

Addressing Solubility through Nano Based Drug Delivery Systems

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Abstract

Developing new techniques to overcome poor solubility in drug development remains a challenge for the pharmaceutical industry. It is estimated that up to 40% of newly discovered chemical entities are insoluble. This has led industry to turn to new approaches such as nanotechnology to help overcome solubility. Nanotechnology based drug delivery systems have been associated with improving therapeutic activity and minimising undesirable side effects of drugs. Nanotechnology is an emerging field that has the potential to change the way illnesses are treated and drugs delivered. Despite enthusiasm amongst formulation researchers that nanotechnology could be the answer to improving solubility and deliverability of drugs, concerns regarding toxicology and long term effects of nanomaterial on the body need to be investigated further. This review describes advance drug delivery systems such as liposomes, dendrimers and polymeric micelles which have been explored as drug delivery vehicles and provides an overview of each system.

Keywords: Nanotechnology; Liposomes; Dendrimers; Polymeric micelles; Drug delivery; Solubility

Introduction

This review seeks to provide an overview of nanotechnology used in the pharmaceutical industry to enhance solubility of poorly soluble drugs. Nanotechnology is used to address concerns regarding solubility of drug compounds and is a growing area of interest and research. Nanotechnology is defined as science and engineering carried out in the nanoscale that is 10^{-9} m [1].

Various strategies are used to improve the solubility of drugs. One of the most common methods is to produce a salt form of the drug but sometimes salt formation cannot be achieved because the compound is non-ionisable. Other strategies can be considered such as micronisation and/or the development of oil-based solutions in gelatin capsules, i.e. soft-gel technology. Other approaches which have been deployed successfully to improve solubility are the use of co-solvents, surfactants, complexing agents such as cyclodextrins, emulsions, micro-emulsions and solid dispersion. These approaches have been successful but far more effective and versatile ways are required to deal with formulation issues associated with poorly water soluble compounds [2].

Nanotechnology can be envisioned as the future of drug delivery technology as it has the potential to produce useful therapeutic and diagnostic tools [3]. Nanotechnology based drug products have the ability to increase the solubility and bioavailability, coupled with desirable effects on pharmacokinetic and toxicological profiles making them very useful for drug delivery [4]. It is predicated that once there is further understanding of how nanotechnology enhances solubility, pharmaceutical companies will use this technique to accelerate more hit compounds into their pipeline resulting in a higher success rate of drugs being taken further into the developmental phase [5].

Nanotechnology Based Drug Delivery Systems

Nanotechnology was first introduced by Richard Feynman in 1959 in his lecture *"There's Plenty of Room at the Bottom"*. The first nanotechnology drug delivery system to be described in the literature was lipid vesicles in the 1960s which later became known as liposomes. This discovery has resulted in increased interest and research into nanotechnology which has been demonstrated by closer collaborations between researchers in the academic, industry, and government sectors. The United States has been at the forefront of advancing understanding into nanotechnology and has formed the

National Nanotechnology Initiative (NNI) which has been given a budget of \$1.05 billion to further explore research and development opportunities in the nanotechnology area. One area of clinical application where nanotechnology and its benefits have been applied is in drug delivery. It is estimated that up to 95% of newly developed drugs have poor solubility, pharmacokinetics and biopharmaceutical properties. This has led researchers to look at new methods which can support the delivery of these poorly soluble drugs to the intended site of action, without harming healthy tissues rather than being discarded at the first hurdle because of solubility issues [6].

The description "nanotechnology" can be applied to any material or system where the size varies from 1-100 nm. Internationally there is no exact or agreed definition of what constitutes nano size. The European Commission recommendations on the definition of nanomaterials admit *"there is no scientific evidence to support the appropriateness of this [upper] value [100 nm]"* [7]. Nano drug delivery vehicles or nanocarriers come in both an organic or inorganic form and their size can range from 1 to 300 nm. Inorganic carriers are small, contain elements such as gold, cadmium or selenium whereas organic carriers are carbon based and have better biocompatibility and drug loading capacity [8]. Nanocarriers are usually made from polymer based materials because of good biocompatibility with the human body and reduced risk of toxicity which equates to a reduced side effect burden for patients therefore increasing compliance to treatment [9]. Toxicity is further reduced because nanocarriers have a high surface area to volume ratio allowing for improved biodistribution of the encapsulated drugs [10]. Furthermore, the selection of polymer is important because depending on the type of polymer used drugs can be entrapped within the polymeric structure or attached (functionalised) to the external surface [11].

