A Review and Classification of Emerging Excipients in Parenteral Medications

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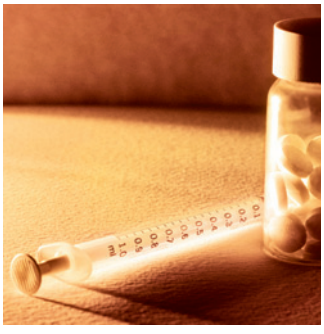

IMAGE 100

As parenteral drug delivery becomes more complex and sophisticated, excipients that can facilitate drug (or gene) delivery to specific therapeutic targets will be required. An overwhelming majority of these excipients are derived from natural sources.

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The International Pharmaceutical Excipients Council defines excipients as "substances, other than the active drug substance of finished dosage form, which have been appropriately evaluated for safety and are included in a drug delivery system to either aid the processing of the drug delivery system during its manufacture; protect; support; enhance stability, bioavailability, or patient acceptability; assist in product identification; or enhance any other attributes of the overall safety and effectiveness of the drug delivery system during storage or use" (1).

This definition implies that excipients serve a purpose in a formulation and contrasts with the old terminology, *inactive excipients*, which hints at the property of inertness. With a literal interpretation of this definition, an excipient can include diverse molecules or moieties such as replication incompetent viruses (adenoviral or retroviral vectors), bacterial protein components, monoclonal antibodies, bacteriophages, fusion proteins, and molecular chimera. For example, using gene-directed enzyme prodrug therapy, research indicated that chimera containing a transcriptional regulatory DNA sequence capable of being selectively activated in mammalian cells was linked to a sequence that encodes a β-lactamase enzyme and delivered to target cells (2). The expressed enzyme in the targeted cells catalyzes the conversion of a subsequently administered prodrug to a toxic agent. A similar purpose is achieved by using an antibody conjugated to an enzyme followed by the administration of a noncytotoxic substance that is converted in vivo by the enzyme to its toxic form (3). In these examples, the chimera or the enzyme-linked antibody would qualify as excipients.

Furthermore, many emerging delivery systems use a drug or gene covalently linked to the molecules, polymers, antibody, or chimera responsible for drug targeting, internalization, or transfection. Conventional wisdom dictates that such an entity be classified as the *active substance* or *prodrug* for regulatory purposes and be subject to one set of specifications for the entire molecule. The fact remains, however, that only a discrete part of this prodrug is responsible for the therapeutic effect, and a similar effect may be obtained by physically entrapping the drug as opposed to covalent conjugation. The situation is further complicated when fusion proteins are used as a combination of drug and delivery system or when the excipients themselves

are not entirely devoid of pharmacological activity. As parenteral drug delivery becomes more complex and sophisticated by adding diverse fields such as gene delivery, immunomodulation, and sustained and targeted release, differentiating the excipient from the active is likely to become increasingly difficult (33,37, 39,41,66,96,106).

Most of the literature on the subject of pharmaceutical excipients consists of compilations of exhaustive lists of conventional small inorganic molecules (4–7). These do not necessarily lend themselves to predicting trends in the evolution of excipients suitable for parenteral administration nor toward an understanding of the structure–property–application relationships that seem to govern the introduction of excipients into the therapeutic armamentarium. Hence, a need for a rationalization scheme exists for emerging excipients that will provide a tool for predicting such trends.

Table I: Natural products, including naturally occuring polymers and derivatives.

