain the same particle size and can be injected intravenously. The particle size of drug-loaded nanogels is ≤ 150 nm. This size is convenient for several reasons, for example: i) it allows the sterilization of drug–nanogel formulations by filtration; ii) particles can penetrate even small blood capillaries; and iii) can readily enter cells by endocytosis. The low buoyant density of nanogels makes them a unique type of drug carrier, with great potential for systemic administration.

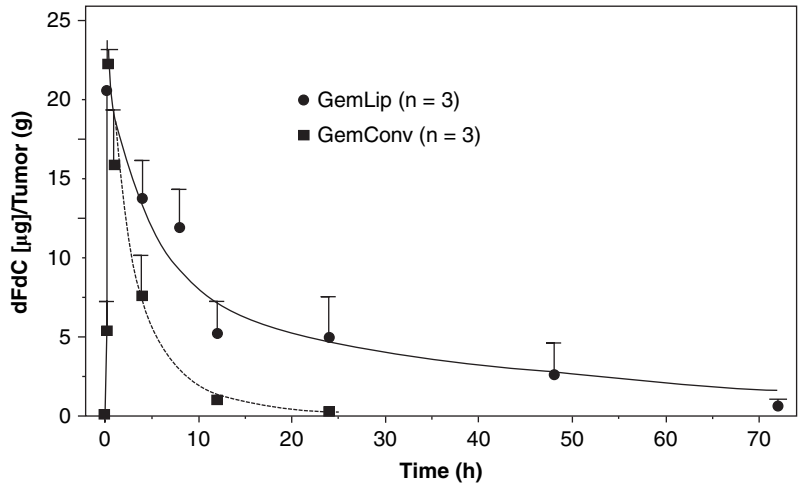

Figure 6. Accumulation of radiolabel (presented as the amounts of dFdC) in tumors after administration of 14C-dFdC given as GemLip (vesicular phospholipid gels) or GemConv (6 mg dFdC/kg each).

This figure was published in [27], reproduced with kind permission of Springer and Business Media.
14C-dFdC: Radiolabeled gemcitabine hydrochloride; dFdC: Gemcitabine hydrochloride.

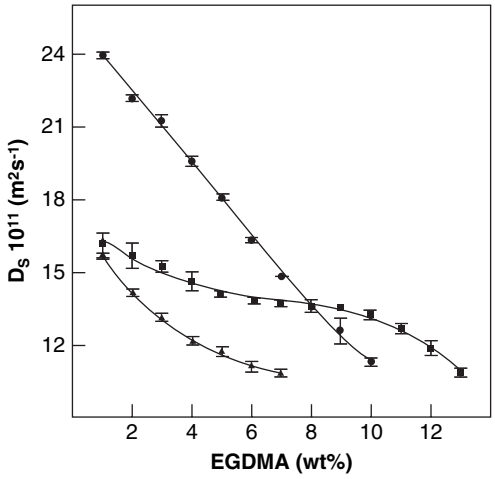

Figure 7. Variation of the apparent diffusion coefficient for saline solution uptake (Ds) in poly (2-hydroxyethyl methacrylate-co-acrylamide) (HEMA/A ± % EGDMA) hydrogels as a function of their percentage of EGDMA at 310 K: (●) 50 HEMA/50 A; (■) 75 HEMA/25 A; and (▲) 90 HEMA/10 A.

Reproduced with permission from [30].
EGDMA: Ethylene glycol dimethacrylate; HEMA: 2-Hydroxyethyl methacrylate.

Furthermore, the membranotropic properties of nanogels loaded with azidothymidine triphosphate, an antiviral NA, have been confirmed *in vitro* and have been clearly demonstrated by various microscopic methods [33]. Following interactions with membranes, drug-loaded nanogels actively release incorporated drug (**Figure 9**). A drug release mechanism triggered by the interaction of the drug-loaded nanogels with the phospholipid bilayer has been proposed and described [33].

5.4 Microparticles

Microparticles are spherical polymeric particles with sizes ranging from 1 to 2000 µm (ideally < 125 µm in diameter). They include microcapsules that are vesicular systems in which a drug can be confined to a cavity surrounded by a polymeric membrane; and microspheres that are matrix systems in which the drug is dispersed throughout the particle [34]. Biodegradable microparticles have been extensively used in pharmaceutical design to obtain delivery systems that allow drug to be released in a sustained manner. For NAs, it is essential to release them in efficient concentrations and for an extended period of time. In fact, most NAs are cell-cycle specific and, thus, their therapeutic efficacy is related to the exposure time of tumour cells to the active agent.

A major obstacle to the particulate formulation of NAs is their high water solubility and low molecular weight, resulting in rapid leakage of the small hydrophilic drug molecules through the thin polymer wall of the particle, giving rise to poor encapsulation efficiency and rapid drug release from microparticles.