Nanocarriers have demonstrated several benefits for drug delivery: (1) improved delivery of poorly water-soluble drugs; (2) targeted

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delivery of drugs in a cell or tissue-specific manner; (3) transcytosis of drugs across tight epithelial and endothelial barriers; (4) delivery of large macromolecule drugs to intracellular sites of action; (5) co-delivery of two or more drugs for combination therapy; (6) visualisation of sites of drug delivery by combining therapeutic agents with imaging properties; (7) real time read on the *in vivo* efficacy of a therapeutic agent [12].

In order for nanocarriers to work they need to remain in systemic circulation over a prolonged period of time. This can prove difficult because the natural defence system distinguishes nanocarriers as foreign particles which results in nanocarriers being opsonised and removed from systemic circulation. Nanocarriers need prolonged circulation times to ensure that the drug can accumulate and be released at the specific target site through the enhanced permeability and retention (EPR), either through active or passive targeting pathways [13]. One strategy that has been successfully used to increase blood circulation times is conjugation of water soluble polymers such as PEG (poly(ethylene glycol)) to the surface of the nanocarriers resulting in decreased interactions with blood components and also increased water solubility and colloid stability. Formulation scientists have recognised that PEG may not be suitable in all cases due to oxidation and the loss of protein repulsive properties. Alternative strategies are being explored looking at nonfouling material and zwitterions (phosphorylcholine (PC), carboxybetaine (CB) and sulfobetaine (SB)) as possible substitutes to PEG as they have been minimal interactions with biological components such as proteins and cells [14].

Developing nanocarriers with high drug loading capacity presents a challenge. The consequence of nanocarriers with reduced drug loading capacity is a sub therapeutic amount of drug being delivered to the body to have an optimal pharmacological effect. Nanocarriers have also been associated with premature release (burst release) phenomena of the entrapped drugs after administration. This can lead to systemic toxicity or release of drug at other points in the body rather than at its intended therapeutic site. Further developmental work is required to design nanocarriers with optimal drug loading efficiency and biocompatibility of carrier material with the human body. To address these issues with nanocarriers interest has shifted towards investigating “squalenoylation technology” which is the construction of nano metal oxide frameworks (NanoMOFs) to improve drug loading and/or reduce the burst release effect [15].

Further investigation is required to determine the safety of nanomaterials in biological systems. This has led to an emerging field known as nanotoxicology, the study of potential undesirable interactions between nanomaterials and biological systems. Data have indicated that nanomaterials could cause toxicological harm to the body by the formation of free radicals and in some cases damaging brain cells. Currently, no internationally agreed toxicological reference standards exist for nanomaterials highlighting a need to develop robust and rigorous toxicological studies [16]. Moreover, a universally accepted method needs to be developed which can quantify the release of a drug from a nanocarrier *in vitro* which will allow comparisons between different classes of nanocarriers such as liposomes, micelles and polymeric nanoparticles [17].

In summary, the ideal nanocarrier for drug delivery should have the following attributes: (i) biocompatible with the human body and biodegradable; (ii) the ability to locate and deliver most of the drug to the desired therapeutic site; (iii) possess optimal biophysicochemical properties allowing for efficient drug loading, and effective circulation time; and (iv) cost effective scale up for commercialisation. The

refinement and incorporation of these qualities in one nanocarrier is the ‘Holy Grail’ of nanomedicine [18].