Name	Use
Squalene, squalane	Vaccine adjuvant
Phosvitin	Protein stabilizer
Phytic acid	Protein stabilizer
Phospholipids	Liposomes
Spermidine	Inhibitor of lipid peroxidation
Hyaluronic acid	Viscoelastic
Sphingomyelin	Component of liposomes
Biopolymer	Protective coating for liposomes
Fibronectin	Protein stabilizer
Spermine: bile acids	DNA transfection agent
Deacylated saponin	Surfactant in intranasal or ocular delivery
α-Hemolysin	Increasing susceptibility of tumor cells to cytotoxic drugs
Galactosylated Histone H1	Liver gene delivery
Sialic acid	RES-avoiding drug delivery
Galacturonic acid or polygalacturonic acid	Contrast medium in MRI
N-Acetyl [Phe^{8}(CH_2-NH)Arg^{9}] bradykinin	Transport across the blood brain barrier
Enoxaparin, heparin	Protein stabilizer
Cyclodextrins	Solubilizer for hydrophobic molecules
Phosvitin–galactomannan conjugate	Emulsifier
Pyridoxal phosphate or derivatives	Paramagnetic metal chelating agent in NMRI
Recombinant fusion streptavidin-mab protein	Transport across the blood brain barrier
Hyaluronic acid: cyclodextrin	Modification of depolymerization kinetics and release
Palmityl-D-glucuronide	RES-avoiding liposomal drug delivery
Transferrin	Ligand in receptor-mediated gene delivery

Because excipients are used to carry out various functions, previous authors have classified them into three categories: those that influence stability, those that influence release and absorption of the active principle, and those that influence manufacturability (8,9). Moreton suggested three types of "new" excipients that could be considered: new chemical entities, new grades of existing materials, and new combinations of existing materials (10). Giorgio et al. attempted to highlight emerging trends in the development of excipients (11). According to them, these trends consist of three main approaches: physical or minor chemical manipulation of materials that are already known, a combination of two or more marketed excipients to reduce unwanted defects, and preparation of new chemical entities.

To the authors' knowledge, a concerted attempt has not yet been made to group or categorize emerging excipients in such a way so that the resulting discernible trends, in their composition and use, can be used toward

- an attempt to rationalize the genesis and evolution of pharmaceutical excipients
- predicting the need and application for new pharmaceutical excipients
- providing a framework within which the necessity of incorporating a new pharmaceutical excipient into a product can be pursued with greater confidence
- identifying the experimental (not yet compendial) chemical compounds, which are already being used as excipients in the literature that fall within the framework of the proposed system.

A classification system such as the system described previously may neither be comprehensive in scope nor unique in concept; indeed, it is not the objective of this article to categorize each and every excipient used in parenteral formulations. The authors refer to several comprehensive reviews that address specific categories, including manuscripts addressing ligands used in receptor-mediated gene delivery (12), cationic lipids for gene delivery (13), dendrimers (14), targeted drug delivery (15), and polymeric biomaterials (16,17,151).This article focuses on a "rationalization by classification" scheme that aims to understand the structure–property–application relationships that seem to govern the introduction of emerging excipients into the therapeutic armamentarium. Excipients could admittedly also be classified on the basis of their function in the formulation such as antioxidants, buffers, and solubilizers. However, conventional molecular pharmacology dictates that "function follows form"; therefore, a categorization based on function alone may be less amenable as a predictor for new excipient molecules.

A review of the literature reveals that most of the emerging excipients can be categorized as the following:

- natural products, including naturally occurring polymers and derivatives
- synthetic polymers
- small molecules
- natural products (or natural polymers) modified with synthetic polymers or vice versa

Table II: Synthetic polymers or modifications.

Name	Use
Tyloxapol	Enhancement of dendrimer-mediated transfection
Hydroxypropyl-methacrylamide	Drug targeting
Histidyl poly(lysine)	Endosomolytic, enhanced transfection
Polyfumaric, polysebacic acid	Enhancing bioadhesive properties of polymers
Steryl poly(lysine)	Terplex nonviral DNA delivery
PLA–POE block copolymer	Modulated drug release, prevention of protein adsorption
PEG–poly(lysine) or polyaspartic acid block copolymer	Polymeric micellar drug carrier
PLGA	Sustained drug delivery
Polyaspartic acid or polyglutamic acid	Hydrophilic analogs of hydrophobic molecules
POE–poly(γ-benzyl L-glutamate) block copolymer	Nanoparticles for delivery of hydrophobic drugs
Poly(amidoamine) dendrimers	Vaccine adjuvant, drug entrapment, transfection

Table III: Small molecules.