Natural polymers such as gelatine [35], albumin [36], chitosan [37] and alginate [38] have been used as matrix materials for the preparation of NA microspheres. These hydrophilic polymers have been shown to efficiently entrap NA molecules. Entrapment efficiencies as high as 70.6 and 65% were obtained for cytarabine-loaded chitosan microspheres [37] and 5-FU-loaded gelatine microspheres [35], respectively. However, the release kinetics of hydrophilic polymer microspheres was characterized by a burst effect during the first hours, followed by a slower release rate. In an effort to resolve this problem, hydrophilic polymer microspheres were coated with a lipophilic polymer film such as poly(lactide-co-glycolide) (PLGA). The total release of cytarabine from chitosan microspheres *in vitro* was detected at

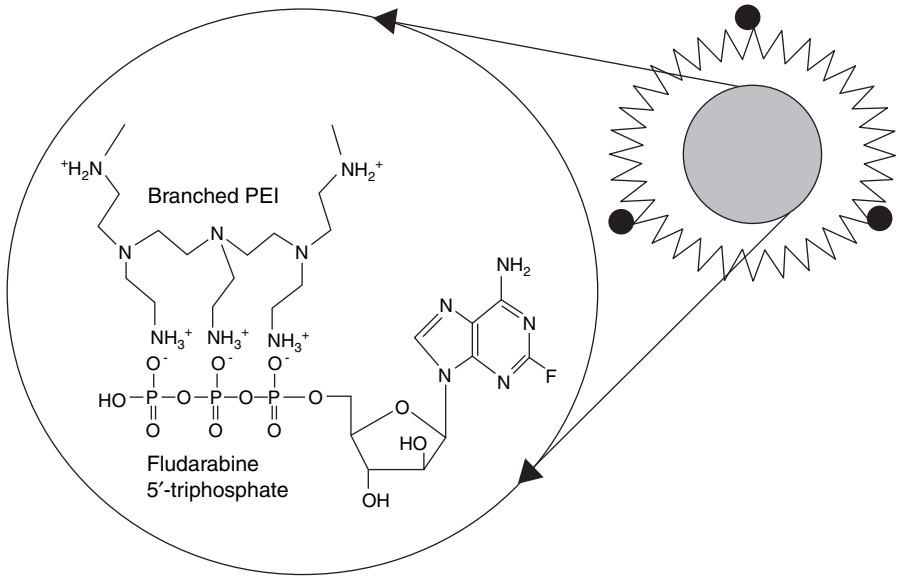

Figure 8. Schematic representation of a nucleoside analogue fludarabine triphosphate-loaded nanogel particle. The particle consists of a condensed core loaded with fludarabine triphosphate/polyethylenimine polyionic complex and a polymer envelope composed of PEG molecules, some of them with attached vector ligands.
This figure was published in [32], Copyright Elsevier (2005).
PEI: Polyethylenimine.

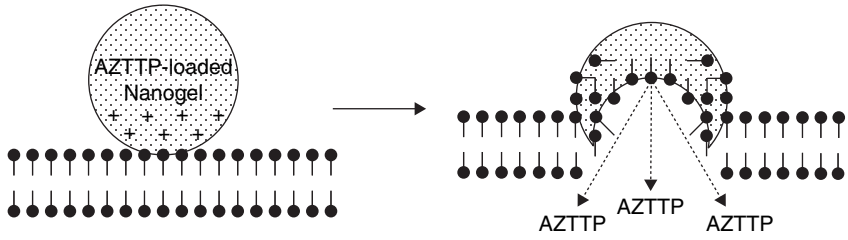

Figure 9. Graphic representation of nanogel fusion with the cellular membrane and substitution of the loaded drug azidodeoxythymidine triphosphate (AZTTP) with the anionic components of the phospholipid bilayer.
Reproduced with permission from [33].

48 h, compared with 80% of cytarabine released within 94.5 h from PLGA-coated chitosan microspheres (**Figure 10**) [37].

Several factors can affect the drug release process from hydrophilic polymer microspheres. Among the different factors, the degree of crosslinking plays an important role. An increase in the crosslinking agent concentration in the preparation of 5-FU-loaded gelatine microspheres produces smaller sized microspheres with lower degrees of swelling, a reduced 5-FU release rate and improved drug loading [35]. However, an increase in the mean diameter of 5-FU-loaded alginate microspheres was observed with an increase in the crosslinking concentration and time of crosslinking [38]. The polymer concentration is also an important factor influencing the morphology and size of microspheres: the lower the polymer concentration, the smaller the spheres produced and the better the microsphere surface obtained [35].

Hydrophobic polymers, such as poly(lactic acid; PLA) and PLGA, have also been used to create microparticles of NAs that are slightly soluble in water, such as 5-FU (~ 10 mg/ml), compared with the high water solubility of other NAs such as cytarabine (~ 148 mg/ml). In one study, 5-FU-loaded PLA-microparticles were prepared using a S/O/W emulsion method. These microparticles contained 5 – 15% w/w of 5-FU and released the drug *in vitro* over a period of 5 days [39]. There was a substantial burst (20 – 40%) of the encapsulated drug from these particles that increased with initial drug loading. To overcome this problem, another type of NA microparticle was prepared from PLGAs of high and low molecular weight, as well as a mixture of PLGAs of different molecular weights, using a S/O/W emulsion method [40]. The resulting microparticles were 50 – 60 µm in diameter, with encapsulation efficiency as high as 75%, and drug

