Expert Opinion

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Drug delivery vehicles with improved encapsulation efficiency: taking advantage of specific drug–carrier interactions

Katarzyna Kita[†] & Christian Dittrich[*]
[†]*Unilever R&D Port Sunlight, Bebington, Wirral, Wirral CH63 3JW, UK and* [*]*Harvard Medical School, Department of Cell Biology, Boston, MA 02115, USA*

Introduction: Drug encapsulation strategies are vital for the delivery of poorly soluble, fragile or toxic compounds. Increasing a drug's encapsulation efficiency in drug carrier particles can achieve a stronger therapeutic effect along with minimized side effects. For these reasons, new encapsulation methods are developed by using new materials and various types of drug–carrier interaction.

Areas covered: Strategies used for drug encapsulation are discussed in this review, focusing particularly on approaches leading to high encapsulation ratios resulting from specific interactions between the drug and the carrier. In the first part, classical encapsulation by hydrophobic self-assembly, its limitations and improvements are briefly discussed. Following this, encapsulation strategies for specific drugs are reviewed, where particular kinds of interaction play a role between the drug and the encapsulating material, which can lead to dramatically increased entrapment. Such specific approaches can be utilized more generically for various classes of molecules with similar properties, with regard to their ability to participate in a given kind of interaction.

Expert opinion: With the focus on delivering a high drug dose precisely to the site of action, high encapsulation efficiency is the first thing to consider in drug development. Academic research shows considerable interest in specific encapsulation, and it seems to be an established trend now to design drug delivery particles to achieve the most favorable properties. The authors believe the research in this area will focus on material properties and interactions between the drug and the carrier to ensure high drug loading into particles.

Keywords: drug encapsulation, drug–carrier interactions, encapsulation efficiency, specific interactions

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1. Introduction

Drug delivery has become a 'hot topic' in scientific research, particularly in the last decade – as evident from SciFinder™, the amount of literature devoted to this field increased dramatically from a mere 4100 reports (including both scientific papers and patents!) before 1990 to 83,000 between 2004 and 2006 alone. Recently, a steady slow-down has been observed in the publication output; however, this probably does not relate to the decreased importance of this area of research. In rich, ageing societies, the demand for new pharmaceutical solutions is increasing, and so are the efforts of many academic research groups and pharmaceutical companies to provide the best therapies and thus meet the needs of the market. This refers in

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Article highlights.

- Drug encapsulation is often necessary to improve solubility, reduce toxicity and protect the drug.
- Passive encapsulation approaches (e.g., liposomes) suffer from poor encapsulation efficiencies (~ 15%); improvements are possible by optimizing processing parameters.
- Loading efficiencies up to 100% are available by considering specific interactions between the drug and the carrier material.
- Covalent bonds and interactions such as hydrogen bonding and electrostatic are most commonly utilized for various classes of molecules.
- Complex formation is most common with metal-containing drugs or drugs rich in π-electrons; electrostatic forces are used for DNA encapsulation, and conjugates for protein delivery.

This box summarizes key points contained in the article.

particular to age- and civilization-related diseases such as diabetes, obesity, or cancer, just to mention a few. As could be expected, after the initial boost of academic research in the mid-2000s, mainly showing proofs of principle of new drug encapsulation strategies, we are now witnessing the 'translational' phase with more disease-focused applications and quite a few formulations in clinical trials [1].

It is, however, a long way before a drug can be sold in pharmacies. Assuming a successful drug discovery process, the issues of encapsulation, targeting, delivery and release need to be tackled, considering the pharmaceutical technologies and manufacturing capabilities available to industry. The complexity of this process has inevitably led to a situation where research teams most often focus on one or two specific problems from this list, partly because different competencies are required for individual subfields of the drug discovery and development research.

In this article, strategies for specific drug encapsulation are focused on. There are a few reasons why drugs may need to be encapsulated – in general, this is done to reduce the effect of their high toxicity, improve solubility, protect the drug from external conditions (pH, enzymes, oxidation), and finally to improve circulation time and availability. The encapsulating vehicle can at the same time act as a targeting platform, to help deliver the drug to specific cells in the body. Targeted drug delivery has been reviewed extensively [2,3] and is not discussed here.

The effect of a pharmaceutical depends on the applied concentration. The possibility to concentrate large amounts of a drug into small packages increases the potential application of the compound. It would be ideal if the encapsulation process led to concentration of the drug; however, 'passive' encapsulation is usually reported, which is frequently limited by the drug concentration in solution during the encapsulation process. On the other hand, specific interactions may exist between the drug and the encapsulating agent(s), which can be taken advantage of in order to increase the drug concentration in the vehicle. Obviously, to make sure that the drug is still able to dissociate from its encapsulating vehicle, the release process needs to be considered when applying this approach.

In the following sections, selected literature examples are discussed that use the specific encapsulation strategy by using different kinds of physical and chemical interactions. From this pool, passive encapsulation by self-assembly that uses hydrophobic interactions (leading to micelles, vesicles and solid particles) is mentioned first. This approach suffers from relatively poor encapsulation efficiencies [4], mostly limited by the drug concentration in solution; however, various improvements have been reported in the literature and are discussed briefly here. The 'specific' encapsulation approach always depends on the pharmaceutical agent; however, classes of drug molecules with similar properties (solubility, charge, ability to form complexes or hydrogen bonds) can benefit from generic encapsulation platforms. These are described in the second part, and the subsections discuss different types of interaction (and classes of molecules that can be involved) and how these can be utilized in drug delivery research.

2. Passive encapsulation

The use of liposomes (spherical lipid bilayers encapsulating an aqueous volume) for medical applications, and in particular for drug delivery, was reviewed as early as in the 1980s [5], and their use in treatment of many diseases had been envisaged quite some time ago [6,7]. It is worth noting that cancer therapy (chemotherapy) solutions using liposomes have been investigated for more than two decades now [8]. This resulted in commercial solutions utilizing micelles, liposomes and colloidal particles; the first liposomal drug formulation for cancer therapy (Doxil) was approved in 1995 [9].

As mentioned earlier, drug encapsulation efficiencies by the passive, hydrophobic self-assembly approach within lipid or polymer particles are fairly poor, especially for water-insoluble drugs, which, when encapsulated in liposomes, have to be associated to the vesicle membrane [10,11]. Therefore, continuous efforts in this field are being devoted to the efficient encapsulation of hydrophobic drugs. In general, this is done by using lipids or polymers, with polarities corresponding best to the polarity of the drug, which ensures maximum synergy during the nano/microprecipitation process, and thus efficient drug entrapment. The solubility parameter (Hildebrand or Hansen) is often used as a helpful estimate of the 'likeness' of the two materials (drug and carrier). Other solutions are also studied, most commonly optimization of process parameters and modifications of the carrier material. A brief summary of the examples discussed below is provided in **Table 1**.

Table 1. Comparison of improvements to passive encapsulation strategies.