Name	Use
DOTMA, polyquaternary ammonium salt lipids	DNA-transfecting agents
Mannosylglycerate	Enzyme stabilizer
Sucrose laurate	Solubilizer
DTPA	Chelating agent for paramagnetic metals in MRI
SAIB	High-viscosity liquid carrier for controlled depot delivery
Tranexamic acid	Solubilizer for nonglycosylated proteins
N-(-β-Hydroxyethyl)-lactamide	Solvent
N-Methyl pyrrolidinone	Solvent
Polidocanol	Enhancing gene transfer
Dimethyl sulfoxide	Solvent for embolyzing composition

- natural products (or natural polymers) modified with small molecules or vice versa
- synthetic polymers modified with small molecules or vice versa.

Natural products, including naturally occuring polymers and derivatives

Natural polymers and derivatives occur ubiquitously throughout the plant and animal kingdoms. Examples of polymers or derivatives that have been used or investigated as vaccine adjuvants are

- individual saponins derived from the South American tree Quillaja saponaria (18,19)
- keyhole limpet hemocyanin (KLH), a nonheme copper containing protein found in anthropods (20)
- MPL, a monophosphoryl derivative of the Lipid A molecule found in gram-negative bacteria
- Leishmania elongation initiation factor (LeIF), a protein produced by the parasite leishmania (21)
- ricin, a potent immunotoxin obtained from the seeds of castorbean plants (22)
- squalene, an isoprenoid found in large quantities in shark liver oil (23).

Intranasal or ocular formulations of insulin that contain a deacylated saponin derivative as a surfactant showed a dose-dependent hypoglycemic response in rats (24). Albumin, gelatin (25), deoxycholic acid, sesame oil (26), and gangliosides (27,28) are substances that occur naturally in the body and would be exquisitely suited as excipients.

The coupling of naturally occuring polyamines such as spermine or derivatives of bile acids allows the formation of facial amphiphiles that result in promising transfection agents (29,30). Spermidine also has been used to inhibit liposomal lipid peroxidation (31). Antifreeze glycoproteins, which are synthesized by fish living in polar regions (149), can be used to inhibit leakage from liposomes undergoing thermotropic phase transitions during lyophilization (32). Hyaluronic acid can be used as an excipient in a dry-powder nucleic acid composition composed of a cationic lipid–DNA complex for delivery to the lung (148). It also has been injected into the knee to restore the elasticity and viscosity of the synovial fluid in patients suffering from osteoarthritis (33). Sphingomyelins occur in the myelin sheaths of nerves and can be used as components of liposomes (34). A glycoprotic biopolymer excreted by a new Gram-negative species of bacteria, *Pseudoalteromonas antarctica* NF3, can be used to coat liposomes to protect the bilayers against the action of nonionic detergents that may be used during manufacture (35).

Histones are basic, small, compact proteins with a high affinity for DNA. They occur naturally attached to the DNA of cell nuclei by ionic links. Their classification is based on relative amounts of lysine and arginine. The galactosylated lysine–rich histone H1 was found to be superior to the H2–H4 histones as a DNA carrier for liver gene delivery (36).

Heparin and heparin-like polyanions are known to have significant stabilizing effects on proteins. Heparin has been used as a constituent of the solution that is used to reconstitute lyophilized proteins (37), and enoxaparin is a low molecular weight fragment of heparin that was found effective in preventing heat-induced protein agglomeration (38). Phosvitin, a

Table IV: Modifications of natural products with synthetic polymers.

Name	Use
Tocopherol–PEG–succinate	Antioxidant or solubilizer
Polyrotaxanes	Drug delivery
Galactosylated poly(lysine)	Gene delivery vector
SP-B: poly(lysine)	Gene delivery vector
α-2-Macroglobulin: poly(lysine)	Gene delivery vector
Carboxyphenoxypropane: sebacic acid copolymer	Implantable drug delivery system
Polyoxyethylated derivative of castor oil	Surfactant, emulsifier
Galactosylated Poly(ethyleneimine)	Lectin-mediated gene transfer to hepatocytes

Table V: Modifications of natural products or polymers with small molecules.