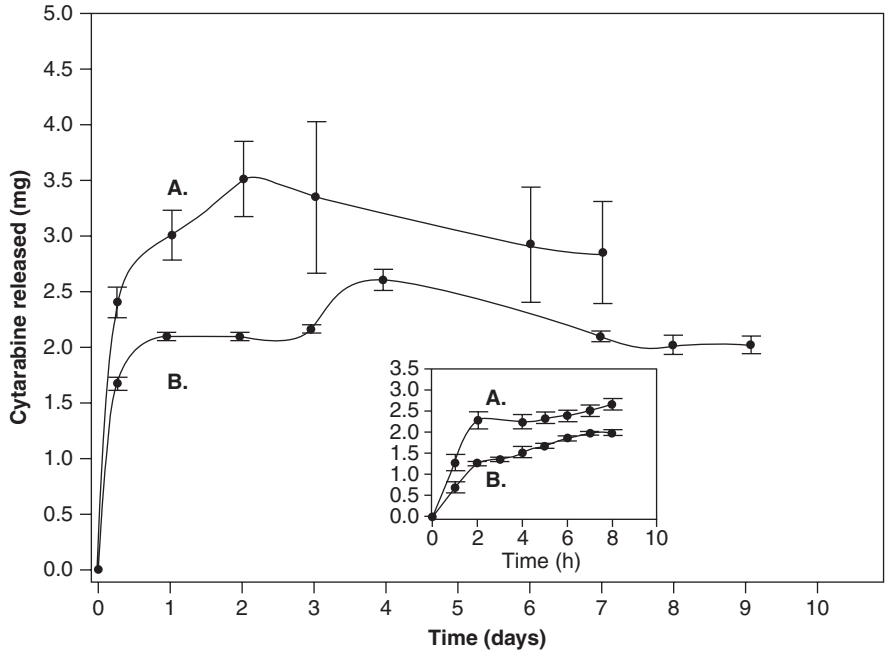

Figure 10. Cytarabine released from A. Chitosan microspheres and **B.** The comatrix, in phosphate buffer, 0.1 M, pH 7.4 at 37°C, by swelling as a function of time.
Reproduce with permission from [37].

loading of 25% by mass. In this case, the initial release was slow and sustained, with no burst, and remained ≥ 3 weeks depending on the PLGA molecular weight. Higher molecular weight polymers yielded formulations with a longer controlled-release duration. After polymer degradation, the remaining drug is released over a period of ~ 1 week. Thus, the release is controlled by both diffusion and polymer hydrolysis rates, resulting in a biphasic release profile.

Similar results have been obtained in a study comparing three types of 5-FU-loaded microparticles prepared from poly(lactide-co-caprolactone), PLA and PLGA, by a spray-drying method [41]. With PLGA microspheres, an increase in the lactide to glycolide ratio resulted in a progressive decrease in the 5-FU release rate from microspheres. Poly(lactide-co-caprolactone) microspheres released 5-FU more rapidly compared with PLGA systems. PLGA of a high lactide to glycolide ratio (85:15) was successfully used to entrap a combination of cytarabine and 5-FU into microspheres for slow drug delivery to ocular tissue [42]. A therapeutic concentration could be maintained for up to 48 h after a single intravitreal injection of these drugs. A lower concentration was maintained for up to 11 days.

In another study, a new material, poly(methylidene malonate 2.1.2), has been used to prepare 5-FU-loaded microparticles yielding a more prolonged release than PLGA microparticles due to its chemical structure (ester bonds in the side chains only) resulting in a long biodegradation time [43]. The authors reported that the percentage of 5-FU released within 24 h could be lowered to 65%, and microspheres were not significantly degraded *in vitro* after 43 days while the release was ongoing.

A pH-sensitive polymer, Eudragit® P-4135F (Degussa), was also used to prepare 5-FU-loaded microspheres by a simple o/w emulsification process. Eudragit P-4135F, pure or in a mixture, was found to retain the drug release at pH 6.8 lower than 35% within 6 h. At pH 7.4, almost immediate release (within 30 min) was observed for pure P-4135F, but mixtures with Eudragit RS100 enabled slightly prolonged release (**Figure 11**). A capsule-like structure, which was established by morphological analysis, caused only slight changes in the release kinetics after the RS100 addition. However, the formulation proved its applicability *in vitro* as a promising device for pH-dependent 5-FU colon delivery [44].

5.5 Nanoparticles

Nanoparticles are submicronic (< 1 µm) polymeric systems. According to the process used for their preparation, nanospheres or nanocapsules can be obtained. Nanospheres and nanocapsules are the morphological equivalents of microspheres and microcapsules, respectively [45]. Many processes can be used for the preparation of nanoparticles, including solvent evaporation, organic phase separation, interfacial polymerization, emulsion polymerization and spray drying. However, only a few are acceptable for NA formulation. As mentioned earlier, the high hydrophilicity and the low molecular weight of these molecules represent a major challenge to particulate formulation because most of