Industrial Perspective on Solubility

The pharmaceutical industry faces significant challenges in improving drug solubility. It is suggested that over 40% of new chemical entities are practically insoluble in water or lack cell membrane permeability resulting in poor bioavailability [19]. This equates to approximately \$65 billion in lost drug revenues for pharmaceutical companies due to suboptimal bioavailability [20]. Lipinski has reported that up to 90% of new chemical entities do not reach clinical trials because of problems relating to their bioavailability in the development phase. This further re-emphasises the importance of careful compound selection early in development [21].

The two main strategies employed by pharmaceutical companies in the discovery phase of drug compounds are: rational drug design (RDD) and high throughput screening (HTS). In both, hit compounds are identified according to screening in a biological environment. RDD generally lead to compounds with high molecular weight resulting in poor permeability. The HTS pathway also leads to high molecular weight compounds but also increased lipophilicity which can hinder the solubility characteristics of the hit compounds [22]. The pharmaceutical industry has increasingly pushed towards a “fail fast/fail cheap” paradigm in an effort to reduce costs in the early development of new chemical entities, so resources can be allocated to alternative programmes with a better likelihood of success. In a research program, early assessment of the efficacy and safety is often dependent upon efficient drug administration to generate reliable *in vivo* results in animal models for a “go” or “no go” decision. However, early drug candidates often exhibit poor pharmacokinetic and physicochemical properties, such as poor solubility, making *in vivo* activity assessment difficult due to low exposure to the drug [23]. Solubility is a key indicator for absorption but still large numbers of newly discovered or developed compounds are relatively insoluble. This could be due to the application of combinatorial chemistries to generate large chemical libraries where the high throughput screening modalities are often undertaken in non-aqueous or mixed solvent media. Additionally, receptor binding studies which are driven by hydrophobic interactions further contribute towards the possibility that drug candidates will have limited aqueous solubility. The target site of where the drug will act, will dictate the design of the drug. If the drug will be acting on disrupting intracellular signalling pathways it needs to be lipophilic to penetrate the cell membrane but highly lipophilic drugs display poor water solubility [24]. Poor solubility is not the sole reason that determines the viability or failure of a new drug compound early on in the project but project failure could be down to poor *in vivo* exposure and performance which indicates marginal efficacy, narrow therapeutic index (TI) caused by limited exposure in dose escalated toxicity studies, expensive or unstable formulation, or severe food effects [25].

The need to improve solubility at the earliest opportunity is one of the key tasks in the drug development process. The degree of solubility or the amount that enters into systemic circulation will influence the level of pharmacological response the new drug compound elicits. To increase the solubility of poorly soluble compounds a number of approaches can be utilised which are dependent on the properties of the drug under consideration, nature of excipients selected and the desired final dosage form. A compromise needs to be achieved between increasing solubility and not simultaneously decreasing the potency of the drug. Medicinal chemists are working to modify

the physicochemical properties of hit compounds so they have more favourable characteristics going forward into the formulation phase. Formulation scientists are investigating the use of nanotechnology to develop nanocarriers to counter the issue of solubility, enabling more compounds to move successfully from discovery, through development and to the patient.

Drug Delivery Systems

Polymeric micelles

Polymeric Micelles (PMs) were first reported over 30 years ago, initially by Ringsdorf as potential drug delivery vehicles to help overcome water solubility problems and reduce systemic toxicity [26]. Their application as a possible drug delivery system for anticancer drugs was introduced in the early 1990s by Kataoka's group who successfully conjugated doxorubicin with a block copolymer [27]. From this success, PMs have been used as drug delivery vehicles for low molecular weight anti-cancer drugs, contrast/imaging agents, proteins, plasmid DNA, antisense DNA, and more recently short interfering RNA (siRNA). Several formulations that utilise PMs structure have now entered into clinical trials [28].