Name	Use
Spermine: cholesteryl carbamate, fatty acids or alcohols	DNA transfection vectors
N-Octyl-glucoside	Proteoliposome preparation using detergent-mediated dialysis
Palmitoyl glycol chitosan	Controlled drug delivery
Glycated chitosan	Immunoadjuvant
N,N,N-Trimethyl chitosan	DNA complexing agent
Chlorogenic acid chitosan	Confer water solubility at basic pH
Digalactosyl glycerol	Component of liposomes
Sulfolipo cyclodextrin derivatives	Vaccine adjuvant
Sulfobutyl ethers of cyclodextrins	Solubilizer with reduced hemolytic potential
Polymers of acylated amino acids (Proteinoids)	Oral protein delivery
DOSPA: histamine- or protamine-derived peptides	Enhancing cationic lipid–mediated gene transfection
Acyl carnitines	Reduction of side effects
3-[(3-Cholamidopropyl)-dimethylammonio] -1-propane sulfonate	Solubilizer

Table VI: Modifications of synthetic polymers with small molecules.

Name	Use
Poly(ethyl acrylic acid), Poly(propyl acrylic acid)	pH-dependent membrane lysis
Long-chain alkyl amine substituted poly(acrylic acid)	Solubilizer
Polypeptides, DTPA	Drug delivery

phosphoprotein isolated from egg yolk, and phytic acid, a naturally occuring phosphorylated carbohydrate that has been shown to suppress the growth of epithilial cancers, also were effective in stabilizing proteins (38,39). Polygalacturonic acid derived from the hydrolysis of pectin has the potential to be used as a contrast medium in magnetic resonance imaging (MRI) (40). *N*-Acetyl [Phe^{8}(CH_2-NH)Arg^{9}] bradykinin (Cereport, Alkermes Inc., Cambridge, MA), is modified bradykinin that increases the permeability of the blood brain barrier to enable delivery of drugs to the brain (41–43).

Cyclodextrins (44) are naturally occuring clathrates obtained by the action of *Bacillus macerans* amylase on starch to form homogeneous D-glucopyranose linked units. Their fluoro analogs can be used to encapsulate extremely hydrophobic compounds (45). Phosvitin can be conjugated to galactomannan to yield an excellent emulsifying agent (46). Dipyridoxyl phosphate and its derivatives can be used as paramagnetic metal chelating agents in nuclear magnetic resonance imaging (NMRI)–contrast agent compositions (47–49). The depolymerization kinetics of hyaluronan (HA) can be modified by direct coupling of β-cyclodextrin to HA carboxylic acid groups. The degree of substitution can be used to advantage to modify the release kinetics of entrapped drugs (50). Streptavidin or avidin genetically fused to an antibody specific for the transferrin receptor can transport biotinylated drugs across the blood brain barrier (51,52).

The expanding area of gene delivery is likely to witness the incorporatation of many novel natural products as excipients. An example is listeriolysin O, a thiol-activated protein. This is a bacterial component of a targeted gene delivery system that, when combined with a polynucleotide and a binding agent, can lyse the endosome of the targeted cell, thereby causing the internalized polynucleotide to be released into the cytoplasm (53,54).

The transferrins are a group of homologous nonheme, iron-binding glycoproteins widely distributed in nature. The transferrin receptor is elevated on some tumors, including gliomas and hematopoietic tumors, and therefore suited to gene delivery (55). Gene transfer to K562 hematopoietic leukemic cells was achieved with a transferrin-polycation (poly-L-lysine or protamine) conjugate (56). Melanoma cells were transfected with the gene for interleukin-2, which resulted in a succesful tumor vaccine (57).

