Expert Opinion

Past and future evolution in colloidal drug delivery systems

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Colloidal drug delivery systems have been providing alternative formulation approaches for problematic drug candidates, and improved delivery for existing compounds for decades. Colloidal systems for drug delivery have all evolved down a similar pathway, almost irrespective of the delivery system, from conception, to the use of safer excipients, PEGylation for passive targeting and attachment of ligands for active targeting. The recent emergence of truly biologically interactive systems represents the latest step forward in colloidal delivery systems. In this article, the maturation pathway and recent advances for the major classes of colloidal delivery systems are reviewed, and the paper poses the question of whether the nanotechnology boom will create a revolution in colloidal delivery, or just the next natural stage in evolution.

Keywords: colloidal drug delivery, dendrimer, emulsion, liposome, micelle, nanoparticle

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1. Introduction

There is an increasing trend in drug discovery programmes towards the extremes of physicochemical properties of lead candidates: on the one hand, drug discovery programmes focussed on potency and high activity of small molecule drugs are often leading to more lipophilic and poorly water-soluble molecules. On the other hand, new biological therapeutics, such as antibodies and peptides, also pose problems in stability and in the ability to deliver the drug in a patient-friendly manner to specific target tissues. Candidates may pose problems in solubility (and hence dissolution), stability, acute toxicity, lack of specificity for the target tissues or in manufacture, leading to a bottleneck in the development process. In some cases optimising the molecular structure may overcome these problems; however, where optimisation programmes have failed, the formulation in a colloidal delivery system has often been useful in alleviating these issues. Existing therapeutic compounds with poor side-effect profiles – anticancer compounds in particular – are also under intense study as candidates for incorporation into delivery systems. Consequently, colloidal drug delivery is an increasingly important field of research that will continue to deliver better medicines.

Colloidal drug delivery systems typically possess a structure on the submicron scale, whether that is as particles, or as matrix systems. The main advantages of colloidal systems that lead to their adoption as delivery systems for problem candidates stem from these attributes and are summarised in **Box 1**. They have been collected into four major classes: self-assembled systems, polymer systems, drug nanoparticle systems (differentiated as they do not comprise a 'carrier' system), and pro-colloidal systems (that typically form colloidal structure that is key to their performance after administration). Their particular attributes are then a function of their size, surface area, surface modification or encapsulation/solubilisation capacity. Schematics of the various colloidal delivery systems discussed in this article are presented in **Figure 1**. Although the particle-based carrier systems (micelles, emulsions, liposomes, polymer

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Box 1. The beneficial attributes of colloidal delivery systems.

Solubilisation of poorly soluble compounds, often without the need for solvents

Protection of labile compounds

Targeting

'Trojan horse' approach to cross biological barriers

Controlled release to reduce acute toxicity

Co-formulation of incompatible compounds or those with very different physicochemical properties

particles) are often considered the mainstay of colloidal delivery systems, other emerging classes of colloidal delivery systems – in particular polymer conjugates and dendrimers – should not be considered any less important.

The decision of which colloidal system to choose for the particular application comes down to selection of the most appropriate delivery system in terms of satisfying the particular attributes required in the product profile. The definition of 'most appropriate' must take into consideration the route of administration, manufacturing costs and complexity, acceptability of excipients, storage and in-use requirements and regulatory issues. In many instances, an increase in drug solubility via a simple micellar system may be sufficient. The protection of labile compounds, or reduced toxicity on administration, may require more complex encapsulation, such as in a liposome; however, this introduces additional concerns over particle size for intravenous formulations, and manufacturing complexity. An increased particle surface area for solid drug nanoparticles may suffice for drugs with too low a dissolution rate. Furthermore, the delivery system may be chosen to provide one of the above functions, but also be designed in order to actively target particular tissues such as tumour cells.

There have already been many reviews written on the various colloidal systems used for drug delivery, and a full review of each of the aforementioned delivery systems and issues is beyond the scope of this article. To assist the reader new to the area, each type of delivery system is briefly introduced below; for further reading, **Table 1** provides a selection of excellent recent reviews on the various types of colloidal delivery systems discussed in this article. The purpose of this article then is to compare and contrast some of the more recent developments between the different types of delivery systems to attempt to clarify future trends and directions in the field.

2. The past and present

2.1 Micelles

The earliest colloidal drug delivery systems were of course developed by nature. The process of fat digestion to produce colloidal micellar species comprising endogenous surfactant and lipid digestion products (bile salt mixed micelles) has evolved as an efficient method for facilitating the absorption of highly insoluble fatty acids, monoglycerides and fat soluble vitamins, such as vitamin E from the gastrointestinal tract [1]. The post-absorption formation of chylomicrons, comprising lipoprotein-stabilised fat globules, allows the distribution of reformed triglyceride molecules from the enterocytes that line the gut back into the bloodstream in the form of submicron emulsion droplets [2]. These delivery systems are essential to life, and in their absence sustenance is only available through the parenteral administration of lipid-soluble nutrients via intravenous total parenteral nutrition [3].

The small size (typically < 10 nm) of micelles, their thermodynamic stability (spontaneous formation on mixing of components) and colloidal stability (lack of particle aggregation) are attractive attributes for their use as drug delivery systems from a formulation and manufacturing/sterilisation perspective. The concept of bile-salt mixed micelles was used in the early 1980s in the formulation of a bile salt–lecithin mixed micellar parenteral delivery system for diazepam [4] and the development of intravenous micellar formulations for a number of therapeutic compounds, including vitamin K1 (marketed as Konakion MM®; Roche) [5]. One limitation of micellar systems is the relatively low hydrophobic volume of the interior of micelles, limiting drug loading. Consequently micellar formulations may often comprise a surfactant and solvent mixture, such as Cremophor EL® (BASF) and ethanol in the injectable Taxol® (Bristol-Myers Squibb) formulation for paclitaxel, to boost drug load in the initial formulation, and provide a transiently high drug concentration on dilution of the formulation in intravenous fluids immediately before infusion [6].

A common progression for intravenously administered delivery systems has been the development of 'stealth' systems using PEGylation approaches. The use of PEGylated lipids provides a stealth-like coating, enabling the particle to avoid uptake by the reticuloendothelial system and hence prolong its circulation time [7]. The increased circulation time can lead to the preferential accumulation of particulate material or macromolecular material in tumour tissue otherwise known as the enhanced permeation and retention (EPR) effect, due to the combination of poor vascular architecture permitting extravasation of substances in these tissues, and poor lymphatic drainage from the site retaining the particles close to tumour cells. This can be exploited to target anticancer compounds to tumour tissue by associating drug with stealth colloids possessing polyethylene glycol (PEG) groups on the surface.