Encapsulant/conditions	Encapsulated drug (compound)	Encapsulation efficiency	Ref.
Egg PC (phosphatydilcholine) + freeze-thawing	Hydrophilic AChE	> 40%	[13]
DMPC, DPPC, HSPC, cholesterol; with $NiSO_4$ > 50°C	Mitoxantrone	> 95%	[14]
PLGA + salt; solvent	Leuprolide acetate	Up to 100%	[15]
PLGA, conjugated to poly-L-lysine + homogenization and sonication	Plasmid DNA	> 90%	[16]
PLGA/various processing	Vincristine sulfate/quercetin	93/33%	[17]
PLGA/various processing	Vincristine sulfate/verapamil hydrochloride	55/70%	[18]
L-α-lecithin-cholesterol-dicetylphosphate	Doxorubicin	Up to 50%	[19]
PEG-PLA block copolymers and oleic acid calcium salt	Cyclosporin A; testosterone enanthate; betamethasone 17-valerate; betamethasone 17,21-dipropionate	Up to 100%	[20]
Hyperbranched poly[(amine-ester)-*co*-(DL-lactide)]	Bovine serum albumin	Up to 60%	[21]
Chitosan-*graft*-polycaprolactone	7-Ethyl-10-hydroxy-camptothecin	Up to 85%	[22]
Polyacrylamide + crosslinking	5-Fluorouracil	Up to 80%	[23]
Pluronic L121 micelles in chitosan-based strip, epoxy-crosslinked	Rapamycin	Up to 95%	[24]
Photo-crosslinked PEG hydrogels	DNA	Up to 75%	[25]
PEG-*block*-poly (ε-caprolactone); worm micelles	Paclitaxel	Up to 65%	[26]
Biopolyester micelles and nanoparticles	Taxanes	Up to 80%	[27]

AChE: Acetylcholinesterase; DMPC: 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine; DPPC: 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine; HSPC: Hydrogenated soybean phosphatidylcholine; PLGA: Poly(lactide-*co*-glycolide); PEG: Poly(ethylene glycol); PLA: Polylactide.

2.1 Process parameters

A very important issue to consider with passive encapsulation is the processing parameters, which can dramatically change the encapsulation efficiencies. Most often investigated are the conditions in solution during particle preparation, including concentrations, pH, temperature, but also stirring rate, sonication and process technologies, as reviewed recently [12].

As shown by Chaize *et al.* [13], it appears that the conditions for the preparation of a liposomal formulation (in this case enzymes were encapsulated) dramatically influence the entrapment efficiency and enzyme activity. Obviously, the increase of lipid concentration leads to the production of a larger number of particles and thus more enzymes will be encapsulated. However, the number of freeze–thaw cycles plays a role too by transforming multilamellar vesicles into unilamellar vesicles, thereby increasing the encapsulated aqueous volume.

Temperature and transmembrane ion gradient as process parameters were found to play a role on encapsulation of mitoxantrone, an antineoplastic agent (used in the treatment of multiple sclerosis, advanced hormone-refractory prostate cancer and acute non-lymphocytic leukemia) [14]. Transmembrane $NiSO_4$ gradient was applied, and despite the fact that Ni^{2+} cannot bind to the drug in solution, it was found to mediate the encapsulation and significantly improve the drug entrapment. The detailed mechanism of this process was not elucidated; however, UV-VIS spectroscopy suggests that a particular kind of a Ni–drug complex may be present, which is stable and membrane-permeable. As expected, increased temperature leads to liposome destabilization and thus lower encapsulation efficiencies.

Precipitated particles, for example poly(lactide-*co*-glycolide) (PLGA) microparticles, prepared by the co-solvent evaporation method are frequently cited as promising materials for drug delivery [15-17]. Precipitation parameters, such as concentration of added salt and the kind of solvent used (in the suspending phase), have been shown to increase encapsulation efficiency of leuprolide acetate, a synthetic peptide analogue of gonadotropin-releasing hormone [15]. Among other process variables studied, it has been shown that the increase in encapsulation by adding sodium chloride is a result of the increased osmotic pressure of the external phase, leading to denser particles. Depending on the solvent combination used for particle preparation, faster or slower precipitation can be achieved, resulting in dense or porous particles, leading to different drug encapsulation efficiencies.

The influence of various processing variables on dual drug encapsulation was investigated by Song and co-workers [17,18]. These authors showed that encapsulation ratios of hydrophilic/hydrophobic and hydrophilic/hydrophilic drug pairs can be successfully increased by optimizing the lactide-to-glycolide ratio of PLGA, initial drug content, solvent volume ratio, the volume of organic solvent, and pH of the aqueous phase. The process essentially relies on the principles of emulsification in in an oil-water system upon solvent exchange, and could be principally optimized for various drug molecules. On the other hand, encapsulation efficiencies do not exceed 70%. Although this is quite high for a passive precipitation process, it can be improved by utilizing more specific interactions.

2.2 Encapsulating material

The particle-forming material can determine the efficiency of drug encapsulation and the release properties. In liposomal encapsulation, drug loading efficiency can be increased, for example, by changing the content of cholesterol in liposomal membranes. This was demonstrated in doxorubicin encapsulation [19]: doxorubicin interacts strongly with phospholipids, and thus it can be expected that different membrane composition will affect liposome loading. In fact, doxorubicin is thought to associate to liposomal lipid bilayers at least partly as a result of electrostatic binding; however, higher encapsulation ratios at smaller cholesterol contents suggest that hydrophobic interactions between the drug and the lipid prevail in this case.

The combination of block copolymers and fatty acid salts has been shown to produce better encapsulation results for poorly water-soluble drugs compared with single-component block copolymer micelles [20]. For a spectrum of drugs including immunosuppressants, anti-inflammatories and hormones, calcium oleate showed a stronger solubilizing effect than methoxy-polyethyleneglycol-polylactide (PEG-PLA). Interestingly, drug encapsulation in mixed particles does not seem to correlate with the drugs' oil/water partition coefficients, which suggests that the encapsulation process depends on multiple parameters and that drug molecules may be located at various positions in mixed micelles. Finally, mixed fatty acid–polymer micelles showed higher drug entrapment efficiency than polymeric micelles, and could also be stabilized by the addition of calcium (calcium oleate adds rigidity to the micellar structures).

For copolymers, enhanced encapsulation can be also achieved by using different polymer architectures. This was demonstrated for hyperbranched poly[(amine-ester)-*co*-(DL-lactide)] (HPAE-*co*-PLA) encapsulating bovine serum albumin (BSA) as a model protein [21]. In particular, higher molecular mass of the polymer leads to increased viscosity, inhibiting BSA diffusion, which may be beneficial for encapsulation efficiency. On the other hand, the two polymer blocks have different interactions with the protein, and thus counter-play during the encapsulation process (nevertheless, the entrapment efficiency increased with increasing mass of PLA). The particles were prepared by the solvent evaporation method, for which improved encapsulations were again observed for increasing copolymer concentration in acetone, showing a dependence on the BSA concentration at the same time.