Bacterial viruses capable of binding mammalian cells expressing the growth factor receptor ErbB2 and undergoing receptor-mediated endocytosis have been engineered to package the green fluorescent protein (GFP) reporter gene that is driven by the CMV promoter. After application to cells, GFP expression occurred only in cells overexpressing ErbB2 (58).

Bacterial pore-forming proteins such as α-hemolysin secreted by *Staphylococcus aureus* can be modified so that pore formation is activated by chemical, biochemical, or physical triggers. Such hemolysins, when targeted to tumors, could increase their permeability and therefore susceptibility to various cytotoxic drugs (59).

Palmitic acid is conjugated to glucuronic acid to form a reticulo endothelial system (RES)–avoiding liposome delivery system (60). Phospholipids such as phosphatidyl choline or phos-

phatidyl ethanolamine are used as constituents of lipid complexes or liposomes (61,62). Table I is a partial list of the names and uses of excipients in this category.

Synthetic polymers or modifications

A plethora of synthetic polymers has emerged as constituents of targeted- or sustained-release drug delivery systems. Their abundant use may be ascribed to the ease with which they can be structurally (and, consequently, behaviorially) modified to suit specific applications. They encompass aliphatic polyesters such as polylactic glycolic acid (PLGA), poly(ε-caprolactone), poly(hydroxy butyrate), poly(vinyl alcohol), poly(ethylene oxide), poly(ortho esters), poly(cyano acrylates), polyamides, polyphosphazines, poly (methyl methacrylates), poly(*N*-alkylacrylamides), and their relevant permutations and combinations.

Polymers that undergo strong conformational changes upon occurence of small changes in the environment (e.g., pH, temperature, and ionic strength) can be used as biomimetic actuators for drug delivery. A classic example includes the development of a glucose-sensitive insulin-releasing system that consists of copolymers of *N*-isopropylacrylamide and acrylic acid or poly[(*N*,*N*-dimethylamino)ethyl methacrylate-co-ethylacrylamide] that collapses or expands depending on gel pH (142–145).

When "smart" polymers are integrated into a microcapsule wall or a liposomal lipid bilayer, the conformational transition of the polymer affects the integrity of the microcapsule or liposome as a result of pH or temperature changes and allows the regulated release of the drugs loaded into the microcapsule or liposome (146,147). PLGA is a polymer of lactic acid and glycolic acid, substances that are found naturally in the human body (63). Polyglutamic acid or polyaspartic acid can be conjugated to paclitaxel to produce its water-soluble analog (64).

Tyloxapol is an oligomer of octoxynol 9 (Triton X-100, Sigma, St. Louis, MO), which is used in respiratory distress syndrome and as a mucolytic in cystic fibrosis (65,66). It also can be used to enhance dendrimer-mediated transfection (67). Anhydride oligomers that enhance the bioadhesive properties of a polymer can be synthesized from dicarboxylic acid monomers such as maleic acid (68). Water-soluble polymer conjugates that are based on *N*-(2-hydroxy-propyl)-methacrylamide (HPMA) are currently in clinical trials (71). One such agent is an HPMA-copolymer conjugate of doxorubicin with a peptidyl spacer cleavable by intracellular lysosomal cysteine protease enzymes, which are found in high levels in certain types of tumors.

Poly(amidoamine) (PAMAM) dendrimers are manufactured by a divergent repetitive growth technique and are typically based on an ethylene diamine core (72). They can be used as an adjuvant for influenza antigen and similar materials (73). Linear PAMAM polymers that have amido and tertiary amino groups along the main polymer undergo pH-dependent conformational changes and thus can be used as potential endosomolytic agents to improve transfection efficiency (74). Poly(oxyethylene) (POE) has been piggybacked onto poly(γ-benzyl L-glutamate) to produce nanoparticles for the delivery of hydrophobic drugs (75,76). Polyanhydrides synthesized from ricinoleic acid half esters with maleic and succinic anhydrides possess the desired physicochemical and mechanical properties for use as drug carriers (77). Poly(phosphoesters) are biodegradable polymers that are being pursued for use in controlled-release delivery systems (78,79).