Stealth micelles have been shown to provide enhanced circulation times [8]. Although the EPR effect is most often acknowledged for its use in delivering anticancer drugs to tumour tissues, it has also been shown to facilitate the accumulation of PEG micelles in heart tissue following

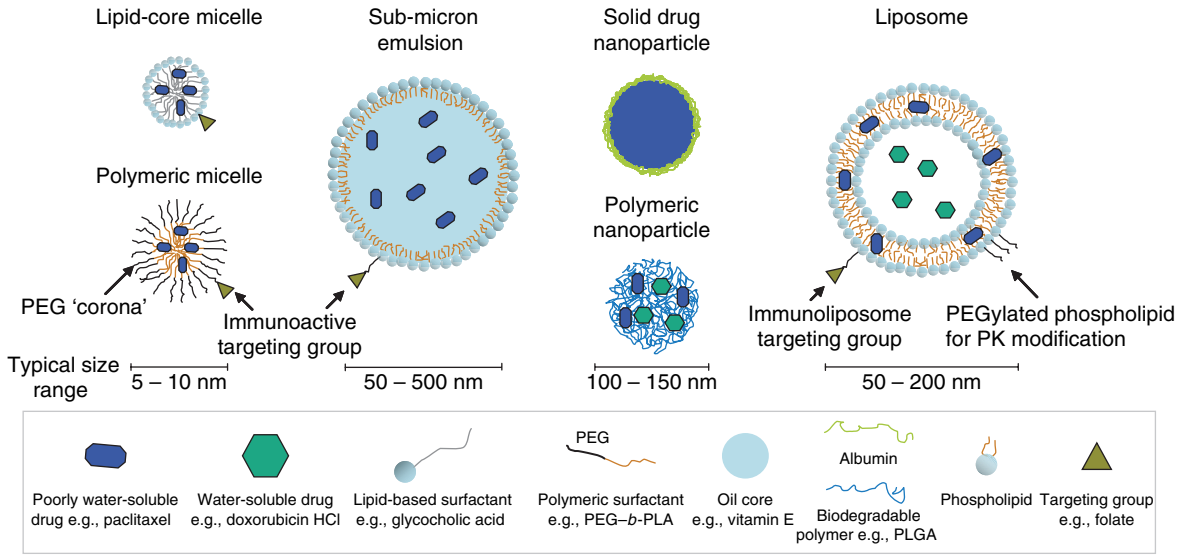

Figure 1. A schematic of the various particle-based colloidal delivery systems discussed in this article.
PEG: Polyethylene glycol; PK: Pharmacokinetics; PLGA: Poly(lactide-co-glycolide).

Table 1. Recent selected reviews for the various colloidal drug delivery systems.

Delivery system	Ref.
Self-assembled lipid systems	
Micelles, immunomicelles	[12,23]
Liposomes, immunoliposomes	[18,130]
Emulsions	[131,132]
Solid lipid nanoparticles	[47]
Polymer systems	
Polymer conjugates	[133]
Polymeric micelles	[13]
Polymeric nanoparticles	[134]
Dendrimers	[135-137]
Drug nanoparticles	[45]
Pro-colloidal systems	
Lipids, self-emulsifying oral formulations	[138]
Liquid crystalline systems	[139,140]

experimental myocardial infarction [9], leading to myocardial protection when loaded with ATP [10]. Drug is maintained within regular micelles by partitioning favouring the hydrophobic core, and drug will immediately leave the structure on dilution in a sink condition such as blood. However, the PEG outer layer or 'corona' (see **Figure 1**) can act as a diffusion barrier for hydrophobic drugs to reduce the burst release characteristic of micelles [11], allowing the EPR effect to deliver encapsulated drug to target tissues.

More recently, polymeric micelles have been developed as an alternative to lipid-based surfactant systems, as they offer a more versatile structure, biodegradability, lower critical micelle concentrations that lead to better *in vivo* stability, and alternative chemistry for the binding of ligands [12]. Self-assembled from block copolymers, they most often comprise a relatively hydrophobic block such as poly-lactic acid, poly-caprolactone and poly-aspartic acid, with a hydrophilic PEG segment. A number of polymeric PEG–micelle formulations have entered clinical trials [13]. One example – the polymeric micelle encapsulation of doxorubicin [14] – has gone through Phase I clinical trial for solid tumours [15] and has also shown use in restenosis by encouraging accumulation in vascular lesions [16]. Trimaille *et al.* have recently developed a series of biodegradable poly-lactic acid polymers that possess lipophilic side chains that self-assemble to form micelles that are somewhat hybrid between polymeric micelles and lipid-based micelles [17]. These polymers self-assemble with relatively low critical micelle concentrations, holding promise for maintaining the micelle integrity under high dilution conditions in *in vivo* applications, whilst retaining the high solubilising power of lipids for very lipophilic compounds.

Passive targeting via the EPR effect, achieved using PEG, is being rivalled by a large amount of effort involving the decoration of particles with antibodies to provide more active targeting to cells expressing particular antigens on their surface [18]. Immunomicelles are micelles that possess covalently attached functional groups for improving the targeting of the micelle to the site of action, such as

tumours [13], in order to deliver higher drug concentrations specifically in target tissues. Researchers have employed a wide range of ligands for targeting micelles, including folate [19], sugar residues [20], proteins such as transferrin [21], and antibodies [22,23]. Roby *et al.* have very recently targeted a poorly soluble porphyrin to lung tumours using immunomicelles for light-activated therapy, and have shown *in vivo* tumour accumulation and tumour growth inhibition in mice [24].

2.2 Liposomes

Liposomes are arguably the most intensively researched colloidal delivery system. An attempt to review liposomes in this article would be superficial – for the interested liposome novice there are excellent books covering all aspects of liposome technology, two useful examples being those by Gregoriadis [25] and Lasic [26]. Liposomes consist of one or more lipid bilayers, usually formed using a phospholipid, surrounding an aqueous interior. The lipid regions can be used to solubilise lipophilic drugs, and the interior can be used to encapsulate and protect hydrophilic drugs. Release of drug from the hydrophilic core can be controlled through alteration of the membrane permeability using cholesterol. The low toxicity of phospholipids has been a key to their utility in intravenous formulations. The formulation of hydrophobic drugs into liposomes is usually straightforward, and involves dissolving the drug, or the drug in the form of a lipid complex, in the phospholipid prior to hydration. The loading of hydrophilic drugs is significantly more challenging, requiring a pH gradient between the internal and external aqueous domains to drive drug into the interior of the liposome by partitioning through the membrane. Free drug on the outside of the liposome then needs to be removed, typically by dialysis methods. A novel promising method for loading liposomes involves the formation of a t-butanol/chloroform/water solution containing the phospholipid, hydrophilic agent and a sugar cryoprotectant, which is freeze dried and rehydrated resulting in high encapsulation efficiencies [27]. A number of liposomal drug delivery systems have been commercialised, including Ambisome® (amphotericin B; Astellas Pharma) Doxil® (doxorubicin hydrochloride; Ortho Biotech) and Visudyne® (verteporfin; Novartis).