Duan *et al.* used the lyophilization method to encapsulate 7-ethyl-10-hydroxy-camptothecin (SN-38; an antitumor drug) in micelles from cationic chitosan-graft-polycaprolactone (CS-*g*-PCL) copolymers [22]. The micelle loading increased strongly (from 64 to 85%) with increasing amount of grafted PCL (1:8 to 1:24), showing that a change in the structure of the encapsulating material plays a role in the entrapment process. Mechanistically, it is not quite obvious what the exact molecular packing is within drug-loaded particles. These should be studied whenever possible, to enable more rational drug carrier design.

Among many attempts to optimize the drug carriers to achieve the best encapsulation results, chemical crosslinking was investigated [23]. Specifically, for polyacrylamide particles loaded with 5-fluorouracil, a chemotherapy drug, encapsulation can be regulated by using a polymer crosslinking agent *in situ*, such as *N*,*N*-methylene-bis-acrylamide or ethylene glycol dimethacrylate. It is found, however, that the addition of a crosslinker improves the encapsulation only for a large amount of added drug. For small drug amounts, slow diffusion and increasing particle density do not seem to favor drug entrapment. A different strategy was presented by Chen *et al.* [24], who encapsulated lipophilic drugs in the hydrophobic micelle core, and further immobilized the micelles in a chitosan matrix, also crosslinked by ethylene glycol diglycidyl ether (an epoxy compound). The crosslinking was used mainly to ensure the stability and a required release profile of the drug, yet the micelles embedded in the hydrogel nevertheless provide very high entrapment efficiencies.

For DNA delivery, photo-crosslinked polyethylene glycol-based hydrogels were applied [25]. These authors used polyester–polyethylene glycol block copolymers and, depending on the polymer chemistry, observed various DNA release profiles. The crosslinking method did not compromise the DNA integrity and activity (the ability of the plasmid to produce the encoded protein); however, the actual efficiency of DNA entrapment is not discussed.

2.3 Other factors

In addition to the material properties and particle processing during the encapsulation process, it has been found that the morphology of the resulting particles has a major influence on encapsulation efficiency. The authors mentioned uni- and multilamellar lipisomes were mentioned before, but when considering polymer particles, other shapes are available, such as worm-like micelles, branched micelles, and so on. For example, PEG-*b*-PCL worm-like micelles possess a larger cargo space per particle and are able to solubilize twice as much paclitaxel compared with spherical micelles [26].

Other factors influencing drug incorporation in particles, such as chemical composition of the core-forming polymer, polymer–drug compatibility as well as physical state of the micelle core, were reviewed recently by Gaucher *et al.* [27]. These authors discuss taxanes encapsulated in polyester-based particles, yet the physical principles and hydrophobic interactions can be translated to different drugs and encapsulating agents.

In summary, passive drug encapsulation is a process that depends on many variables, and is mostly dictated by the particle formation process. Self-assembly of amphiphilic molecules such as lipids or polymers allows for production of different types of particle, and drug loading can be improved by optimizing processing parameters or the material properties (molecular mass, polymer architecture). Specific interactions

between the drug and the carrier material offer the promise of more efficient encapsulation: selected examples are discussed in the following section.

3. Specific drug encapsulation

Specific encapsulation of cargo compounds is driven by high specificity between the loaded drug and its container. This may include donor–acceptor (coordination complex formation, where the donor, also known as ligand, shares its electron pair), electrostatic attraction, covalent bonding and hydrogen bonding, and ultimately result in higher drug encapsulation efficiencies. Practically, they will very frequently be linked and appear simultaneously with hydrophobic interactions. There may also be examples where one drug is capable of interacting with the carrier in many ways: this property allows for using various carriers that will be equally efficient at encapsulating the same molecule. Although extremely specific, biological ligand–receptor interactions are not utilized for drug encapsulation, but are, however, quite common for particle targeting in the drug delivery process.

Below, interesting literature examples that utilize these interactions, leading to drug carriers containing very high payloads, are reviewed briefly. Although the section is structured by the type of interactions, it should be remembered that they are closely related to the types of drug molecule that can, owing to their chemical structure, form a particular kind of bond or complex.

3.1 Complex formation

Coordination complexes are usually formed by metals and inorganic or organic ligands that donate an electron pair. The complex-forming properties of transition metals are particularly interesting for encapsulation purposes: platinum compounds, for example, are established in therapy of various cancers.

The use of electron donor–acceptor interactions to improve liposome loading was reported in the 1970s [28], where charge transfer complexes were formed between 8-azaguanine (leukemia drug) or 6-mercaptopurine (immunosuppressant) and chloranil (see **Figure 1** for chemical structures of the drugs). The introduction of chloranil leads to some improvements in encapsulation efficiencies for both drugs at pH 7: from 0.2 to 31% for 6-mercaptopurine and from 0.9 to 5.7% for 8-azaguanine. Advantageous for drug delivery applications is the fact that the complexes decompose to their donor and acceptor parents on uptake; however, toxicity of chloranil does not allow for their pharmaceutical use. Nevertheless, the paper demonstrates very clearly how specific interactions can boost encapsulation efficiency.

Kataoka *et al.* reviewed the formation of block copolymer micelles [29], considering also medical applications such as drug delivery. In this context they (Kataoka *et al.*) pointed out that structure matching between the polymer and the drug should be taken into account. One example includes doxorubicin encapsulation in poly(ethylene glycol)–poly (*b*-benzyl-L-aspartate) micelles, where π–π interactions have a stabilizing effect on the micelles and the drug itself [30]. It should be mentioned here that often one drug is able to build various interactions with the carrier. For example, doxorubicin can be stabilized by complex formation, but it can also be delivered in the form of conjugates with polymers [31].

Metal complex formation is discussed considering *cis*-diamminedichloroplatinum(II), known as cisplatin. This well-established cancer drug is a platinum complex, in which, for the encapsulation process, chlorine ligands can be replaced with a polymer's carboxylate groups. This leads to a stable formulation from which cisplatin can be recovered in physiological conditions. Such a strategy is supposed to overcome the solubility problems of cisplatin and improve encapsulation efficiency (versus, for example, passive liposomal encapsulation that does not exceed 18% [8]).

An interesting approach using complex formation to achieve increased drug stability in conventional liposomes was presented for indium and gadolinium-based drugs [32]. Briefly, empty liposomes are loaded first with the chelating agent, and just before administration the drug is formed *in situ* by incubation with another metal complex of smaller binding constant. This leads to 90% encapsulation of ^{111}In [33] and ^{67}Ga [34] in liposomes.

Host–guest complexes were prepared using cyclodextrins for encapsulation of oxaprozin, an anti-inflammatory drug [35]. Even though the complexes are formed by using physical methods, their stoichiometry (1:1) and stability constants suggest high specificity owing to the size compatibility of the guest molecule and cyclodextrin cavity. Complex formation between drugs and carriers is not reported extensively in the literature; the above are a few selected examples where improved encapsulations were achieved. This approach, however, seems promising for the encapsulation of enzymes possessing metal active sites, and the delivery of compounds rich in π-electrons.