Stearyl-poly(L-lysine) is a constituent of a terplex nonviral DNA delivery system (80). Poly(lysine) can be partially substituted with histidyl residues, which become cationic upon protonation of the imidazole groups at pH $<$6.0, and therefore can be used to improve transfection efficiency by endosomolysis (81). To prevent proteins from adsorbing on homopolymers such as poly(lactic acid) (PLA), hydrophilic polymers such as POE have been used to cover the hydrophobic PLA surface. A further modification resulted in microspheres that were based on POE–PLA triblock copolymers (i.e., PLA–POE–PLA) for drug delivery. Piggybacking POE onto PLA also modified the degradation kinetics of the PLA block (82). Polymeric micelles based on block copolymers have been used to conjugate or physically entrap anticancer drugs or used as gene carriers (83,84). Table II is a partial list of excipients in this category.

Small molecules

A whole generation of cationic lipids modeled after the naturally occuring bilayer-forming phospholipids has emerged as DNA-complexing agents. Quaternary ammonium salt lipids such as dioleoylpropyl-trimethylammonium chloride (DOTMA) were proposed as DNA delivery agents (85). Further substitution with alkylene alcohol or alkylene amines resulted in compounds that displayed enhanced transfection activities (86,87). Polyquaternary ammonium salt lipids with decreased cytotoxicity or enhanced gene transfer have been proposed (88–90). Amidinium moieties, including guanidinium and polyguanidinium groups, have been introduced into cationic lipids to yield "second-generation" transfection agents (91,92).

Sucrose acetate isobutyrate (SAIB) is a sucrose molecule esterified with acetic and isobutyric acid moieties (93). SAIB is a high-viscosity liquid carrier material that is insoluble in water and significantly decreases in viscosity when mixed with a solvent to enable the controlled delivery of a substrate in vivo. Sucrose laurate has been investigated as a solubilizer for hydrophobic drugs (94). Diethylene triamine pentaacetic acid (DTPA) has been used as a chelating agent in MRI contrast-enhancing i.v. preparations (95).

An ingenious approach has been applied to the formulation of recombinant reteplase injection in which tranexamic acid, an antifibrinolytic agent, has been used as an excipient to increase the solubility of the nonglycosylated plasminogen activator (96,97). *N*-(-β-Hydroxyethyl)-lactamide, a clear, colorless, syrupy, water-miscible liquid, has been used in Europe as a solvent for oxytetracycline injectables (98,99).

1-Methyl-2-pyrrolidinone is the subject of a patent in which it is used to dissolve the lactone stable form of highly lipophilic camptothecin derivatives (100). This solution, upon further dilution with a suitable parenteral vehicle, provides an i.v. infusion of the drug.

Dimethyl sulfoxide is used to solubilize an embolyzing composition containing a biocompatible polymer that is insoluble

in blood (101). Capric–Caprylic acid is conjugated to propylene glycol to form the ester used in an intraspinal sustained-release delivery system (102). Fluorosurfactants are modifications of phospholipids that contain hydrophobic fluorinated fatty acid chains and phosphatidyl amines as a polar hydrophilic head group (103). Mannosylglycerate has been used as an excipient to stabilize enzymes against thermal stress and freeze drying (104). The nonionic detergent sclerosing agent, polidocanol, has been used to modify the epithilial barrier in nasal airways in an attempt to enhance adinoviral gene transfer (152,153). Table III is a partial list of excipients used in this category.

Modifications of natural products with synthetic polymers

Tocophersolan polyethylene glycol (PEG) succinates are combinations of tocopherol (vitamin E), PEG, and succinic acid used as antioxidant–solubilizers or used to inhibit *P*-glycoprotein-mediated multidrug resistance (105,106). Polyrotaxanes are interlocking, self-assembling molecular systems, originally envisaged as improved solid polymer electrolytes because of their ability to function as "molecular shuttles" (107). They can be made biodegradable by "threading" cyclodextrins onto a single PEG chain capped with bulky endgroups and either conjugated with drug molecules or physically entrapping them (108,109).