Liposomes are increasingly being conjugated with functional moieties to alter their *in vivo* behaviour, as discussed above for micelles. The use of PEGylated lipids to modify liposome pharmacokinetic behaviour is routine and originally gave rise the term 'stealth' to described particles that have a PEGylated surface to avoid the reticuloendothelial system [28]. Again, the literature on PEGylated liposomes is vast; however, one relatively recent development, which is a potential limitation to the use of PEGylated liposomes, is the complement activation that can lead to anaphylactic response – in one study close to half the patients receiving PEGylated doxorubicin-containing liposomes experienced hypersensitivity reactions [29]. The effect appears to be mediated by the zwitterionic nature of PEG–phospholipids (and non-PEGylated phospholipids with an intact zwitterionic functional group) [30]. PEGylated liposomes are also susceptible to accelerated clearance on readministration at certain doses, so far observed in rodents and monkeys, due to the production of anti-PEG–lipid antibodies (see schematic of this process in **Figure 2**) [31], with the magnitude of the reduction in clearance being inversely proportional to the mass dose of PEGylated lipid [32]. The use of poly(hydroxyethyl-L-asparagine)-coated liposomes has been shown to some degree to overcome this effect [33]. Together with the potential for anaphylactic reactions, these effects may have important negative consequences in a clinical setting.

Immunoliposomes have been studied for the last decade as a means to enhance drug delivery to target specific tissues [18], and across biological barriers such as the blood–brain barrier [34], particularly for gene therapy, using similar approaches to those for immunomicelles. The targeting ligand can be attached directly to the anchoring phospholipid or attached to the terminal end of the PEG chain on PEGylated lipids, most commonly using a thioether or disulfide linker [35]. Derivatisation of cholesterol with galactose or mannose residues has also been useful in targeting liposomes to hepatoma cells [36] and macrophages [37], respectively. Methods of producing useful quantities of antibody fragment conjugated to PEG–lipids to GMP standard for incorporation into liposomes has been demonstrated [38].

2.3 Emulsions

Submicron emulsions have been used as intravenous drug carriers for lipophilic drugs for several decades. They are often stabilised using lecithin and a non-ionic surfactant such as polysorbate, and their lipophilic core acts as a reservoir from which drug can be instantly released by partitioning due to short diffusional distances and high surface area, resulting in a fast-acting formulation, with higher solubilising capacity compared with an aqueous solution. These properties make injectable emulsions well suited for the delivery of anaesthetics and sedatives, such as diazepam and propofol (e.g., Diazemuls® [Pfizer] and Diprivan® [AstraZeneca], respectively). Emulsions are also used to provide total parenteral nutrition, as mentioned earlier, in products such as Intralipid® (Fresenius Kabi).

The development of submicron emulsions has followed a similar path to micelles and liposomes, with both pharmacokinetic modification via PEG attachment to surface lipids [39] and antibody-mediated targeting being studied *in vitro* and *in vivo* [40]. In a recent study by Goldstein *et al.*, the uptake of a submicron emulsion comprising HER-2 receptor ligands into cells using the SK-BR3 cell line has been demonstrated for cationic and anionic emulsions of equivalent size, indicating that receptor-mediated endocytosis of emulsions can occur [41].

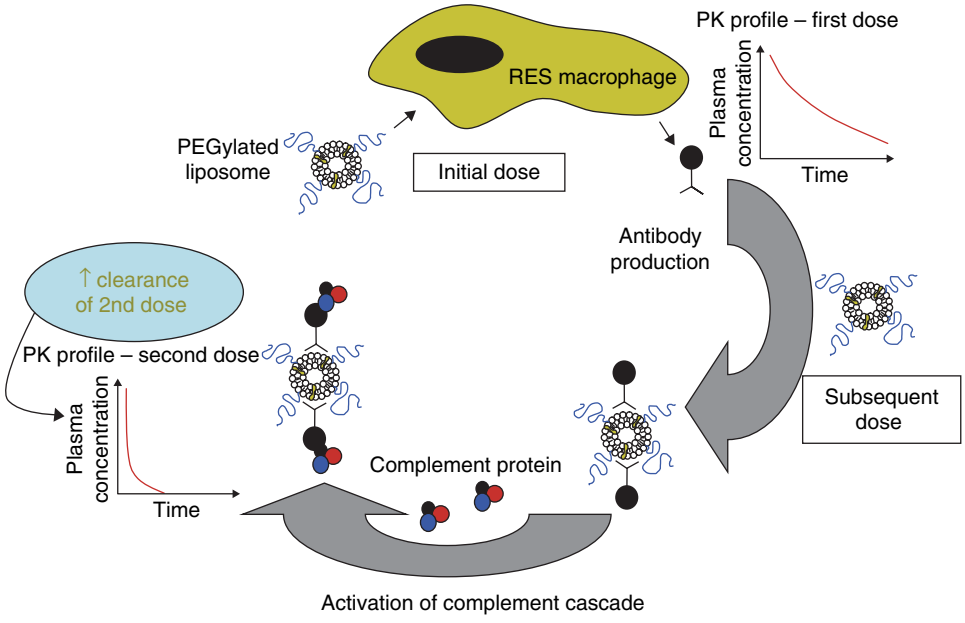

Figure 2. Proposed mechanism of accelerated blood clearance (ABC) of PEGylated liposomes. The first dose initiates the production of anti-PEG IgM antibodies, while the second dose activates the complement cascade that opsonises the second dose, targeting it for rapid blood clearance and uptake by the liver and spleen.

Information from [120].

PEG: Polyethylene glycol; PK: Pharmacokinetics; RES: Reticuloendothelial system.

However, traditional pharmaceutical emulsions do suffer the disadvantage that they are not thermodynamically stable and will eventually aggregate over time, and that they are not amenable to filtration sterilisation and hence require aseptic manufacture. In addition, problems have arisen with bacterial contamination in multi-use injectable emulsions, which has somewhat tarnished their reputation [42]. Furthermore, the determination and description of particle size, and detection of very few large particles in an emulsion, which have the potential to form emboli, can also be problematic [3]. Nevertheless, the discovery that paclitaxel has a moderate solubility in D-α-tocopherol (vitamin E) has led to the recent development of an injectable submicron emulsion (Tocosol®; Sonus Pharmaceuticals) as an alternative to the Taxol Cremophor/ethanol micellar formulation [43]. Clinical trials are being undertaken and have shown that the formulation can be administered using a short 15-min infusion, compared with 3 h for Taxol, and has shown activity against ovarian, lung and bladder cancer in Phase II trials, providing greater drug exposure compared with the micellar formulation [44].