PMs are designed to mimic natural carrier systems like viruses and are composed of relatively bio-inert materials which allow them to be used as therapeutic drug delivery systems. PM comprise of two separated functional segments: inner core and outer shell. The outer shell consists of a hydrophilic polymer (corona) determines the *in vivo* pharmacokinetic (PK) behaviour, while the core, made up of a hydrophobic polymer, is responsible for drug loading, stability and drug release behaviour [29]. The hydrophilic part has a brush like architecture which protects the hydrophobic core from biological invasion by enzymes and minimises protein adsorption on to the PM thereby ensuring the size of the PM is above the threshold for renal clearance resulting in prolonged circulation times [30]. The hydrophilic block, in all cases, comprises of poly (ethylene glycol) (PEG), due to its favourable biocompatibility profile and its unique "stealth" characteristic that limits the number of interactions of the hydrophobic block with serum proteins and cellular components. Moreover, the conformations of the hydrophobic blocks differ extensively, so can be manipulated to entrap drugs of varying degrees of lipophilicity, chemical structure and charge, thereby contributing to the versatility of polymeric micelles as drug carriers. The most commonly studied block polymers include PEG-poly(amino acids), PEG-poly(D, L-lactide) (PEG-PLA), PEG-poly(ϵ -caprolactone) (PEG-PCL), PEG distearoylphosphatidylethanolamine (PEG-DSPE) and PEG-poly(propyl oxide)-PEG (PEG-PPO-PEG, Pluronic[®]) [31].

PM nanostructures are formed by self-assembly of amphiphilic block polymers in aqueous solution. PM are formed when the concentration of the block copolymer increases above a certain concentration referred to as the critical aggregation concentration (CAC) or critical micelle concentration (CMC). At the CAC or CMC, hydrophobic segments of block copolymers start to associate to minimise the contact with water molecules, leading to the formation of a vesicular or core shell micellar structure [32]. When favourable thermodynamic conditions (CMC) have been established, multiple monomers spontaneously assemble so that the hydrophobic tail groups arrange inwardly to repel water, thereby creating an inner lipophilic core that can be occupied by lipophilic drug micelles. Meanwhile, micellar head groups, which are polar, protrude outward in water, creating a shell (or corona) to shield the stored lipophilic drugs within the micellar cores [33].

The CMC is an important factor in characterising the self-aggregation of amphiphilic compounds. At low concentrations, copolymer molecules exist in aqueous solution as individual molecules (monomers) self-assembly occurs when copolymer concentration reaches the CMC. CMC is dependent on the relative sizes of both the hydrophobic and hydrophilic domains. A larger hydrophobic domain will result in a lower CMC, and a higher hydrophilic domain area will result in a higher CMC. The optimal diameter for a drug delivery vehicle is in the range of 10 and 100 nm which is formed when the CMC value is low. PM with large diameters, above 100nm, lack penetration and accumulate in tumours with hypovascular and hypopermeable characteristics and do not make ideal drug delivery vehicles [34].

The primary mode of action of PM is the enhanced permeability effect (EPR) creating a passive targeting system. The EPR effect is based on the pathophysiological characteristics of solid tumour tissues; hypervascularity, incomplete vascular architecture, secretion of vascular permeability factors stimulating extravasation within cancer tissue and absence of effective lymphatic drainage from tumours, which impedes the efficient clearance of macromolecules accumulated in solid tumour tissues [35].

Successful application of PM for drug delivery is dependent on various factors. PM have the optimal size distribution profile for systemic drug delivery and contributes towards their overall stability. The hydrophobic core of PMs provides a reservoir where hydrophobic drugs or multiple drugs can be entrapped allowing the PMs to be used for combination treatment. The outer surface of PMs can be used to attach specific anticancer targeting molecules helping to promote specificity of these nanocarriers [36]. Despite these promising characteristics, the synthesis of block polymers and incorporation of drugs into PMs on a large industrial scale in a highly reproducible manner remains problematic. Increased risk of chronic liver toxicity has been associated with PMs because drugs incorporated into PMs are metabolised slowly compared with free drug since access of metabolic enzymes to drugs is inhibited because of conjugation and incorporation [37].