3.2 Electrostatic interactions

3.2.1 Encapsulation of genetic material

Electrostatic attraction, apart from hydrophobic forces, is by far the most widespread drug–carrier interaction leading to enhanced drug entrapment [36]. Recently, particular attention has been devoted to gene delivery, as oligonucleotides can be 'compacted' by polyelectrolytes, especially polymers [37,38], and the resulting particles can subsequently be delivered to cells. For example, block copolymer micelles from PEG-polylysine have been shown to compact DNA in their collapsed cores [29]. At the same time, PEG is a strongly hydrophilic polymer and protects the micelles from unwanted interactions with proteins in the bloodstream, thus increasing the systemic circulation time. It was noted that the stability of polymer/DNA complexes influenced the degradation rate of DNA, which dramatically decreased after complexation with the block copolymers. Also, the degradation rate of DNA

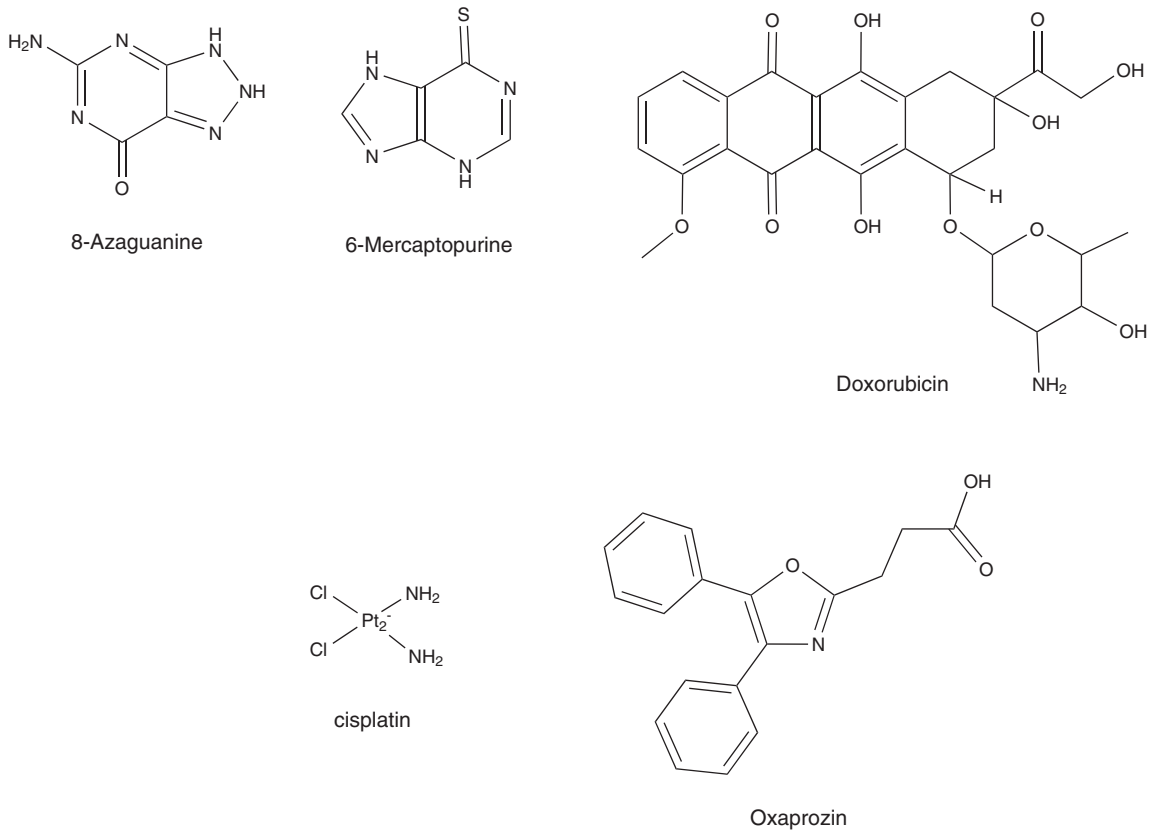

Figure 1. Drugs encapsulated by the formation of coordination complexes.

decreased with an increase in the degree of polymerization of the polylysine segment.

Polyethyleneimine (PEI), conventionally used for complexing DNA, can be used in its hyperbranched form to improve particle loading [12]. The possibilities to regulate the molar mass, introduce functional groups and control the degree of functionalization also broaden the applicability of PEI polymers in the gene delivery field – these may be important for cell targeting and transfection. On the other hand, the capacity of cationic polymers to associate and co-precipitate with DNA increases with the polymer's molecular mass, which also increases the cytotoxicity of these delivery platforms. For that reason, block copolymers of PEI with poly(L-lactide), poly(ethylene glycol) and poly (*g*-benzyl-L-glutamate) are investigated with the hope of reducing the toxicity.

Oligonucleotides, owing to their relatively small molecular mass, pose a problem when encapsulated in polyionic micelles as they diffuse out at fast rates. The attempts to encapsulate and stabilize oligo-DNA species so far have involved micelle crosslinking, for example by disulfide bonds that can dissociate after the micelle has reached its delivery destination [29]. Semple *et al.* [39] used ionizable aminolipids as an alternative to cationic lipid to produce liposomes with improved encapsulation ability. Cationic liposomes, apart from their toxicity, have unfavorable clearing properties in the bloodstream and show poor accumulation in diseased tissues. The particles described here, in turn, are cationic at the time of preparation at acidic pH, yet remain neutral at the time of administration (at physiological pH). It is shown that using 1,2-dioleoyl-3-dimethylammonium propane (DODAP) markedly changes the encapsulation of oligonucleotides from a mere 5% at 0% DODAP to 80% at 30% DODAP in the lipid mixture at pH 4.

Plasmid DNA encapsulation at 42% was achieved using liposomes composed of cationic (low amount) and fusogenic (high amount) lipids [40]. The molar ratio of DNA-to-lipid was adjusted to yield polyplexes with net negative charges, where encapsulation takes place by Coulomb attraction, whereas the transfection properties are provided by the excess amount of 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE). In such a system, the authors were able to decrease the liposome size (compared with commonly used Lipofectamine) in order to improve particle circulation time and accumulation in tissues. In a different study, modification of PLGA with positively charged polylysine led to particles with plasmid DNA encapsulation efficiency of 90% [16]. For structural stabilization, particles were prepared from mixtures

of PLGA and PLGA–polylysine, the ratio of which also influenced the release kinetics.

Apart from genetic material, charged drug molecules can be very efficiently encapsulated by electrostatic attraction with the carrier. A few examples are discussed below, and the drug structures are shown in **Figure 2**.

3.2.2 *Negatively charged drugs*

Retinoic acid is an example of a negatively charged drug: it is used to treat skin conditions such as acne, and is known commonly to cause skin irritation. To reduce this side effect, Castro *et al.* prepared nanoparticles containing cholesterol and stearylamine, encapsulating high payloads of retinoic acid by ionic interactions [41]. The addition of positively charged stearylamine remarkably improves the entrapment from 13% (without stearylamine) to > 90%. As with other examples, the ion pairing increases the lipophilic properties of the drug, making it easier to incorporate into the lipid matrix. This also improves the stability of nanoparticles, and the formulation produces reduced skin irritation in mice as compared with commercial drugs.