2.4 Solid nanoparticles

Solid nanoparticles as drug delivery systems (i.e., particles with a more or less homogeneous and usually solid core) may be further classified into three major groups: solid lipid nanoparticles (SLNs), polymeric nanoparticles and solid drug nanoparticles [45]. The interest in these systems is rapidly growing, particularly in the cancer therapy and imaging fields [46].

SLNs are analogous to submicron emulsions, but are prepared using lipids that solidify at room temperature [47,48]. The distinction between 'solidify at room temperature' and 'solid at room temperature' is subtle but important, as SLNs have been observed to undergo significant supercooling effects, meaning that SLNs often need to be cooled well below the melting point of the comprising oil [49]. The perceived advantage of SLNs is the encapsulation of the drug in the solid matrix for protection and controlled release purposes. However, SLNs can suffer from drug-loading limitations, which are further exacerbated by exclusion of the drug from the interior of the particle on solidification of the core [50,51]. This can lead to significant burst release on dilution, and has been a limitation to further development.

Polymeric nanoparticles such as those prepared using biodegradable polymers such as poly(lactide-co-glycolide) (PLGA), polylactic acid (PLA) and chitosan have also received significant attention in the literature, as the materials are well characterised, and have precedence for use in parenteral sustained-release products such as Eligard® (sanofi-aventis). Polymeric nanoparticles have been derivatised with PEG to extend circulation times [52], and the resulting influence on particle biodistribution and opsonisation has been recently reviewed [53]. Of course, the active targeting of polymeric nanoparticles is a very active research

field, as for other colloidal delivery systems. PLGA–PEG nanoparticles have been conjugated to A10 RNA aptamers specific for prostate membrane antigen and have demonstrated targeting of the particles to the prostate in mice, relative to non-conjugate equivalent, as a generic delivery platform [54]. The attachment of HER-2-targeting ligands with subsequent targeting to tumour cells has also been reported for polymeric nanoparticles [55,56]. Polymer nanoparticles bearing a galactose surface have also been shown to target hepatocytes via receptor-mediated endocytosis for the delivery of retinoic acid [57]. As is the case for SLNs, polymeric nanoparticles are often limited in their use by low drug loading; for example, paclitaxel loading in PLGA nanoparticles is only ~ 1% [58].

Solid drug nanoparticles are useful in oral delivery to enhance the drug dissolution rate for poorly water-soluble compounds [59,60] and hence are a particularly useful approach for those compounds with a bioavailability that is limited by solubility. Drug nanoparticles are produced by three main methods: nano-milling, homogenisation or solvent evaporation [61]. The nano-milling approach has been commercialised under the name Nanocrystal® by Elan, and the homogenisation approach has been commercialised as DissoCubes® (SkyePharma). Nab–paclitaxel (albumin-stabilised paclitaxel nanoparticle) on the other hand is produced by a solvent-evaporation process [62].

For colloidal intravenous drug formulations, drug loading into the carrier is often a major limitation, and hence, in cases where the carrier would only be required for solubilisation, the alternative approach of formulation as a drug nanoparticle makes good sense. Issues around preparation to a tight particle size specification, Ostwald ripening to produce large particles in suspension, and regulatory difficulties are all hurdles to the development of such dose forms. Kipp has recently reviewed the application of drug nanosuspensions in parenteral applications, and describes the various antibiotic and steroidal formulations available for injection as submicron suspensions [63]. In light of the potential advantages in terms of drug loading, it is somewhat surprising that so far only one drug nanosuspension for intravenous administration has been brought to the market. An injectable suspension of paclitaxel has recently been developed and approved for use against metastatic breast cancer under the trade name Abraxane®, marketed by Abraxis Bioscience. The formulation consists of 130-nm colloidal paclitaxel particles stabilised by albumin at the surface, so-called 'nab–paclitaxel'. The Phase III trials showed an improved tolerability and efficacy compared to Taxol [64,65]. As it is the first alternative (Cremophor-free) paclitaxel product to market, it is in an enviable position among other colloidal systems for paclitaxel in development.

Although one function of the albumin coating on nab–paclitaxel is to provide colloidal stabilisation and a high concentration of drug in the formulation, this formulation is more advanced than a mere dose-enhancing system. One function of albumin in the body is to act as a carrier for poorly soluble nutrients, delivering those nutrients beyond the blood via transcytosis mechanisms involving gp60 (which sequesters the albumin particle at the endothelial surface) and caveolin-1 (which transcytoses the albumin particle across the cell to the interstitium, see **Figure 3**) [66]. Tumours have evolved systems to take advantage of this process by upregulating the gp60, to sequester the nutrients essential for their growth. Once in the interstitium, SPARC (Secreted Protein, Acidic and Rich in Cysteine [67]) which is also over-expressed in tumour cells [68], sequesters the albumin particle and localises it to the tumour membrane, resulting in the delivery of paclitaxel (in place of nutrients) directly to the tumour cell. Thus, by designing the nanoparticle in this way, the tumour's biology works against itself, and thus is an extremely elegant demonstration of the potential of nano- and biotechnology to have a great impact on therapy. Importantly, this approach is not specific for paclitaxel – it could in theory be applied to the delivery of any poorly water-soluble anticancer compound with an affinity for albumin, such as camptothecins.

2.5 Dendrimers

Dendrimers are macromolecules prepared by a stepwise synthetic approach that adds multivalent layers or generations (G1, G2 etc.) to a core structure. The physical size of dendrimers (usually described by a hydrodynamic diameter) may vary widely (1 – 100 nm), but is typically regarded as being between that of a small molecule and that of a colloidal particle. As such, dendritic macromolecules share properties in common with both typical small molecules and larger colloidal species. The various advantages and disadvantages of dendrimers as drug delivery systems are listed in **Box 2**. The ability to prepare dendrimers as unique molecular entities provides an important point of differentiation from a regulatory and manufacturing/quality perspective, when compared with self-assembled systems. The molecular versatility of dendrimers is almost infinite, and the structure of the core and additional multivalent monomers, the number of layers and the terminal surface groups can all be altered (**Figure 4**) [69]. This provides for variation in size, molecular weight, surface charge, symmetry of molecular shape and hydrophilicity, all of which may impact on the physicochemical and biopharmaceutical behaviour of dendrimers. Dendrimers have been studied as drug carriers in which the drug is either covalently bound to the dendrimer surface, or by drug solubilisation within a hydrophobic core or pocket within the dendrimer [70,71]. Dendrimers have also been developed where the dendrimer itself has antiviral/anti-microbial properties [72] or antiscarring capability [73]. Entire issues of the journals *Advanced Drug Delivery Reviews* (Volume 57, Issue 15), and *Molecular Pharmaceutics* (2005, Volume 2, Issue 4) have been dedicated