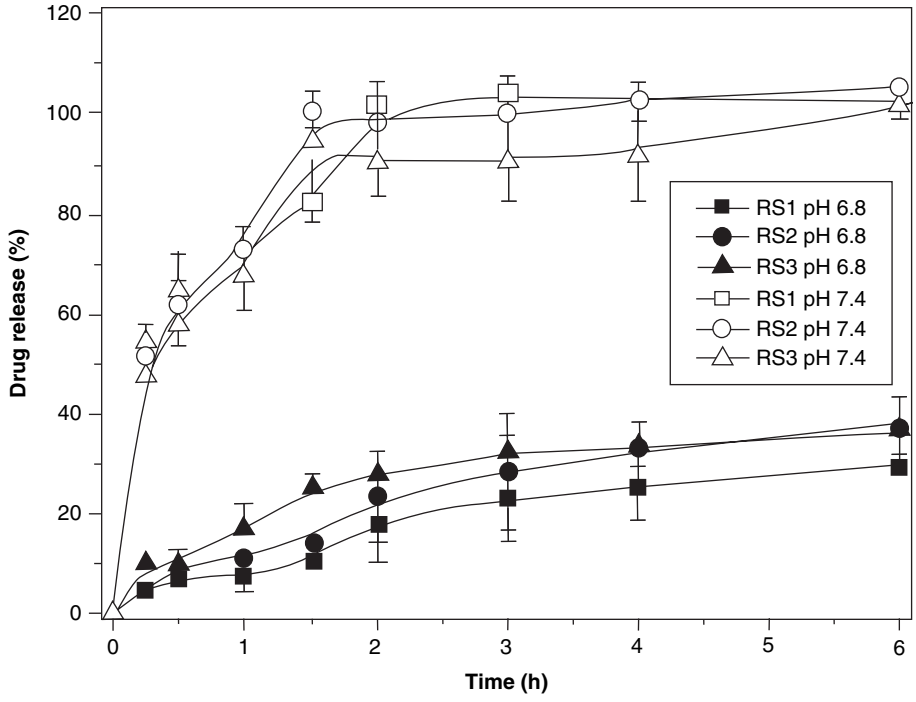

Figure 11. Cumulated 5-FU release versus time from microspheres composed of mixtures of Eudragit P4581F and Eudragit RS100, where RS1 represents the mixture 9:1, RS2 8:2, and RS3 7:3. All batches were tested in phosphate buffer systems of pH 6.8 and 7.4 (n = 3).
Reproduce with permission from [44].

the typical processes for nanoencapsulation are based on the affinity of the drug for the lipophilic phase of an emulsion or for the polymer.

Nanoparticle formulation of NAs offers numerous solutions to NA chemotherapeutic treatment problems. In particular, it provides NA sustained release, tumour targeting and improved cellular uptake because of their small size. Therefore, it would not only increase the NA therapeutic efficiency, but also would enable clinicians to reduce the amount of administered drug and hence minimize NA pronounced side effects [46,47].

Numerous polymers have been used or are available as matrices for nanoparticles. Most are biodegradable, for example polyesters such as poly(D,L-lactide), poly(D,L-lactide-co-glycolide), poly(orthoesters), polyanhydrides, poly(alkylcya noacrylates). Others are not-biodegradable, such as (methyl methacrylate), polystyrene and polyamide [48].

Most research performed on NA micro- and nanoencapsulation have been done with polyesters, especially PLGA. In fact, long experience of the use of co-polymers of lactic and glycolic acid has demonstrated their biocompatibility and biodegradation to toxicologically acceptable products. The second reason for their use for NA encapsulation is that particles of PLGA may be obtained by a solvent evaporation process, which is, in spite of some limitations, compatible with the handling of NAs. This has been demonstrated for 5-FU-loaded PLGA nanoparticles, which were prepared by a nanoprecipitation-solvent displacement technique [49]. Under optimized conditions, the encapsulation efficiency was as high as 78.30%, suggesting that 5-FU might be entrapped and adsorbed on the nanoparticle surface. *In vitro* drug release from the PLGA nanoparticles in phosphate buffered saline (pH 7.4) was suggested to be controlled by a combination of diffusion and slow and gradual erosion of the particles (**Figure 12**) [49].

In another study, efficient NA passive targeting to the intestinal mucosa, and a remarkably enhanced cellular uptake was possible using nanoparticulate systems prepared from poly(alkyl cyanoacrylate) by an emulsion-polymerization method. This is the case of poly(isohexylcyanoacrylate) nanospheres, which have been shown to be an efficient vector for the targeting of the antiviral NA, azidodeoxythymidine (AZT), to the gastrointestinal mucosa and the associated lymphoid tissues [50]. No similar example of anticancer NA-loaded nanoparticles is reported in the literature. In this study, the drug was added to the polymerization medium, resulting in encapsulation efficiency as high as 50%. The release profile was strongly dependant on the presence of esterases in the release medium. An initial burst release of 35% of loaded AZT was observed in water or in USP XIII simulated gastric medium, followed by a prolonged plateau

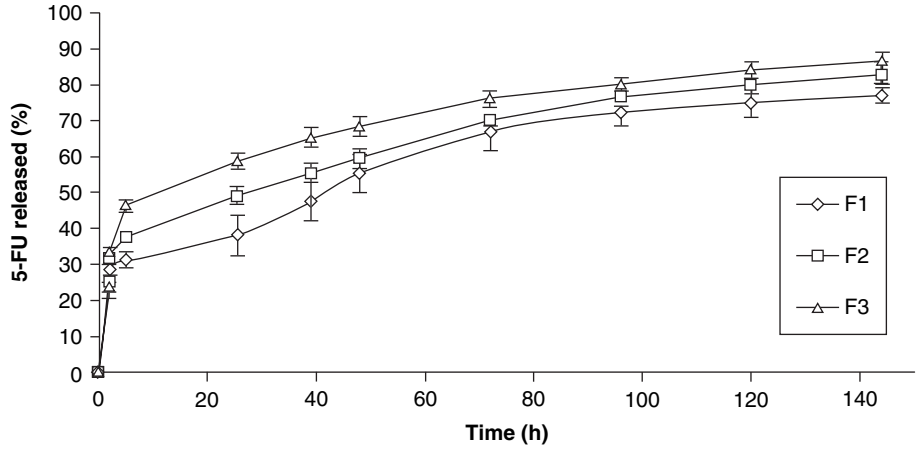

Figure 12. *In vitro* release of 5-FU from nanoparticles (n = 3).
Reproduced with permission from [49].