Methotrexate (MTX) is a negatively charged antimetabolite and antifolate drug. Its most common uses include the treatment of cancer and autoimmune diseases, and it is also used for medical termination of pregnancy. The drug was recently encapsulated in inorganic nanolattices (layered double hydroxides [LDH]) from sodium, magnesium and aluminum [42]. Electrostatic stabilization of the drug has been demonstrated by Fourier transform infrared (FTIR) spectroscopy. No analysis of the encapsulation efficiency has been presented, but nevertheless it has been shown that this approach allows the drug resistance in MTX-resistant cells to be overcome, helps inhibit cancer cell proliferation, and improves cellular uptake and intracellular retention.

3.2.3 *Positively charged drugs*

Encapsulation of cationic drugs such as cisplatin (**Figure 1**) can be improved by using anionic liposomes containing phosphatidylserine [8]. In this case, electrostatic interactions were demonstrated by zeta potential measurements that showed differences depending on whether liposomes were incubated with mannitol or NaCl. In mannitol, where positively charged platinum complexes are present, zeta potential increased compared with control liposomes. Moreover, it was more difficult to recover cisplatin from this batch compared with NaCl-incubated vesicles, which contained neutral platinum species.

Cationic propranolol, a beta blocker also used in the treatment of hypertension, was condensed in calcium alginate beads using negatively charged magnesium aluminum silicate (MAS) [43]. It was shown that with increasing amounts of MAS the encapsulation efficiency can reach above 60%, versus ~ 15% without MAS. The formation of drug–MAS electrostatic complexes formed in the dispersion enhances the drug entrapment efficiency, and also, thanks to the presence of sodium alginate, drug loss from the beads is reduced. Inversely, the encapsulation reversely depends on the calcium chloride concentration in solution, showing that the bead formation process is sensitive to the preparation conditions, but can be optimized to achieve high entrapment ratios.

3.2.4 *Zwitterionic and amphiphilic drugs*

When encapsulating tioguanine, an amphiphilic drug used for treatment of leukemia, charge–charge interactions proved to influence markedly the encapsulation efficiency, in addition to other factors such as pH and sonication time [44]. It was shown that tioguanine in its zwitterionic form interacts with charged and zwitterionic lipids when encapsulation takes place in liposomes. For a membrane-inserting drug as described here, the charge displayed by the liposomal membrane is an important physicochemical parameter regulating the insertion process in membranes, and thus encapsulation efficiency.

Hydrophobic drugs, such as cyclosporine A (an immunosuppressant), can be encapsulated at high rates using polyelectrolytes such as polyethyleneimine-based polymers [45]. Here, the drug itself is not charged, and rather becomes stabilized in the polymer micelles by hydrophobic interactions; however, the charged moieties (quaternary ammonium groups) massively stabilize the internal micelle core networks, yielding particles with high encapsulation efficiencies and enhanced oral absorption. On a similar note, weak self-assembling polyelectrolytes (containing poly[2-(diisopropylamino)-ethyl methacrylate] as the core-forming block) show considerable improvements in encapsulation of charged, hydrophobic drugs containing carboxylic groups [46]. Acid–base pairing leads to almost 100% w/w drug entrapment, compared with controls not displaying such specific interactions that show only ~ 10% encapsulation. Polyacrylic acid contained in multicomponent polymer networks was also shown to help increase encapsulation of vancomycin, a glycopeptide antibiotic [47].

3.2.5 *Proteins*

Pharmaceutically active proteins are something of a challenge for drug delivery research. The encapsulating system must ensure the delivery of proteins in their native folding state to sustain their biological activity. Also, maintaining the structure and thus activity needs to be taken into account if the protein is going to have any pharmaceutical activity at the site of action. As reviewed by Yeo and Park [48], charged polymers seem to perform better as encapsulating agents for proteins compared with end-capped polymers, as long as electrostatic interactions are concerned in the encapsulation process. As an alternative, proteins (enzymes) can be used as building blocks for polyion micelles, which facilitate their shielding and further delivery [29]. Block copolymers containing polyaspartate were used as carriers to entrap