Liposomes

Liposomes are vesicular nanostructures that form cell membranes through the self-assembly of phospholipids and cholesterol molecules to form structures on the 50-250nm scale [38]. The internal structures of liposomes are highly hydrophilic, forming an aqueous phase allowing for the delivery of water soluble drugs. The outer bilayer membrane of the liposome allows the entrapment of poorly soluble drugs but the loading capacity is limited due to membrane destabilisation effects [39]. Interactions between water molecules and the hydrophilic phosphate groups of the phospholipids, cause the lipid bilayer to close in on its self in a spontaneous fashion producing amphiphilic bilayer phospholipids [40]. The observation of spontaneous self-association by Alec Bangham (1965) marked the beginning of research into liposomes as potential drug delivery vehicles. The similarity of liposomes to biological membranes allowed drugs to be delivered into cells or their sub-cellular compartments while simultaneously protecting entrapped drug molecules from external degradation [41]. The amphiphilic nature of liposomes and their biologically inert profiles reduces the chances of antigenic or toxic reactions in patients [42]. Although the morphology of liposomes mimics human cell physiology, for application as drug delivery vehicles two conditions need to be met: liposomes must demonstrate a high degree of stability and the structure of liposomes must be pH sensitive before and after administration [43].

The classification of liposomes is based on several aspects: the method of preparation (eg reverse phase evaporation vesicles or vesicle

extruded technique), size (small, intermediate, or large) and lamellarity (uni, oligo, or multilamellar vesicles). The type of liposome formulated will affect the type of drug compound that can be encapsulated in the liposomal structure. Single lipid bilayers (Uni Large Vesicles (ULV), 50-250 nm)) are suited to encapsulate hydrophilic drugs, whereas two or more bilayer liposomes measuring 1-5 μm are superior in their ability to accommodate lipid soluble drugs. The different bilayer arrangement of liposomes also impacts on the release characteristics of the entrapped drug, e.g. ULVs display a much faster release rate compared with multilamellar large vesicles [44].

Drug encapsulation into liposomes can be achieved by two methods, passive or active loading. Passive loading is the preferred methodology for water-soluble drugs and it involves dissolution of dried lipid films in aqueous solutions. The drawback of this method is low drug loading efficiency. The active loading method results in higher intraliposomal concentrations and minimal wastage of drug material. This is achieved by ensuring that there is a transmembrane pH gradient that causes ionisation of drugs, allowing them to enter the liposome and become entrapped inside the bilayers. Entrapment or encapsulation also affects pharmacokinetic and pharmacodynamic properties of drugs by reducing systemic toxicity and increasing potency of the drug [45].

Liposomes need to evade detection and clearance by the mononuclear phagocytic system (MPS) in the liver and spleen, so they can remain in systemic circulation to have an effect. This requires special modifications to the phospholipid surface [46]. To avoid rapid clearance by the MPS and ensure optimal circulation times in order to provide a sufficient level of accumulation of the drug at the target site, liposomes are masked by modifying (grafting) their surface with water-soluble polymers such as polyethylene glycol (PEG). PEG slows down the opsonisation process, a step that precedes macrophage uptake and the clearance of the liposome from the bloodstream [47]. The PEG also improves the pharmacokinetics and bio-distribution of a drug. This is evident from PEGylated liposomal doxorubicin (also referred to as stealth liposomes) which reduces the volume of distribution of doxorubicin from $\sim 1,000$ litres/ m^2 in the free drug form to 2.8 litres/ m^2 by limiting the distribution within the plasma. Furthermore, the liposomal formulation has demonstrated a reduction in toxicity to healthy tissues and has shown increased accumulation of drug in tumours through the enhanced permeability and retention effect [48]. Research into stealth liposomes was originally done by Abuchowski and McCoy who found that attaching PEG to proteins increased their circulation half-life. This idea was then extended to liposomes and several research papers appeared to show that grafting of PEG to the liposome surface resulted in substantial reductions in the rapid clearance of liposomes into the MPS unlike 'classical' liposomes with no PEG grafted to the surface [49].