(40% released after 8 h). In the pancreatin-supplemented medium, AZT release was more progressive and reached almost 80% after 8 h, which could possibly be attributed to the progressive enzymatic degradation of the polymer by esterases contained in pancreatin [50].

Because of their specific affinity for the intestinal mucosa, poly(isohexylcyanoacrylate) nanospheres were able to concentrate at least 4.4- and 5.9-times more of AZT in the gastrointestinal tract compared with the free drug control solution after 30 and 90 min, respectively [50]. After oral administration, the poly(isohexylcyanoacrylate) particles were captured efficiently by the mucosa. This capture was due to the glycoproteic gel constituting the mucus, which acted as a porous adsorbent in which small nanospheres can diffuse and be immobilised until mucus renewal. In turn, because of immobilization, an increase in the contact time would result in an enhanced uptake.

In the emulsion polymerization method, less loading efficiency is generally obtained when the drug is added after the formation of nanoparticles, probably because only the surface of the polymer is available for drug adsorption. This was shown in the encapsulation of stavudine (an antiviral NA) in poly(butylcyanoacrylate) nanoparticles [51]. In this study, it was demonstrated that the larger the nanoparticles, the smaller the loading efficiency, as the specific surface area for stavudine loading of small particles is normally higher than that of large particles.

Magnetic nanoparticles represent very interesting carriers, allowing the active targeting of NAs to their site of action. Many attempts have been directed to this purpose. Gemcitabine-loaded magnetic nanocapsules have been successfully prepared, using poly(ethylcyanoacrylate) by an interfacial polymerization method [52]. The gemcitabine loading capacity was rather poor (9.37% w/w). An initial burst effect was observed in the early stage of the *in vitro* release study. This behaviour was probably due to the small amount of poorly encapsulated drug bound to the nanoparticle surface and/or to residual drug from manufacturing and handling. A smaller loading capacity for gemcitabine (7.6% w/w) has been achieved with poly(ε-caprolactone; PCL) magnetic nanospheres [53], even though similar release behaviour of gemcitabine from PCL nanospheres was observed.

NA tumour-targeting has also been approached, using pH-sensitive polymer nanoparticles because the environment of tumour cells shows a decreased pH value due to their hypoxic metabolism. Accordingly, pH-sensitive 5-FU nanoparticles were prepared to achieve selective drug release to tumour tissues [54]. These nanoparticles were synthesized from a polymer of amphiphilic nature (pullulan acetate/sulphonamide conjugate) by a diafiltration method. The nanoparticles showed good stability at pH 7.4, being equal to that of normal body fluid, but shrank and aggregated below pH 6.8, being close to tumour pH value. The release profile was heavily pH-dependent around physiological pH, and the release rate was significantly enhanced at pH values < 6.8.

5.6 Dendrimers

Dendrimers are emerging as a rather new class of polymeric nanosystems with increasing applications in drug delivery. These systems are built from a series of branches around an inner core, providing products of different generations, and offer intriguing possibilities in this regard. Dendrimers are synthesized from monomers using either convergent or divergent step growth polymerization. They can be synthesized from almost any core molecule and the branches are similarly constructed from any bi-functional molecule [55]. The distinctive architecture (star-shaped) of dendrimers has attracted interest in loading drug molecules to be either encapsulated into the interior of dendrimers or chemically attached or physically adsorbed onto the dendrimer surface, with the option to tailor the carrier properties to the specific needs of the active material and its therapeutic applications [56].

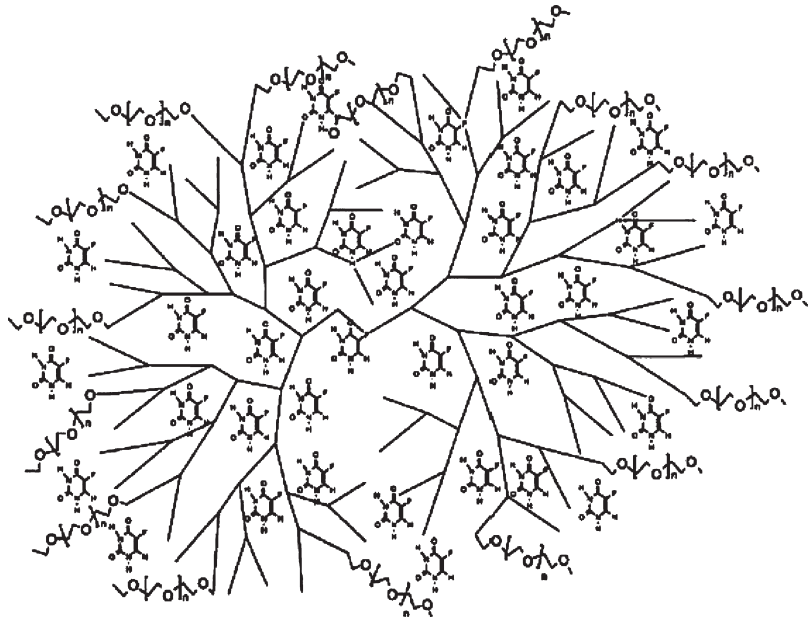

Figure 13. Schematic presentation of the encapsulation of 5-fluorouracil (right) into PEGylated third- and fourth-generation polyamidoamine dendrimers.