One of the constituents of pulmonary surfactant (surfactant protein B [SP-B]) can be covalently attached to poly(lysine) to enable efficient transfection of DNA to the lung (110). Carboxyphenoxypropane is copolymerized with sebacic acid, an endogenous fatty acid, to produce a biodegradable polymeric implant (111, 112). Cremophor is a polyoxyethylated derivative of castor oil (113). Similarly, fatty acids also can be polyoxyethylated to produce surfactants (114).

Galactosyl residues coupled with poly(ethyleneimine) (PEI) gave an efficient vector that could selectively transfect hepatocytes by using the asialoglycoprotein receptor–mediated endocytosis (115,116). Galactosylated poly(L-lysine) and the protein α-2-macroglobulin conjugated with poly(lysine) have been used to deliver genes to the liver (117,118). Table IV is a partial list of excipients in this category.

Modifications of natural products (or polymers) with small molecules

Chitosan (deacylated chitin) can be modified by the addition of a mono or oligosaccharide side chain to its free amino groups. Such glycated chitosans can be used as immunoadjuvants in laser- or sensitizer-assisted immunotherapy (119). Chitosan can also be quaternized to form a polyelectrolyte complex with DNA and galactose residues attached to the resulting molecule for increased cellular recognition (120,121). Palmitic acid has been covalently attached to glycol chitosan, a water-soluble derivative of chitosan, to yield an

amphiphilic polymer that forms a hydrogel as a result of the hydrophobic interaction of the palmitoyl groups (150). This gel can serve as a matrix for controlled drug delivery.

Carnitine is an essential cofactor of fatty acid metabolism and is a constituent of striated muscle and liver. Acylcarnitines have been used as absorption enhancers from the gastrointestinal tract (122). They also have been used to reduce the side effects associated with angiotensin-converting enzyme inhibitors used in the treatment of cardiovascular disorders (123).

A bilayer-forming galactolipid, digalactosyldiacylglycerol can be used in liposomal drug delivery systems (124). Chlorogenic acid is enzymatically grafted onto chitosan to confer water solubility to the polymer under both acidic and basic conditions (125).

Cyclodextins can be modified to less-hemolytic sulfobutyl ethers (127) or to sulfolipo derivatives, which, when administered in conjunction with squalane-in-water emulsion, demonstrated strong adjuvanticity, low reactogenicity, and good stability as a vaccine adjuvant (128).

A sulfobetaine derivative of cholic acid was originally proposed as a new nondenaturing, deaggregating, and electrically neutral detergent for membrane biochemistry (129) and may be used as a solubilizer for hydrophobic molecules. Proteinoids are condensation polymers of modified (acylated) amino acids (130) and are used as microspheres for oral delivery of proteins.

Polyamine spermine, which occurs naturally in the nucleus, plays a role in DNA compaction during cell division. The introduction of a carboxyl-spermine analog into a lipopolyamine to yield dioctadecyl amidoglycyl spermine afforded increased DNA transfection efficiencies compared with quaternary ammonium salts (131). A series of linear polyamines comprising spermine or spermidine derivatives linked to hydrophobic groups such as cholesteryl carbamate, fatty alcohols, or fatty acids displayed an increased activity for transfection of pulmonary epithilium cells (132,133). Structural variations of these polyamines have been proposed, including the modification of the polyamine, the spacer, the lipid moiety of the polyamine, and the introduction of a side chain suitable for targeting (134,135). Polyamine has been combined with quaternary ammonium salt to form *N*-[2-({2,5-bis [(3-aminopropyl) amino]-1-oxypentyl} amino)ethyl]-*N*,*N*-dimethyl-2,3-bis(9-octadecynyloxy)-1-propanaminium trifluoroacetate (DOSPA), which, after formulation with dioctyl phosphatidyl ethanolamine, yields the highly efficient transfection agent Lipofectamine (Invitrogen Life Sciences, Carlsbad, CA) (136).