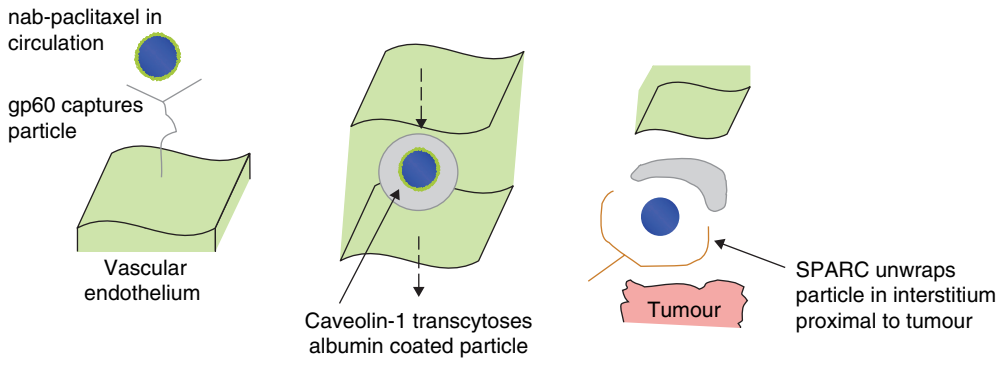

Figure 3. The mechanism of delivery of nab–paclitaxel (Abraxane). The formulation is intravenously administered and circulating nanoparticles are sequestered by gp60 protein overexpressed at the endothelial cell surface in tumours (left). The albumin-coated particle is then chaperoned across the cell in caveolae formed by the caveolin-1 protein, and exits the cell in the interstitial space (middle). SPARC (Secreted Protein Acidic and Rich in Cysteine) proteins secreted by the tumour cells then localise the nanoparticle close to the tumour cells (right) where the cytotoxic paclitaxel dissolves and distributes into the cell.

Box 2. The key features of dendrimers as drug delivery systems.

Advantages

Single well-defined molecular species (regulatory advantage)

Highly versatile structure for manipulation of pharmacokinetics/targeting

Stable to dilution compared with, for example, micelles

Biodegradable (depending on building blocks)

Drug liberation can be triggered using labile linker groups

Dendrimer itself may have therapeutic activity depending on functionality

Do not show accelerated blood clearance on subsequent administration

May be filter sterilised in manufacturing process

Drawbacks

Complicated, possibly expensive, synthesis

Potential limitations in payload

Analytically challenging

to dendrimers in drug delivery, so only the most pertinent points and recent developments will be highlighted here.

The vast majority of studies have investigated dendrimers using poly-amidoamine (PAMAM), poly-ethyleneimine and poly-L-lysine as the multivalent building blocks. In a recent novel approach, Tang *et al.* prepared dendrimers using salicylic acid–glycerol–succinic acid building blocks [74]. Other types of dendrimers are receiving increasing interest, such as poly-glycerol and polyester dendrimers [75]. The cationic nature of most of the building blocks has also led to their use as DNA condensation carriers for gene delivery [76], with apparent advantages over linear polymer counterparts [77]. TAT-peptide-conjugated dendrimers have been prepared for enhancing cellular entry [78]. However, the cationic dendrimers are cytotoxic [79], and must be modified for drug delivery applications. For classical drug delivery applications, the surface of dendrimers has been modified for three main purposes: i) to modify the pharmacokinetics using PEG groups, to increase circulation time to take advantage of the EPR effect [80]; ii) for the direct targeting of dendrimers to particular tissues, using, for example, folate [81] or mannose groups [82]; or iii) to mask cationic charges using, for example, acetyl groups [83] or sugar residues such as galactose [84]. Lipids have also been conjugated to dendrimer surfaces to reduce cytotoxicity [79], and also provide an apparent improvement in oral absorption [83].

2.5.1 *In vitro studies into dendrimers as drug delivery systems*

Dendrimers that comprise a relatively hydrophobic interior and polar exterior have been termed 'unimolecular micelles'. Although dendrimers with hydrophilic cores may also be synthesised [85], the hydrophobic core has specific use for the delivery for poorly water-soluble drugs. There are a plethora of publications describing the use of dendrimers for drug solubilisation and *in vitro* drug release [86-98]. Dendrimer composition, size and surface functionality all impact on the amount of drug that can be encapsulated. However, the passive loading of practical levels of drug into the interior of the dendrimer is limited, even with the use of a hydrophobic interior, ion-pairing sites and ligands [96]. The release of drug is then likely to be under diffusion control, which for a small particle is usually extremely rapid in a sink condition environment [99]. As a consequence of the loading and release limitations of non-covalent drug–dendrimer systems, the field has

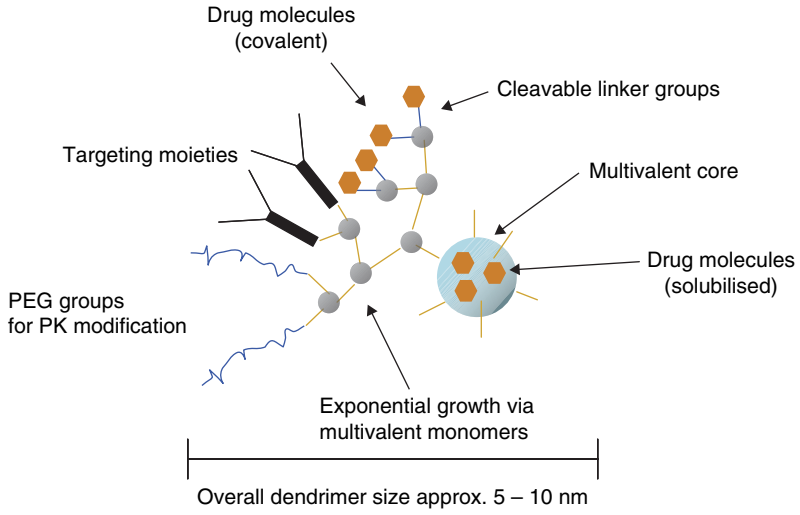

Figure 4. A schematic representation of a dendrimer-based therapeutic, illustrating pharmacokinetic modification and targeting groups, with covalent and non-covalent drug conjugation. The dendrimer size is theoretical size, and may be larger depending on solution conditions and aggregation.
PEG: Polyethylene glycol; PK: Pharmacokinetics.

shifted almost exclusively to the design and characterisation of covalently bound complexes.