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Furthermore, the high density of surface groups allows the attachment of targeting groups, as well as groups that modify the solution behaviour or toxicity of dendrimers [57].

This technology was applied to NA delivery in an attempt to produce sustained release nanosystems. For this purpose, 5-FU was covalently attached to polyamidoamine dendrimers. Some of the NH_2 groups on the outer layer of dendrimers were acetylated. The acetylated dendrimers were then reacted with 1-bromoacetyl–5-FU to form dendrimer–5FU conjugates [58]. These conjugates release 5-FU on incubation in phosphate-buffered saline. This system may reduce 5-FU toxicity, due to slow release.

In a recent *in vivo* study, 5-FU was encapsulated into G4 polyamidoamine dendrimers with carboxymethyl PEG5000 surface chains (**Figure 13**). This system revealed reasonable drug loading, reduced release rate and hemolytic toxicity compared with the non-pegylated dendrimer [59].

In another study, cytarabine (Ara-C) was covalently linked to PEG. The hydroxyl functions of PEG were functionalised with a bicarboxylic amino acid, and Ara-C was conjugated directly to the peripheral carboxylic acid groups, providing the branching unit of the dendron [60]. This prodrug strategy was found to improve the blood residence time of the drug, to increase its stability towards degradation and to reduce the Ara-C toxicity when compared with the free drug.

Ara-C-loaded PEG–dendrimers hybrids can be synthesized in another way. The branching of termini can be accomplished via aspartic acid to form PEG–aspartic acid. Complete conjugation of dendritic acid with Ara-C has been achieved, via its amine group, by the use of spacers that allowed a greater separation of the branches to accommodate several large Ara-C molecules in proximity to one another [61]. Similar results were obtained for this Ara-C prodrug in terms of increased stability and reduced systemic toxicity, with one drawback: the low payload of the carried drug.

5.7 Polymeric micelles

Biodegradable colloidal nano-micelles are novel targeting drug delivery and controlled release systems, which could prolong the biological half-life and reduce the toxicity of NA anticancer agent, and offer acceptable biocompatibility [62].

5-FU has been entrapped into biodegradable nano-micelles formed from the amphiphilic copolymer polylactide-grafted dextrans (DEX-g-PLA), consisting of a hydrophobic block (PLA) and a hydrophilic block (DEX) [63]. Nano-micelles showed high stability both *in vitro* and *in vivo*. The encapsulating efficiency was ~ 9.3%. The 5-FU release from DEX-g-PLA nano-micelles was sustained for longer time than that of the naked drug. The *in vitro* inhibition rate of cell growth was similar in the 5-FU/DEX-g-PLA group and the naked 5-FU group; but interestingly, the *in vivo* inhibition rate of tumour growth was significantly higher in the 5-FU/DEX-g-PLA group than in the naked 5-FU group.

Drug release from nano-micelles is generally controlled by both drug diffusion and polymer degradation. Therefore, the molecular architecture of biodegradable polymers can be exploited to adjust polymer degradation and erosion rates. A series of four-armed block copolymers with different molecular weights and lactic acid/ethylene oxide ratio have been synthesized and evaluated as drug delivery nanocarriers for 5-FU and paclitaxel, and compared with nano-micelles derived from diblock and triblock copolymers [64]. The

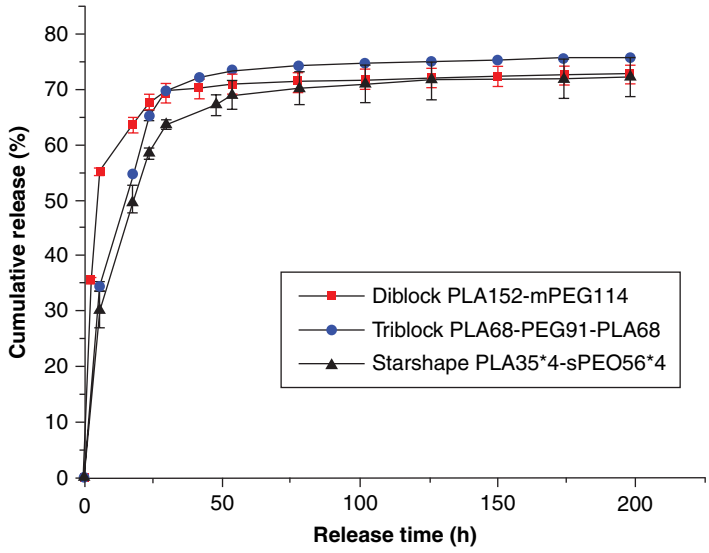

Figure 14. Release profile of 5-fluorouracil from nanoparticles of triblock PLA_{68}–PEG_{91}–PLA_{68}, diblock PLA_{152}–$mPEG_{114}$ and four-armed block copolymer PLA_{35*4}–$sPEO_{56*4}$ with a polymer:drug ratio of 10:2.
Reproduce with permission from [64].

micelles from the star-shaped branches showed more complete release of drug than the diblock copolymers; also, the lower hydrodynamic radius of star-shaped polymers may result in better clearance of the carrier polymer from the body. 5-FU was released *in vitro* over 300 h. Both diblock and star-shaped derived nano-micelles showed similar release patterns for 5-FU, with no control over the release (**Figure 14**).