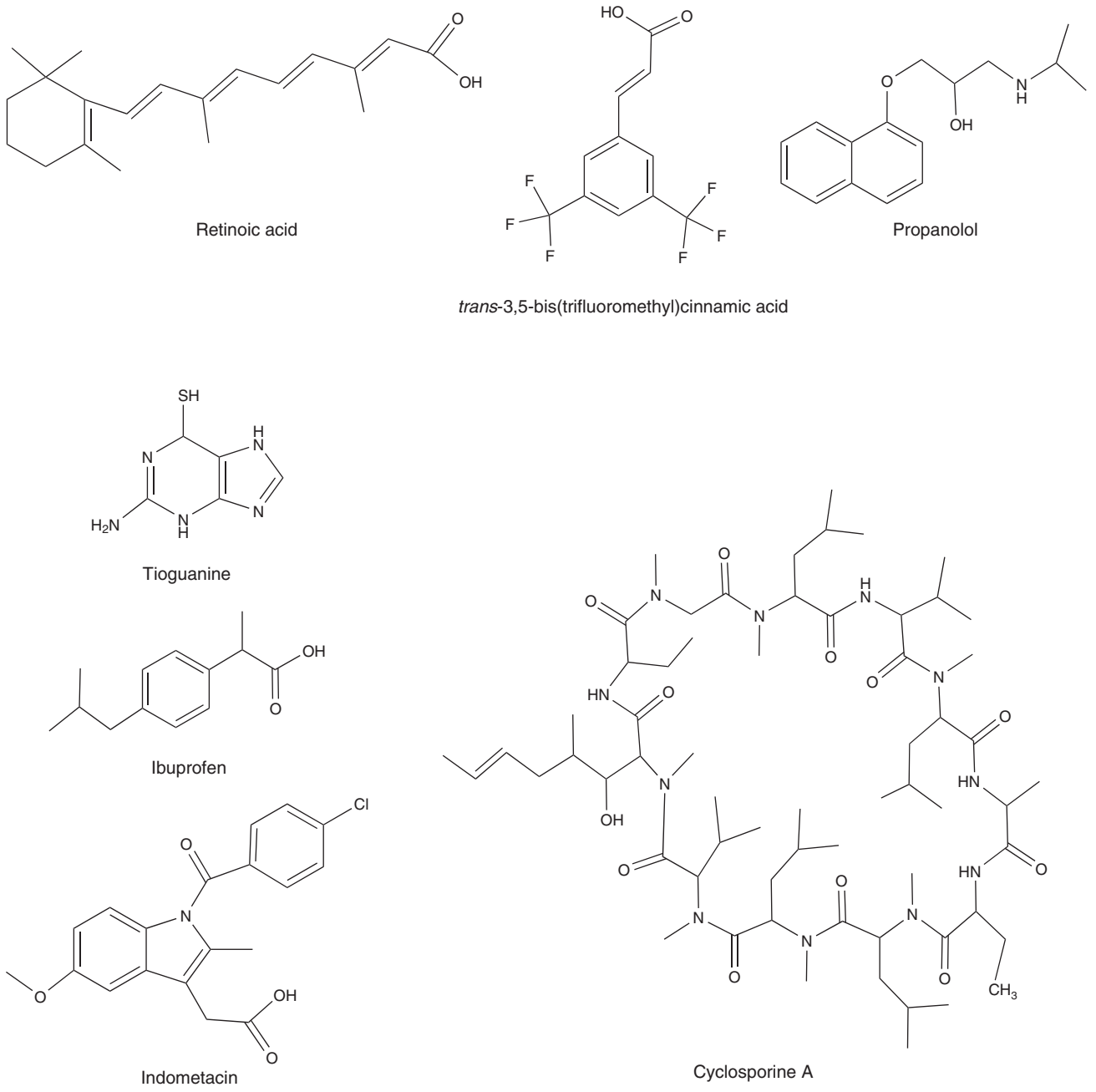

Figure 2. Charged drugs, encapsulated by electrostatic interactions with the carrier.

lysozyme successfully, without affecting its enzymatic activity. In fact, the authors observed increased activity of the enzyme within the micellar core, which is explained by the increased substrate concentration in the micelles. On the other hand, polyethylene oxide corona of the aggregates prevents undesired effects such as lysozyme degradation in cells – the release can be fine-tuned by pH and salt concentration in the surrounding medium, to which electrostatically bound micellar cores respond very strongly.

3.3 Covalent bonding

Covalent attachment of drugs to carrier materials is commonly known as the drug-conjugate approach, and used mostly for the purpose of increasing the solubility and circulation time, and reducing drug toxicity in the body. The drug release from such conjugates proceeds by means of, for example, hydrolysis or enzymatic cleavage of the covalent bond. However, it has been pointed out [36] that the number of drugs for which this method can be applied is limited

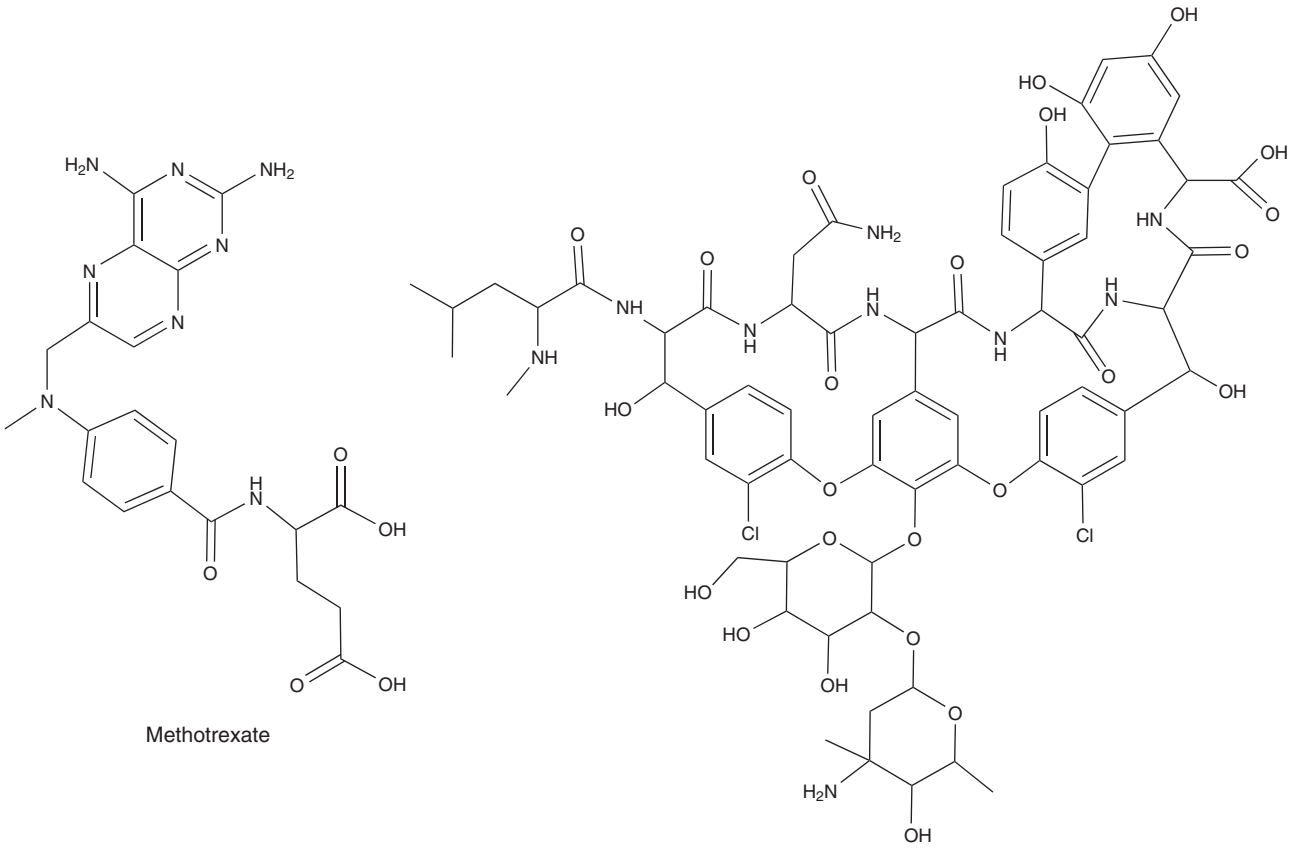

Figure 2. Charged drugs, encapsulated by electrostatic interactions with the carrier (continued).

owing to the required (and often difficult) chemical synthesis. Nevertheless, the pharmaceutical industry follows this approach for drugs that would not be deliverable otherwise [49]. The relevant conjugation strategies have been reviewed in the literature previously [50-53].

For covalently bound drug-carrier conjugates, the encapsulation efficiency can be discussed only when particles are formed, which is not always the case. Non-particle conjugates can still be used owing to the aforementioned benefits and, sometimes, targeting properties (see **Figure 3** for the molecular structures of selected drugs that were delivered by conjugation to the carrier). For example, Meijer *et al.* [54] used the low-molecular-mass protein lysozyme as a carrier for delivery of various drugs, including antibacterial agents and albumins derivatized with various sugars for delivery of the anti-inflammatory drug naproxen. Depending on the attached sugar, they observed specific delivery to various types of cell.