One drawback of conjugating drug molecules to the terminal groups of the dendrimer is the reduction in sites available for the attachment of groups for active targeting and/or for passive targeting/modification of pharmacokinetics via the EPR effect. Nevertheless, there are a number of recent studies demonstrating the effectiveness of targeting drug to particular cell types and their cytotoxicity when bearing anticancer compounds. The laboratory of Baker and co-workers have reported a number of PAMAM-based dendrimers that possess drug (methotrexate), a targeting function (folate) and an imaging group (fluorescein isothiocyanate) [100]. This dendrimer conjugate was shown to target KB cells, and had > 100-fold cytotoxicity compared with free drug *in vitro* [101]. The same construct design carrying paclitaxel in place of methotrexate also showed uptake-related cytotoxicity in KB cells [102]. Tansey *et al.* have also demonstrated the *in vitro* targeting and biodegradation of a poly-glutamic acid dendrimer using folate groups [103]. Succinic acid dendrimers bearing camptothecin molecules have shown improved cellular uptake [98]. Gurdag showed that the binding chemistry of methotrexate to PAMAM dendrimers is important in dictating their *in vitro* cytotoxicity – hypothesised to be due to differences in lysosomal drug release [104]. In non-cancer-related applications, a PAMAM dendrimer has been shown to facilitate the rapid uptake of ibuprofen into A549 lung epithelial cells when incubated as a complex, compared with the free drug alone [105], and Fernandes *et al.* have reported the antithrombigenic properties of a streptokinase–polyglycerol dendrimer conjugate as a fibrinolytic surface [106].

In the case of dendrimers in which drug is associated by covalent conjugation, drug must be released from the superstructure in order to act in target tissues. This can be achieved through the use of a variety of cleavable linker groups that are pH, hydrolytically or enzymatically labile (see **Table 2**). Najlah *et al.* [107] have shown the relative release kinetics of naproxen from a dendrimer scaffold when coupled via a range of linker groups in various biorelevant media (PBS, plasma, acid) [107]. They showed that lactic acid ester librates naproxen more slowly than a diethylene glycol ester on incubation in plasma, illustrating the potential to tailor drug release rates *in vivo* by selection of linker group. Ihre *et al.* have demonstrated the pH-dependent release of doxorubicin from a polyester dendrimer [89].

2.5.2 *In vivo behaviour of dendrimers*

In contrast to the large number of studies into dendrimer synthesis, drug conjugation and targeting to cells *in vitro*, there are relatively few *in vivo* examples of drug delivery using dendrimers.

The derivatisation of dendrimers with lipophilic groups appears to facilitate the oral absorption of dendrimers. Florence *et al.* have performed a biodistribution study on an orally administered poly-L-lysine dendrimer with lauroyl chains at the surface (to increase lipophilicity) in rats [83]. The bioavailability was measured to be 26.4% after 6 h, indicating rapid uptake of the dendrimer from the gastrointestinal tract. Tripathi *et al.* have reported the encapsulation of 5-fluorouracil in a PAMAM dendrimer with palmitic acid surface groups, and a further phospholipid coating [108]. Oral administration to rats resulted in a

Table 2. A summary of some potential linker chemistries to be employed in the construction of dendrimer–drug composites.

Linker type	Summary	Ref.
Hydrazone	Stable at physiological pH but hydrolyse in the acidic microenvironment of solid tumours	[89,141-144]
Ester	Ester cleavage strongly related to structure and the number of cleavable sites, (monoesters are more stable than diesters). In general, esters less stable than amides and more stable than disulfides. Orthoester cleavage is pH dependent and may be used to provide pH sensitive release profiles	[145,146]
Peptide	Typically provide relatively nonspecific enzyme-cleavable linkers. Their stability depends largely on the molecules to which they are attached and to the peptide sequence; however, tumour-specific peptide linkages have been described	[147-152]

higher and sustained plasma concentration of the drug, compared with free drug at the same dose, and very high lymph concentrations, also illustrating the potential of these systems for lymphatic targeting.

Most *in vivo* studies where dendrimers have been administered by intravenous infusion have been for the purpose of biodistribution studies of dendrimers conjugated with heavy metals such as gadolinium, with a view to their use as imaging agents. Kobayashi and co-workers have been particularly active in determining the impact of dendrimer size, charge, core chemistry and PEGylation on biodistribution for imaging [109-112]. The ability to subtly tune the dendrimer size and distribution properties provides great opportunities for selectively contrasting vasculature of particular organs. Several general trends have been ascertained in the literature aimed at imaging applications that may be useful in designing dendrimers for application in drug delivery, in particular that:

- Liver accumulation increases with size for both cationic [109] and anionic [113] dendrimers
- PEGylation significantly increases the half-life and decreases liver accumulation [110]
- Dendrimers with a size greater than G6 tend not to be filtered through the glomerulus [110]; lower generations accumulate in the kidney before excretion [114].

The derivatisation of dendrimers with PEG and other moieties also successfully enhances blood residence time [80,115]. As an alternative to PEG, galactose-coated poly-L-lysine dendrimers have also been shown to avoid *ex vivo* macrophage uptake, although their *in vivo* behaviour is not yet reported [84]. In one study, intravenous injection of an HPMA dendrimer decorated with antibodies and doxorubicin was highly efficacious against human colorectal tumours in mice [116] by virtue of active targeting, and a cisplatin–PAMAM dendrimer conjugate injected intravenously showed high accumulation in tumour tissue due to the EPR effect, and also showed lower toxicity compared with free cisplatin [117].

As mentioned earlier in this article, PEGylated liposomes have recently been shown to exhibit accelerated blood clearance (ABC) on subsequent administration in a number of species, due to the production of anti-PEG IgM (**Figure 2**) [118,119]. It has been proposed that the lack of clinical evidence for the ABC effect in humans is likely due to the cytotoxic payload in commercial PEG liposome formulations inhibiting macrophage function in producing the antibody [120]. An important attribute of PEGylated dendrimers is that they do not show the ABC effect [121,122]. The absence of the ABC effect for PEG–dendrimers compared with PEG–liposomes is illustrated in the pharmacokinetic profiles in **Figure 5**, in which repeat dosing of PEGylated liposomes results in substantially more rapid removal from the circulation after intravenous administration to rats, compared with repeat administration of PEGylated dendrimer. Dendrimers therefore have a potential advantage over liposomes as stealth particles in terms of the possibility of the clinical implications of the ABC effect.

Important recent studies demonstrating an improved therapeutic effect using PEGylated dendrimers has recently been reported. Gillies and Frechet have recently reported new dendrimer geometry, based on the coupling of two dendrons to form a bowtie-shaped dendrimer [123]. This approach has been used to prepare PEGylated and radiolabelled dendrimers across a range of molecular weights, and their biodistribution has been determined in mice. In the case of mice bearing subcutaneous tumours, high levels of dendrimer accumulated in the tumours in mice via the EPR effect [124]. When conjugated with doxorubicin via a hydrazone pH-sensitive linker, the median survival time was significantly greater than for both free doxorubicin, and the commercially available Doxil liposome formulation at equivalent dose. This is an important *in vivo* outcome demonstrating the potential of these systems to provide improved therapeutic outcomes compared with the available 'gold standard' therapy.