5.8 Cyclodextrin – nucleoside analogue inclusion complexes

The ability of cyclodextrins to form inclusion complexes with many guest molecules by taking up a whole molecule, or some part of it, into their cavity places cyclodextrins in a unique class of encapsulation and controlled release techniques [65].

NA inclusion complexes are of interest in order to decrease the volume of the solution required for their administration and to improve their bioavailability, as with NAs with low water solubility, such as 5-FU (1% at room temperature) or NA hydrophobic prodrugs, and to increase their *in vitro* and *in vivo* stability. However, the complexation of NA molecules is difficult, as they lack hydrophobic groups that can interact with the cavity of cyclodextrin.

The preparation of inclusion complexes of 5-FU with β-cyclodextrin has been attempted, but no inclusion complex could be isolated, and the association constant was low [66]. However, 5-FU inclusion complex was successfully prepared using a cyclodextrin derivative bearing a free α-amino acid group [67]. The amino acid group might close the primary end of the cavity. Capping the primary alcohol side, or both sides, was shown in some cases to improve the inclusion property, explaining the possible inclusion of 5-FU. On the other hand, the preparation of inclusion complexes of NA hydrophobic prodrugs was easier. The inclusion complexes of a cytarabine mononucleotide prodrug were prepared, and their cytotoxic activities were investigated on leukemic murine cells [68]. Inclusion complexes exhibited more cyctotoxicity to leukemic cells resistant to Ara-C, compared with free Ara-C.

Table 1 shows an overall summary of the described NA delivery systems, with examples for each system.

6. Conclusion

The clinical use of nucleotide analogues in cancer treatment is limited by their poor stability in biological media, resulting in short half-lifes and low bioavailability. Furthermore, the important hydrophilic character of nucleotides also strongly limits their intracellular uptake, due to the low membrane permeability of these substances.

Many promising NA delivery systems are being developed, ranging from microcarriers, such as microparticles and DepoFoam particles, to nanocarriers, such as nanoparticles, liposomes, polyplex nanogel particles, dendrimers, polymeric micelles and cyclodextrin inclusion complexes. Exciting results can be obtained in terms of reduced systemic toxicity and improved efficiency, encouraging clinicians to investigate some of these systems in clinical trials, for example cytarabine-loaded DepoFoam particles (DepoCyt), which has been approved in several countries for the intrathecal treatment of lymphomatous meningitis. However, the problem of low drug loading still constitutes a major obstacle toward the clinical use of these NA-loaded carriers and, thus, requires more investigation in order to be overcome.

Table 1. Examples of recent research works in nucleoside analogue delivery systems.

Drug delivery system	Size	Nucleoside analogue molecule	Ref.	Advantages
Stealth® liposomes (ALZA)	50 – 250 nm	Gemcitabine Cytarabine	[20] [21]	Efficient encapsulation of all nucleoside analogue molecules (suitable for water-soluble drugs encapsulation); Effective reducing in system toxicity; Passive targeting
DepoFoam™ particles (SkyePharma)	1 – 100 µm	Cytarabine Fludarabine mono-phosphate	[23] [70]	Greater encapsulation efficiency than uni- and multi-lamellar liposomes; Greater stability *in vitro*; Sustained release for periods extending from a few days to a few weeks
Vesicular phospholipid gel	50 – 250 nm	Gemcitabine 5-Fluorouracil	[71] [72]	Higher stability *in vitro* than conventional liposomes
Polyplex nanogel particles	100 – 200 nm	Fludarabine tri-phosphate Azidothymidine tri-phosphate	[32] [33]	Encapsulation of tri-phosphate active form of nucleoside analogues; Overcome of drug resistance related to decreased nucleoside kinase activity
Microparticles	1 – 100 µm	5-Fluorouracil Cytarabine	[35] [36]	Sustain localized drug delivery for extended periods of time by controlling their size and porosity
Nanoparticles	< 1 µm	5-Fluorouracil Azidothymidine Stavudine Gemcitabine	[49] [50] [51] [52]	Sustained drug release, targeting the site of action, reduced toxicity, increased therapeutic efficacy
Dendrimers	< 10 nm	5-Fluorouracil Cytarabine	[59] [60]	Can be efficiently loaded by hydrophilic nucleoside analogue molecules, High density of surface groups allows attachment of targeting groups as well as groups that modify the solution behaviour or toxicity of dendrimers
Polymeric micelles	< 100 nm	5-Fluorouracil	[64]	Prolonged biological half-life, lightening of nucleoside analogue toxicity
Cyclodextrin inclusion complexes	< 10 nm	5-Fluorouracil	[67]	Controlled drug release; Higher *in vitro* and *in vivo* stability of the drug

7. Expert opinion

Despite the discovery of many new cytotoxic agents that are potential candidates for the treatment of cancer, this life-threatening disease still causes > 6 million deaths worldwide every year, and the number is growing.