Poly(ethylene glycol)–poly(α,β-aspartic acid) block copolymer was conjugated with doxorubicin [29] by condensation of the acid's carboxylic groups with the drug's glycosidic primary amino groups. This resulted in the attachment of doxorubicin molecules to ~ 50% of aspartic acid monomers, and changing the properties of the poly(α,β-aspartic acid) block to hydrophobic, which resulted in micelle formation in aqueous medium. pH-sensitive release from doxorubicin-polymer conjugates was also achieved by using poly(ethylene glycol)-poly-aspartate, to which the drug was attached by hydrazone linkers [31]. Similarly, paclitaxel [55] and haloperidol [56] have been covalently conjugated to terminal groups of polymers such as PLGA or PEG-PLA/PLGA, forming particles with slow release properties. Regarding peptide and protein drugs, the conjugate formation with shielding polymers, which probably also play a role in structure preservation, a few are already present on the market or in clinical trials [49]. The most commonly used polymer here is polyethylene glycol, yet new formulations using dendrimers have also been presented, as discussed below.

A few reports discuss the successful use of dendrimers for drug conjugation [57]. In particular, conjugation of trastuzumab (a monoclonal antibody against epidermal

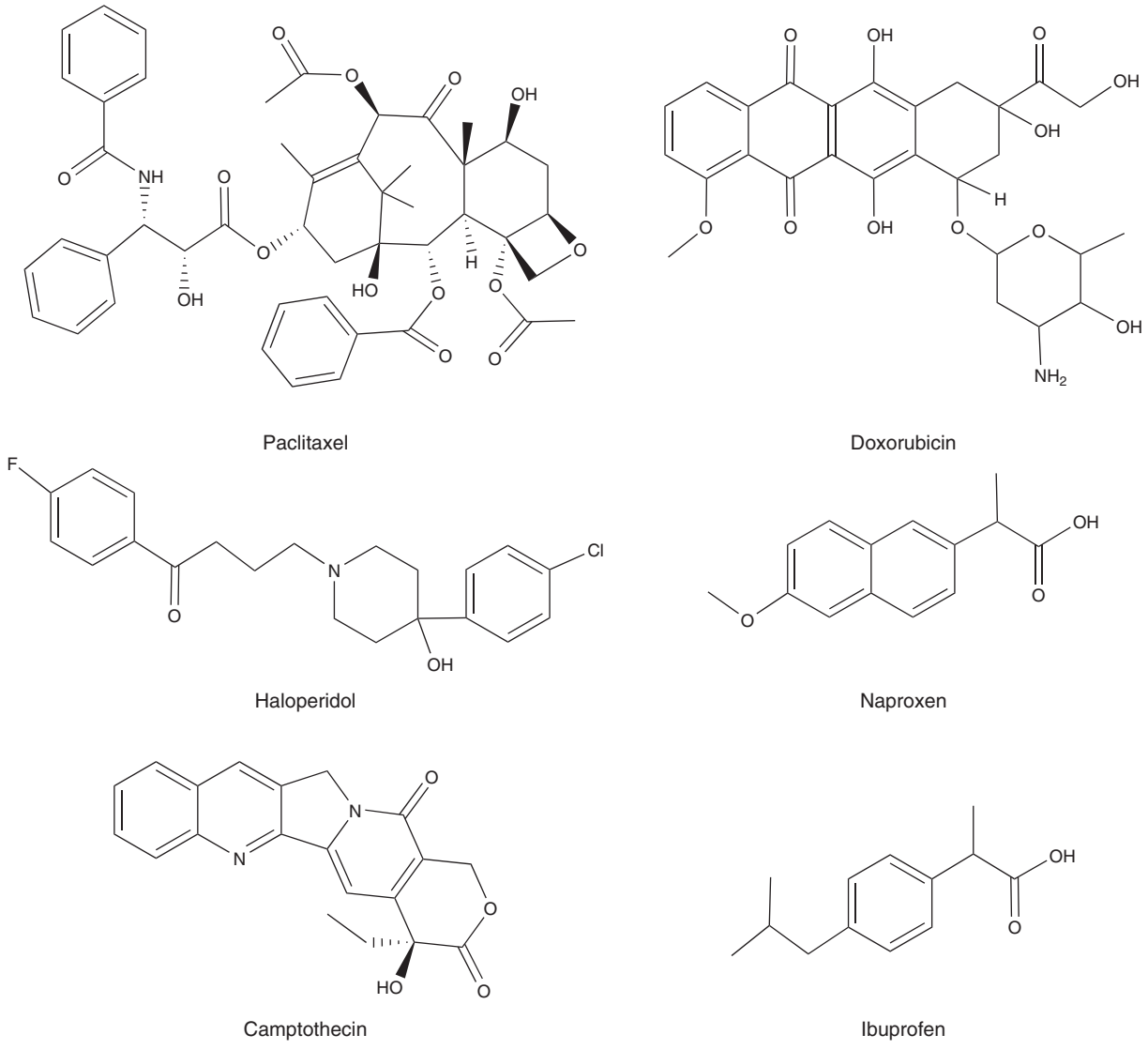

Figure 3. Drugs delivered after conjugation to carrier material.

growth factor receptor 2) to dendrimers enhances the drug loading capacity and allows for specific targeting [58]. These authors developed low-toxicity, biocompatible amino acid-based dendrimer by surface modification of the sixth-generation lysine dendrimer with glutamate. Finally, they linked trastuzumab to glutamate by means of thioether bonds, reaching the efficiency of 1:1 (molar) drug to polymer.

Dendrimer-bound drugs have been shown to improve encapsulation rates for ibuprofen [59] after attachment to polyol and polyglycerol via dicyclohexilcarbodiimide coupling: the average of 50 drug molecules per dendrimer is reported from NMR data (corresponding to 42 and 70% efficiency for polyol and polyglycerol, respectively). In addition, the coupling improves drug stability, but does not seem to influence the therapeutic activity. Similarly to doxorubicin, which can be encapsulated by using different interactions with the carrier, ibuprofen was also shown to be feasible not only for building conjugates, but also for achieving high encapsulation ratios by stabilization with hydrogen bonds (see below).

Protein entrapment by dendronized polymers was demonstrated by two very interesting systems, combining covalent attachment of a ligand to a polymer, and non-covalent, specific binding of a protein to such a functionalized polymer. In the first case, the polymer used was Tetronic™ (tetra-functional block copolymers based on ethylene oxide and propylene oxide, from BASF, Germany) to which heparin was attached by means of a polycaprolactone segment [60]. Such a functionalized polymer forms micelles, which can further bind basic fibroblast growth factor (bFGF) by means of its specific interactions with heparin, reaching loading efficiency of 70%. Similarly, biotin-functionalized polymers