There have also been studies of the use of dendrimer therapeutics in non-cancer-related areas. Chandresaker *et al.* has demonstrated the active targeting of folate–dendrimer conjugates to inflamed tissues for site-specific delivery of the anti-inflammatory drug indometacin [81,125] A ketoprofen–PAMAM dendrimer has been shown by Man *et al.* to provide a prolonged antinociceptive effect in the acetic acid-induced writhing model in mice [126]. Taite and West synthesised dendrimers for the release of nitric oxide to regulate vascular cell proliferation and inhibit platelet

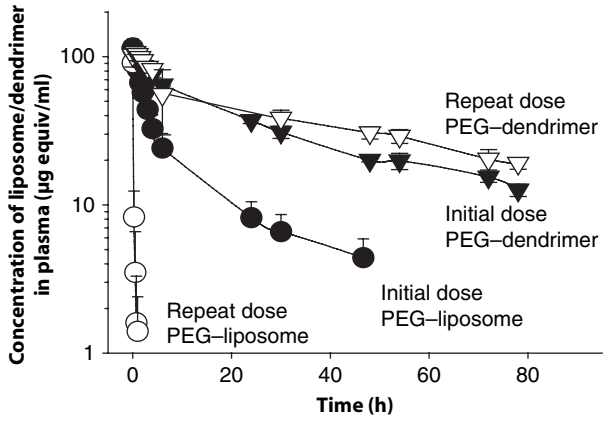

Figure 5. Pharmacokinetic profiles adapted from Kaminskas *et al.* [122] for PEGylated liposomes (circles) compared with PEGylated dendrimers (triangles) after a single intravenous dose (filled symbols) or after a second equivalent intravenous dose administered 7 days after the initial dose to rats (open symbols). Formulations were administered at 5 mg/kg to male Sprague Dawley rats (n = 3; mean ± SD). The liposome composition was identical to those used by Ishida *et al.* [153], and were traced using ^{14}C-labelled cholesterol. The dendrimer was a fully PEG-capped, generation 4 poly-L-lysine dendrimer, with ^{3}H-labelled lysine core.

PEG: Polyethylene glycol; PK: Pharmacokinetics; SD: Standard deviation.

adhesion to thrombogenic surfaces [127]. Kaminskas *et al.* have studied the effect of dendrimer size and surface charge on the pharmacokinetics and biodistribution of antimicrobial aryl sulphonate-capped dendrimers [128].

2.5.3 *Dendrimer metabolism*

Understanding and demonstrating the ultimate fate of the drug carrier after drug has been delivered is a critical aspect in obtaining regulatory approval for colloidal drug delivery systems – accumulation of the residual structure in specific organs after drug release is not viewed favourably. There is a conundrum between, on the one hand, enhancing circulation time by increasing dendrimer size to limit filtration through the glomerulus to try to access the EPR effect, and on the other hand avoiding organ accumulation, which may lead to toxicity and regulatory problems. Some dendrimers, such as PAMAM dendrimers, are not degraded *in vivo*, and consequently excretion is the only route of elimination. Constructing the dendrimer from amino acids is one way to achieve both aims, as the dendrimer can be ultimately degraded *in vivo* into its original building blocks, which can then be excreted or in the case of endogenous monomers such as lysine, incorporated back into bio-pathways. The present author and co-workers have recently shown the inherent biodegradability of poly-L-lysine dendrimer cores *in vivo*, and that the degradation can be blocked using an outer layer of non-natural D-lysine [129].

3. The future – evolution or revolution in colloidal drug delivery systems?

It is often claimed that the advances in nanotechnology are stimulating a 'revolution' in colloidal drug delivery. However, as this review has hopefully demonstrated, even the most recent developments are best described as a necessary step in 'evolution' rather than a 'revolution'. The evolution of colloidal delivery systems over the last five decades has followed a reasonably clear path, illustrated schematically in **Figure 6**. Initially, colloids were employed to enhance solubilisation and dissolution using simple self-assembled systems, which in the case of parenteral delivery allows sufficient drug to be dispersed in an aqueous medium suitable for administration. These systems were low on functionality but provided a useful means of delivering a therapeutic dose, but often with drawbacks of toxicity and with lack of selectivity. The micellar Taxol formulation of paclitaxel is a great example of these systems – although it has significant drawbacks with toxicity and potential drug precipitation [6], it has, until recently, been the only means to deliver paclitaxel at therapeutic levels in a dissolved form.

The next stage in the evolution of colloidal systems was to make them either safer or smarter; in the former case, the replacement of toxicologically unacceptable synthetic components with better excipients has led to improve outcomes. Again drawing on the paclitaxel example, the recent development of Tocosol as an injectable emulsion form of paclitaxel, with significantly lower toxicity than the Cremophor-based Taxol formulation, is a good example. The replacement with biodegradable polymeric micelles of lipid-based micellar systems that often contain haemolytic amphiphilic compounds is another example.

The development of PEGylated lipids and their use in modifying the pharmacokinetics of colloidal systems, such as liposomes, to enhance blood circulation times has led to smarter colloidal systems. These systems are certainly an advance on the relatively uncomplicated systems described above, but would still be classified as 'passive' carrier systems – somewhat low on the evolution scale being conveyed here. Actively targeted systems with ligands attached to the surface should also be included in this class, as although the interaction with cellular surfaces may be novel and the molecular biology required to isolate and use the ligand may be technically advanced, they are on the whole not truly interactive. Systems with 'non-specific' drug release function, such as hydrolysis or pH labile linker groups, such as orthoesters or hydrazones, could also be classified into these smarter systems.

Most recently, there has been an emergence of colloidal systems that not only provide the benefits of earlier systems in terms of drug solubilisation, avoidance of clearance processes and targeting, but also are biologically responsive. Rather than avoid biological processes, they evade the deleterious influence of the reticuloendothelial system and,

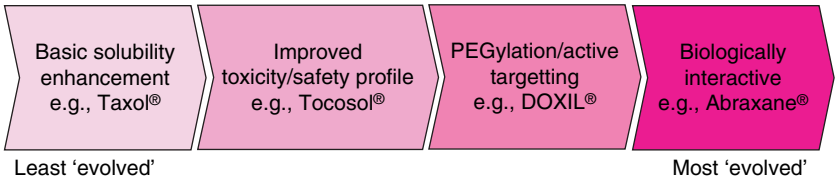

Figure 6. The generic pathway of 'evolution' of colloidal delivery systems.

once at the site of action, interact with biology to gain some beneficial effect. Such relatively simple systems would comprise enzymatically cleavable systems that release drug molecules due to interaction with enzymes localised in the target tissues. The most developed of such interactive systems that has made it through development to a marketed product is the paclitaxel nanoparticle formulation Abraxane.