There is emerging evidence that patients receiving PEGylated liposomes have an increase risk of hand-foot syndrome due to the long circulation time of the liposome [50]. Clinical reports have also suggested that PEG-phospholipids may cause activation of the complement system causing pseudo allergic reactions in patients, again due to the extended circulation half-lives [51]. Research conducted by Stefanick and colleagues has found that the length of PEG used (e.g. PEG_{2000/350/550}) affects the cellular uptake efficiency of liposomes *in vitro*. The 'gold standard' for liposomal formulation has been PEG₂₀₀₀ which has been used as a traditional standard rather than a choice based on functional optimisation whereas Stefanick and colleagues have demonstrated in an *in vitro* cell-based system liposome uptake efficiency was higher when PEG₅₅₀ was used. *In vitro* results need to be

interpreted with caution and it is not clear whether this effect can be replicated in *in vivo* models [52].

The first liposomal drug delivery formulation, Doxil, was approved by the Food and Drug Administration (FDA) in 1995 for the treatment of Kaposi's sarcoma. This led to a further 13 liposome based drug formulations being approved and a further 16 are enlisted in clinical trials. A key therapeutic area that has benefitted from liposomal formulations is oncology. Products that have been approved or enrolled in clinical trials include DepoCyt (liposomal cytarabine), DaunoXome (liposomal daunorubicin), Myocet (liposomal doxorubicin, approved in Europe and Canada), Doxil/Caelyx (liposomal doxorubicin), Sarcodoxome (liposomal doxorubicin), Marqibo (liposomal vincristine), and Lipusu (liposomal paclitaxel, approved in China) [53]. The challenge faced by pharmaceutical companies in bringing liposomal drugs to market relate to stability of the preparations. Liposomal formulations on the market need to be stable for 1.5 to 2 years. To achieve this liposomes must remain in suspension form, through either chemical or physical manipulation, which can affect the overall liposome stability and therapeutic index of the drug [54].

The biocompatible and biodegradable composition of liposomes helps to reduce systemic toxicity, making them a suitable option for drug delivery. Stealth liposomes with PEG coating have significantly enhanced plasma half-life thereby increasing circulation times of drugs but also notably the PEG coating prevents phagocytosis through the mononuclear phagocyte system. The release mechanisms of entrapped drugs can be governed by manipulating pH and temperature which is a useful attribute for a drug delivery vehicle [55]. Despite these positive attributes of liposomes limitations still exist. Problems with stability and industrial reproducibility, difficulties in sterilisation, the oxidation of phospholipids, and the limited control of drug release by the conventional formulations are factors that need to be investigated further for optimal drug delivery. The industrial reproducibility and scalability of producing liposomes is reliant up on bulk-scale synthesis which results in limited uniformity and size variability. The size and uniformity of liposomes can influence the drug dosage, targeting, cellular uptake, circulation time and clearance of the liposome. Microfluidic-based techniques are now being used in the synthesis stage to produce liposomes with tuneable size and low polydispersity [56]. Development work on liposomes is still continuing and innovations in this area are still appearing such as remote drug loading methodologies based on pH or ionic gradient, PEG coated long circulating liposomes, cationic liposomes for nucleic acid delivery, pH-sensitive liposomes for cytosolic drug delivery and targeted liposomes for selective delivery to tumour cells or endothelium [57]. New generations of liposomes are being developed which allow drugs to be released from the liposome using behaviour strategies including thermo-pH-sensitive and ultrasound triggered drug release [42].

Dendrimers

There is very little research into dendrimers as potential drug delivery systems compared to the application of dendrimers in pharmaceutical and medicinal chemistry where extensive literature can be found [58]. Vogtle, Denkewalter, Tomalia and Newkome were the first research groups to characterise dendrimers and Vogtle named them "cascade" molecule which was later changed to "dendrimers" to emphasise the tree like structure of larger dendritic molecules [59]. Research has indicated that dendrimer nanocarriers have the potential to revolutionise the ability to deliver anti-cancer drugs to localised cancerous tumour sites [60].

Dendrimers consist of repeating units, comprising of a central core, internal cavity (void space) and peripheral groups. The peripheral groups are highly branched and well-defined macromolecules which govern the globular structure and monodispersity of dendrimers giving them a tree like branched appearance. The tree-like structure, nano-size range (e.g. have the ability to undergo extravasations through vascular endothelial tissues), high physical stability and the potential for chemical modification of peripheral groups make dendrimers potential drug delivery vehicle option [59].