Cytotoxic NAs and nucleobases were among the first chemotherapeutic agents to be introduced for the medical treatment of cancer, with potential activity in solid tumours and malignant disorders of the blood. However, various challenging problems with their clinical use have to be overcome. The most encountered difficulty toward an efficient clinical use of NAs is an adequate delivery of necessary therapeutic concentrations to the tumour target tissue. It is, therefore, of importance to develop novel micro- or nano-carrier technologies that can be used for targeted drug delivery to tumours and, thereby, improve the therapeutic index of the carried drugs.

In nearly all the carriers developed, researchers have been faced with general low NA-loading yields and an initial burst release effect (with the exception of DepoFoam particles), reaching a NA encapsulation efficiency of 82% and a prolonged release extending from few a days to a few weeks.

Several studies have approached NA targeting to cancerous tissues. Passive targeting using Stealth liposomes yielded a prolonged circulation time of these systems and thereby an enhanced cytotoxic activity. Active targeting using magnetic and pH-sensitive polymer nanoparticles achieved the selective delivery of NAs to targeted cancerous tissues and cells, respectively. Although promising results were obtained, many studies should be done to show the extent to which these delivery systems can be used for clinical applications.

From our point of view, an optimal NA carrier should meet the following criteria:

- Easy and efficient drug loading
- Safety, by excluding the use of toxic agents, such as monomers or crosslinkers of the formulation
- Nanoscale size, in order to facilitate an efficient NA transport into cells

- Efficient protection of encapsulated drug in biological media
- Possibility of vectorization of drug carriers for site-specific delivery
- Intracellular efficient drug release.

In this context, polyplex nanogels appear to be the most efficient NA carriers developed so far, allowing NAs to be administered in their active tri-phosphorylated form, thereby circumventing cellular resistance to NAs, being related to deoxycytidine kinase decreased activity. Their small size and the possibility to bind ligands to their surface should help solve the problem of poor cellular uptake and the poor tumour cell selectivity of NA, respectively. However, this developed system is based on the use of positively charged polymers, such as the polyethylenimine. The residence time of these positively charged complexes in the systemic circulation tends to be short, as interactions with plasma proteins lead to the formation of large polymer–protein aggregates, which are rapidly cleared from the bloodstream by phagocytosis.

DepoFoam particles constitute a safe NA carrier, with good drug loading, prolonged release and an efficient protection of the loaded drug, which is surrounded by numerous interconnected phospholipids bilayers. According to their large size, DepoFoam particles constitute a reservoir or a depot of NA and, thus, do not constitute a vector system capable of selectively targeting cancerous cells.

Nanoparticulate systems are vectors that could solve several problems related to NA treatments. More specifically, these systems provide NA tumour targeting and improved cellular uptake because of their small size. Therefore, they should not only increase the therapeutic efficiency of NAs, but also allow the amount of administered drugs to be reduced and, hence, minimize their side effects. Unfortunately, the important hydrophilic character of NA molecules and their low molecular weight result in poor drug loading and a burst release profile, as the nanoencapsulation process is based on the affinity of the drug for the lipophilic phase of the emulsion or for the polymer. Furthermore, nanoparticles prepared by an emulsion-polymerization method, although having good drug loading values, still contain traces of toxic initial monomers which are used in the preparation procedure, in spite of several steps of washing following nanoparticle preparation.

Other polymeric nano-sized systems, such as dendrimers and nano-micelles, are presently being developed on the basis of prodrug strategies and amphiphilic copolymers, respectively. Good results have been obtained in terms of increased stability and reduced systemic toxicity of the loaded NA. Nevertheless, the major limitation of this approach appears to be the low level of drug loading.

Although many of these carriers feature highly promising properties, only relatively few have been tested in clinical trials, and even fewer on the market. Clearly, this is because of the insufficient drug load, which might be sufficient for animal models, but certainly not for humans. Moreover, researchers should pay more attention to the fact that not all the animal studies performed, involving highly toxic anticancer agents, can be reliable to reflect expected drug responses in humans.

Nanoparticles prepared from biocompatible preformed polymers, such as PCL, PLA or PLGA could constitute an ideal NA delivery system, if the problems of poor drug loading and burst release are resolved. A few approaches are now available to circumvent these challenges. For example, the use of various water-soluble macromolecules in the formulation, such as dextran or chitosan, as with increasing the molecular weight of adjuvants, may retain NA small molecules within the aqueous compartment during nanoparticle preparation and thereby improve the encapsulation efficiency and slow drug release. This approach has been investigated by Hillaireau *et al.* [69]. In addition, polymeric micelles responsive to external stimuli, such as light, heat or ultrasound may exert the activity of the loaded drug in a site-directed manner, ensuring the effectiveness and safety of the nanocarrier-mediated targeting NA therapy, and thereby lower the necessary efficient doses and so the required drug loading. Thus, polymeric micelle-based nanocarriers could be promising, once applied to NA delivery. Furthermore, combining cancer imaging with targeted NA delivery would ultimately lead to a powerful system capable of identifying malignant cells, delivering necessary efficient NA therapeutic doses, and monitoring the extent of cell death in real time.

Clearly, the field of NA delivery systems is moving towards increasingly complex nanocarrier compositions, as well as sophisticated targeting and release devices, which many research teams, will undoubtedly pursue and hopefully achieve.

Acknowledgements

We would like to acknowledge the publishers, Elsevier, Springer Netherlands and ACS, as well as the authors who granted their permission to use previously published scientific data and schematics as referenced in the figure captions.

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