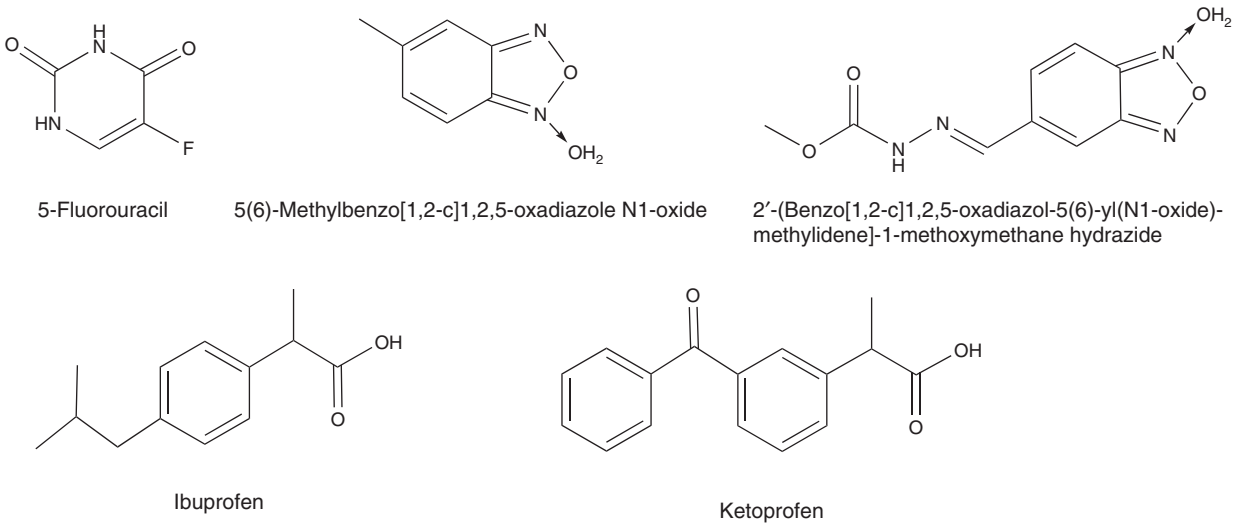

Figure 4. Drugs encapsulated by using hydrogen bonds.

bind avidin owing to very strong and specific interactions [61]. In this latter case, the authors do not comment on the efficiency of their approach, yet given a very high binding constant for the biotin–avidin pair, the entrapment efficiency can be very high, providing a high degree of polymer functionalization. Such combined approaches are indeed promising for the delivery of various molecules, with the potential of even higher and more specific entrapment than achieved so far.

3.4 Hydrogen bonds

Hydrogen bonds are relatively weak compared with covalent attachment; however, they can play an important role when present in abundance. The advantage of this type of interaction in drug encapsulation is the fact that they respond strongly to pH and salt concentration, therefore the loading and release processes can be optimized by taking into account the environmental conditions where the drug-carrying vehicles are prepared and where they will be unloaded. From the literature review, however, it appears that not many drug encapsulation platforms are stabilized by hydrogen bonds (see **Figure 4** for examples of drugs that bind to the carrier by hydrogen bonds).

Bhadra *et al.* [62] reported dendonized poly(amido amine) polymers, which can be coated with a PEG corona. Such polymers form small particles that can encapsulate drugs such as 5-fluorouracil. Drug complexation is believed to be facilitated by a hydrogen bonding-type interaction with dendrimers, leading to drug binding, as shown by light absorption experiments. Importantly, the drug entrapment in PEGylated dendrimers increased 12 times, owing to more sealing of dendrimeric structure by the PEG coat, which prevented drug release. In another study, poorly soluble antichagasic drugs were investigated, also complexed within similar PAMAM dendrimers with various terminal groups [63]. One of the compounds, capable of forming hydrogen bonds, showed increased solubilization (10 times) selectively within the dendrimers containing carboxylate groups. This proves that although weak, these bonds can add significantly to the mere lipophobic–lipophilic interactions to improve drug encapsulation efficiencies.

In solid dispersions of ibuprofen and ketoprofen with poloxamer (PEO-PPO-PEO), hydrogen bonds were proven by vibrational spectroscopy [64]. By altering the drug–carrier ratio, the authors observed different forms of solid dispersions. At any rate, these dispersions improved the dissolution properties of ibuprofen compared with drug alone or in a physical mixture with the poloxamer. These few examples demonstrate that hydrogen bonds can be used in the design of drug delivery vehicles, and should not just be of particular importance for small molecules such as ibuprofen, but could play a role when encapsulating peptide or protein drugs.

4. Expert opinion

This article has described the commonly applied strategies for encapsulating various classes of drugs, depending on their capability to take part in specific interactions. As shown, these can dramatically influence (increase) the encapsulation efficiency in particles, and improve the drug solubility, stability and viability when considering single-molecule drug-polymer conjugates. Although with passive encapsulation the methods to boost entrapment efficiency are essentially limited to processing parameters, interactions of a more specific nature can lead to even better results. With the focus of modern nanomedicine on using personalized therapeutics that deliver a high drug dose precisely to the site of action, high encapsulation efficiency is the first hurdle to overcome on this path.

The drug encapsulation solutions on the market at the moment are for the most part based on lipids (micelles and

vesicles) and polyethylene oxide as conjugating polymer. In the case of lipid particles, only low encapsulation efficiencies can be achieved, owing to the fact that this process is thermodynamically controlled by amphiphilic self-assembly only. As such, the dynamic nature of the particle formation process and the fact that the particle morphology relies strongly on the amount of foreign material (drug) within the particle constitute a limitation that does not allow for high loading efficiencies. Research in this field, however, seems to have moved in the recent years towards polymeric nanoparticles, which can be built from biocompatible materials. This solution is not ideal either, because polymer particles are also formed in the process of self-assembly, with the extra polydispersity parameter. On the other hand, their dynamics is slower and therefore kinetically trapped structures can still be utilized. Additional advantage is the fact that polymer chemistry can be optimized to some extent to include groups or blocks with the possibility of specifically interacting with drug molecules. This leads to improved encapsulation, and as shown in numerous reports also to drug stabilization and release control. Considering conjugated drugs, chemical synthesis is an obstacle in many cases, yet again it appears to be more favorable with polymers than with lipids. How 'difficult' the chemical linking will be always depends on the type of drug.

For the time being, academic research is showing considerable interest in specific encapsulation, and it seems to be an established trend now to design drug delivery particles to achieve the most favorable properties. After the exploratory phase, it became obvious that not every mixture of any polymer with any drug can be sold under the 'drug delivery' banner, and only those efforts are promoted nowadays that hold a true promise for the translational phase into clinics. In the years to come we are likely to witness more clinical trials with new drug delivery platforms, and none of them can be any success when good encapsulation rates are not achieved. For this reason, the authors believe the research in this area will focus on specific material properties to ensure successful drug loading into particles, and in particular it will be interesting to see the new strategies for protein and peptide drugs, the delivery of which seems to be slightly more complex than that of small drug molecules. On the other hand, with the knowledge accumulated in recent years, the clinically successful solutions for high encapsulation efficiencies are bound to arrive soon.

Declaration of interest

The authors declare no competing financial interests, and were not paid for submitting this article.

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Affiliation

Katarzyna Kita[†1] & Christian Dittrich[*2]
[†,*]Author for correspondence
[1]Unilever R&D Port Sunlight,
Bebington, Wirral CH63 3JW, UK
Tel: +44 756 401 0250;
E-mail: katarzyna.kita@mail.com (formerly at University of Leeds, School of Chemistry, Leeds LS2 9JT, UK)
[2]Harvard Medical School,
Department of Cell Biology,
240 Longwood Ave.,
Boston, MA 02115, USA