The use of dendrimers in drug delivery was first proposed in 1982 by Maciejewski who used dendrimers as molecular containers. The size and shape of dendrimers resemble biomacromolecules such as proteins (biomimics) giving them good biocompatibility with the body. The large size (usually several nanometres) ensures that dendrimers are not renally excreted, which can be the case with other nanocarriers. Solubility of poorly soluble drugs can be enhanced through the hydrophobic/hydrophilic internal core allowing for physical complexation or encapsulation of a drug resulting in increased bioavailability and water solubility [61]. Encapsulation of drugs occurs in the interior layers of dendrimers which consist of repeating units of polymers. In addition to the interior layers where drugs can be encapsulated the multivalent surface of dendrimers can accommodate a large number of functional groups such as drug molecules, targeting moieties and solubilising groups. These properties of dendrimers make them potential drug delivery vehicles and currently have been used to deliver anti-inflammatory, antimicrobial, and anticancer drugs [62].

Dendrimers display a particular architecture, which consists of three distinct domains: (i) a core at the centre of dendrimer (ii) repeating units of branches which are covalently attached to the centre core and protrude outwards organised in a geometrical progression that results in a series of radially concentric layers called “generations (G_n , where n is 0, 0.5, 1, 1.5...); (iii) terminal functional groups, located at the surface of dendritic architecture and (iv) empty spaces which can be filled by drugs [63]. They are two methods which can be used to formulate dendrimers; one is the “divergent method” pioneered by Tomalia in the 1980’s where dendrimers were formed by extending layered branches from a central core molecule which involves assembling monomeric modules in a radial, branch-upon-branch motif according to certain dendritic rules and principles. The drawback of this approach is that multiple reactions are required to be performed on each dendritic molecule which increases the possibility of defects, especially with high generations. The second method is “convergent method” established by Hawker and Frechet’s in 1990’s which was designed to overcome the defect problem where branched dendritic polymers are connected to each other via the central core-mediated reactions. This method allows the formation of dendrimers by assembling surface units with reactive monomers resulting in inward growth. However the drawback of using this method is the poor final yield specifically relating to the higher generations which could be due to increased steric crowding at higher generations [64]. Using either of the two methods above (step-wise synthesis) as opposed to polymerisation process for preparing dendrimers ensures consistent molecular weight distribution, and that a defined number of peripheral groups and regular branching dendrimers are produced which can be used as versatile drug delivery platforms [65]. Other approaches have been used to synthesise dendrimers such as “Hypercore and branched monomers”; “Double exponential”; “Lego chemistry” and “Click chemistry” but these methods need to be refined further to ensure the cost effectiveness of these strategies for successful commercialisation of this technology [66].

There are several different classes of dendrimers depending on their synthesis pathway such as polyamidoamine (PAMAM) which is a mixture of amines and amides, polypropyleneimine (PPI), poly (glycerol-co-succinic acid), poly-L-lysine (PLL), melamine, triazine, poly (glycerol), poly [2,2-bis(hydroxymethyl)propionic acid] and poly(ethylene glycol) (PEG). In addition to these, carbohydrate and citric acid based dendrimers have been used for drug delivery purposes. However the most widely investigated dendrimers are the PAMAM and PPI-based [67]. The PAMAM dendrimers were first synthesised by Tomalia using the divergent method of synthesis and were the first commercialised and extensively investigated dendrimer family due to their unique and well defined structures [68]. PAMAM dendrimers consist of an ethylenediamine nucleus and branches based on methyl acrylate and ethylenediamine. Half generations of PAMAM possess carboxyl surface groups, while complete full generations have amine or hydroxyl groups. The surface groups are responsible for their high solubility and reactivity, and internal cavities can be used in encapsulation of small molecules [69].