This review demonstrates that the evolutionary trend described above has been more or less preserved for all of the major colloidal delivery systems, and will continue with newer systems such as dendrimers. The use of the term 'evolution' has been selected rather than 'complexity' in **Figure 6** to reflect the reality that Abraxane, although being a truly bio-interactive system, is also quite simple when compared with the synthetic complexity of, for example, antibody-targeted, enzymatically labile delivery systems. Although dendrimers have not yet advanced to the stage of actual interaction with biological function to enhance drug delivery, such as with Abraxane, it is only a matter of time before similarly advanced functionality is built into these new constructs.

4. Expert opinion

The present intense interest in nanotechnology is resulting in large amounts of research funding into the use of nanoparticles and nanostructured materials as potential drug delivery systems. For example, polymeric nanoparticles, self-assembled particles, layer-by-layer polymeric shells structures, drug nanoparticles and many others, are being investigated for their formation and properties, and research grant applications are replete with claims of the potential of new nanoparticles as having applications in drug delivery. However, in many cases the new technologies are likely to only improve incrementally on the practical and clinical applications of existing drug delivery systems, so that although the technologies themselves may be clever, innovative, well-controlled systems, the case they mount for further investment is often not sufficiently compelling. Consequently, few products are being progressed into formal development programmes and clinical trials. In fact, despite three decades of liposome research and at least 10 years of nanoparticle research, there are very few products based on colloidal delivery systems presently on the market that are commercially viable and ultimately provide an advantage to patients.

The lack of translation from research interest to viable pharmaceutical product worthy of development investment perhaps highlights the gap between the often disconnected realms of material and colloid scientists, and the realities of pharmaceutical development. In order for new technologies to be taken up by the pharmaceutical industry, they need to either: i) provide substantial improvement in therapeutic outcome for the patient at the same or lower cost than conventional treatment; or ii) provide a means to more economically produce the same therapeutic outcome and overcome some existing issue with existing products that can be used to mount an argument as to why the regulatory body should allow clinical trials with new material to commence.

Most colloidal delivery systems provide either enhanced solubilisation, or improved pharmacokinetics (reduced acute drug activity and/or targeting). Self-assembled colloidal delivery systems (liposomes, micelles, emulsions) have dominated the colloidal drug delivery arena. In part this is due to the use of relatively biocompatible excipients such as phospholipids and endogenous surfactants such as bile salts – an important point from a regulatory perspective – and the lipophilic and poorly water-soluble properties of problematic drugs that require solubilisation for effective delivery inherently results in an affinity for membrane-structured delivery systems. The capacity to functionalise the surface with, for example, PEG groups to enhance blood circulation times for better tumour uptake, and specific targeting groups to concentrate the drug carrier in the vicinity of specific cellular structures have yielded both exciting potential drug delivery systems, as well as products already on the market.

The relatively positive regulatory opinion towards lipid-based self-assembled drug carriers is in contrast to novel polymeric systems, whose toxicological profile may be completely unknown – the often polydisperse nature of the excipients can hinder a demonstration of product quality control, and the multitude of potential breakdown products that require some level of *in vivo* characterisation, if not full toxicological profiling, complicate the approval process immensely.

Nevertheless, there are substantial problems that also confront the development and approval of self-assembled

systems. Micellar and liposomal drug products are often extremely complex to manufacture. There is a huge cost and development time penalty to pay in the development of processes required to provide well-defined lipid-based products, usually in a lyophilised state, that reconstitute to provide a consistent particle size at the time of use with sufficient colloidal stability to enable administration under unpredictable clinical conditions. The manufacture often involves a number of discrete stages that may be conducted in different facilities, even in different countries.

Once the manufacturing and quality control processes are ultimately in place, the process of approval of such a product is more problematic than for a small molecule drug product, due to the inherent uncertainty about the nature of the colloidal structures carrying the drug. The regulatory body may require answers to questions such as 'what is the exact composition of the carrier, and does this change with time?' and 'does the composition of the carrier influence the pharmacokinetic, pharmacodynamic and clinical outcomes, and if so should each individual carrier then be classified as a separate entity?' The self-assembled nature of these systems makes it almost impossible to answer these questions with absolute confidence because the composition of individual carriers within the product and their individual behaviour in terms of absorption, distribution, metabolism and elimination of both the carrier and the drug will always be a statistical average of the properties of the individual species present in the self-assembled mixture.

Thus, self-assembled drug carrier systems, although arguably more attractive than novel polymeric carrier systems, have significant issues that require time and money to resolve in order to get a product to market based on these technologies. The recent approval of a paclitaxel nanoparticle formulation for intravenous administration suggests that this approach may become more popular, and may indeed overcome some of the aforementioned limitations of other colloidal system; however, issues around colloidal stability, and the complex manufacture and sterilisation requirements still apply to this drug delivery approach. Hence there is still an opportunity for a drug carrier system that provides the benefits of the colloidal carrier systems, without the drawbacks highlighted for both the self-assembled systems and novel polymer-based systems such as nanoparticles.

It is the opinion of this author that dendrimers will increasingly be developed as an alternative colloidal drug delivery systems, and may, in fact, replace products that are already on the market. The geometry and size of the core, together with the molecular weight of surface groups can be modified to manipulate renal excretion behaviour. The surface can be modified with PEG groups to prolong plasma residence for targeting purposes. Drug can be covalently bound to the dendrimer using labile linker functionality to allow for the selective release of drug in target tissues. Dendrimers can be prepared from biodegradable polypeptides, such as poly-L-lysine and polyesters, and the breakdown products are likely to be readily excreted or utilised by the body in other natural processes. From a developmental and regulatory perspective, dendrimers have a huge advantage over self-assembled or particle-based delivery systems, as synthetic approaches have been refined to the point that even fully functionalised dendrimers can be synthesised in commercial quantities and proven to comprise a single molecular entity.

The development of the first dendrimer-based therapeutic (Vivagel™, a vaginal microbicide for the prevention of HIV infection developed by Starpharma, an Australian dendrimer therapeutics company), which has successfully passed Phase I trials and has received fast track status with the FDA, together with the features described above, is proving that dendrimers certainly present an exciting new class of delivery system, with the potential to become a major platform for drug delivery in the coming decades that may ultimately replace presently used particle-based technologies, at least in a number of delivery applications.

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Declaration of interest

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