Precompacting DNA with Histone H1 or protamine-derived peptides has been shown to enhance cationic lipid–mediated gene transfer in vitro and in vivo in models of Lewis lung carcinoma (137,138). Protamine, when added to plasmid DNA solution before the formation of DNA–lipid

vesicle complexes, enhanced transfection efficiency and expression level to selected cell lines, presumably by protecting DNA from deoxyribonuclease degradation and by promoting delivery to the nucleus (139).

The nonionic surfactant *N*-octyl-glucoside has been used to incorporate proteins into liposomes using detergent-mediated dialysis (140,141). Table V is a partial list of excipients in this category.

Modifications of synthetic polymers with small molecules

Polyacrylic acids have been substituted with long-chain alkyl amines to yield substantially nonhemolytic solubilizers for hydrophobic drugs (69). Poly(ethyl acrylic acid) or Poly(propyl acrylic acid) can be engineered with a varying degree of substitution of the pendent ethyl or propyl groups to efficiently disrupt eukaryotic membranes within defined and narrow pH ranges (70). Long polypeptides can be unfolded by a large degree of substitution with stearic hindrance molecules such as diethylene triamine pentaacetic acid to enhance drug delivery to tumor tissue (126). Table VI is a partial list of excipients in this category.

Conclusion

If individual components of emerging drug delivery systems are accepted as excipients according to the definition in reference 1, a majority of these excipients could be included in the categories previously described. This inclusion would imply that the majority of the objectives of drug formulation and delivery are being met by chemical compounds that are natural products (polymers), synthetic polymers, or small molecules and their various permutations and combinations.

Natural products and polymers (natural and synthetic) or their modifications may assume increased importance as the excipients of tomorrow. As is usually the case, naturally occuring molecules may be improved upon (i.e., in areas such as reduced toxicity or enhanced drug delivery or stability) by subunit selection or epitope identification to the point where they eventually transmute into semisynthetic or synthetic moieties. Some examples of such transitions include synthetic cationic lipids which have evolved from naturally occuring phospholipids to nuclear localization signal sequences (NLS), which are small peptide epitopes of naturally occuring proteins that facilitate nuclear cell trafficking.

The investigative use of new excipients is driven by the need to deliver drugs to specified therapeutic targets. Excipients that have never been used before must pass formidable regulatory requirements before being incorporated into approved dosage forms. Such requirements include, but are not limited to, comprehensive toxicology (including acute, chronic, and reproductive) and pharmacokinetic and carcinogenic studies as outlined in the ICH S7, S3A, S3B, S2B, and S5A and the US Pharmacopeia. The need for streamlining procedures and for formulating consistent guidelines to expedite regulatory approval of novel excipients should be, and is being, addressed. Delivering drugs (genes) to ever more specific (and inaccessible) cellular therapeutic targets may eventually require delivery systems to mimic natural cellular trafficking mechanisms or to evade degradation or misdirected delivery through the body's myriad built-in defense mechanisms. Intuitively, such delivery systems or excipients would best achieve this objective if they were composed of natural products or their modifications. An overwhelming presence of excipients in this category lends credence to this observation. Using natural products composed of complex proteins, antibodies, chimera, or toxins in lieu of small inorganic molecules as excipients for parenteral drug delivery represents a paradigm shift in the introduction of emerging excipients in the therapeutic armamentarium.

It seems conceivable that in the near future, kilogram quantities of fusion proteins, fibronectin, poly(lysine), or α-hemolysin could become available as off-the-shelf excipients or as designer excipient kits. This scenario is even more plausible considering that moieties that were unheard of a decade ago are now routinely available for use as excipients or in biochemical research (e.g., Lipofectamine, poly(lactide-co-glycolide), PAMAM dendrimers, tocopherol PEG succinate, etc.).

References

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