Several areas of PAMAM dendrimer behaviour are incompletely understood e.g. toxicological profile, biocompatibility, biodistribution, biodegradation and unpredictable action and pharmacokinetics. Once researchers have managed to control the composition of dendrimers this will radically help overcome challenges to absorption, distribution, metabolism, excretion, and toxicity but simultaneously capitalise on dendrimers ability to participate in intracellular drug delivery, cross biological membranes, circulate in the body, and target specific cellular and tissue structures [63]. Although, the interest in dendrimers has increased in the past decade, only a few dendrimers have entered clinical trials. The US Food and Drug Administration (FDA) in July, 2003 authorised the first clinical trial for a dendrimer based drug formulation, Vivagel™ which is used for the prevention of HIV infection in women. Another dendrimer undergoing preclinical study is the multiantigenic peptide PHSCN-lysine dendrimer which is being applied in a metastatic murine cancer model [70].

Research into dendrimers and their future clinical applications in the biomedical, pharmaceutical or biopharmaceutical fields is still in its infancy. Understanding toxicity and what causes it, remains a very important question. It has been suggested that modifying the dendrimer structure could reduce toxicity [71]. Further research is still required to increase understanding of dendrimers in the pharmaceutical field where the following areas need to be addressed:

- reducing toxicity associated with PAMAM dendrimers by doing further *in vivo* and *in vitro* studies
- understanding how dendrimers interact with blood components and the effects that arise
- the effect of dendrimers on the immune system
- understanding the effect of dendrimers on cell functioning and how they interact with the vessel wall
- creating dendrimers that have low clearance rates and low plasma half-lives
- ascertaining the most efficient and safest route of administration for PAMAM dendrimers

Conclusion

Advances in science and nanotechnology based drug delivery systems have been used to improve the pharmacological and

therapeutic properties of drugs by increasing the bioavailability, solubility and permeability of many poorly drugs which otherwise would have been discarded in the development phase. One of the favoured approaches used in nanotechnology based drug delivery vehicles has been encapsulation. Encapsulation has many advantages: foremost, it protects a drug against degradation from the external environment of the body and specificity allows the release of the active drug at an identifiable therapeutic site. The size of nanocarriers allows them to penetrate and cross the blood-brain-barrier (BBB) and operate on a cellular level. The small size also has drawbacks particularly around aggregation of nanocarriers which makes physical handling very difficult. Other limitations of nanocarriers concern their very low drug loading capacity and efficiency, and poor uniformity of size.

Although effort has focussed on using nanocarriers to reduce toxic effects and limit side effects, it has become apparent that nanocarriers themselves may exert toxic and harmful effects to the body and a more detailed understanding is needed to ascertain how nanocarriers interact with living cells, organs and organisms to produce these toxic effects. To increase understanding of toxicology it requires a more integrated and collaborative approach between cell biologists and formulation scientists which would help to ensure ideas and concepts on the most optimum and safe nanocarriers become more commercially viable. To overcome toxicological concerns natural polymers (e.g chitosan or alginate) have been used to formulate nanocarriers which have been associated with lower toxic effects. Compared to conventional drugs, optimal nanocarriers, with their selective targeting, accumulate at the required therapeutic sites for drugs to have an effect therefore limiting the build-up of drug in healthy tissue, reducing toxicity and adverse side effects that would be associated with conventional chemotherapeutics.

Nanotechnology remains an area of interest which can be evidenced through the number of products undergoing preclinical evaluation. Despite this, only a small number of drug products have reached the pharmaceutical market (eg liposomal conjugates Doxil® (doxorubicin) or DaunoXome® (daunorubicin). Such is the interest in nanotechnology many countries like the United States, Japan and the European Union have set up dedicated research centres or initiatives to explore this area further for uses not only in healthcare but other areas of domestic life. The area of nanotechnology will keep on expanding and especially as the pharmaceutical industry becomes a lot more interested as they see the commercialisation of nanocarriers which will result in closer collaborative working between academic institutes and the pharmaceutical industry. The potential for nanotechnology to give an alternative approach to drug delivery, compared with current methods is very important for the development of poorly-soluble drugs, but this requires work in material design and formulation to be more closely aligned than currently. This harmonised working effort will ensure challenges such as scaling problems can be resolved through closer communication between nanofabrication engineers and drug delivery scientists.

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