PULSATILE RELEASE COMPOSITIONS AND METHODS FOR ENHANCED INTESTINAL DRUG ABSORPTION

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Publication Classification

(51) Int. Cl7 .......................... A61K 48/00; A61K 9/50
(52) U.S. Cl. ........................................... 424/499; 514/44

ABSTRACT

Delayed release oral pharmaceutical formulations and methods for enhanced intestinal drug absorption. The formulation comprises a first population of carrier particles comprising a drug and a penetration enhancer which are released at a first location in the intestine, and a second population of carrier particles comprising a penetration enhancer and a delayed release coating or matrix. This penetration enhancer is released at a second location in the intestine downstream from the first location and enhances absorption of the drug when it reaches the second location.
Fig. 3A
Immediate Release

Fig. 3B
Pulsatile

Lumen PE concentration
Time
Maximal Effect Level
PULSATILE RELEASE COMPOSITIONS AND
METHODS FOR ENHANCED INTESTINAL DRUG
ABSORPTION

FIELD OF THE INVENTION

[0001] The present invention relates to compositions and methods that enhance the intestinal absorption of drugs, particularly oligonucleotides. More particularly, the invention relates to oral pharmaceutical formulations that deliver a first pulse of drug combined with a penetration enhancer and a second pulse of penetration enhancer to promote absorption of drug which is not absorbed upon release with the first pulse of penetration enhancer.

BACKGROUND OF THE INVENTION

[0002] Advances in the field of biotechnology have led to significant advances in the treatment of diseases such as cancer, genetic diseases, arthritis and AIDS that were previously difficult to treat. Many such advances involve the administration of oligonucleotides and other nucleic acids to a subject, particularly a human subject. The administration of such molecules via parenteral routes has been shown to be effective for the treatment of diseases and/or disorders. See, e.g., Draper et al., U.S. Pat. No. 5,595,978, Jan. 21, 1997, which discloses intravital injection as a means for the direct delivery of antisense oligonucleotides to the vitreous humor of the mammalian eye. See also, Robertson, Nature Biotechnology, 1997, 15, 209, and Genetic Engineering News, 1997, 15, 1, each of which discuss the treatment of Crohn’s disease via intravenous infusions of antisense oligonucleotides.

[0003] Oral administration of drugs, including oligonucleotides and other nucleic acids, offers the promise of simpler, easier and less injurious administration without the need for sterile procedures and their concomitant expenses, e.g., hospitalization and/or physician fees. However, the absorption of orally administered drugs is often poor. One approach to enhancing the absorption of orally administered drugs is pulsatile release formulations in which multiple doses of drug are released from a single formulation by the use of delayed release coatings. (U.S. Pat. Nos. 5,508,040, 6,117, 450, 5,840,329, 5,814,336, and 5,686,105, the entire contents of which are incorporated herein by reference). There is a need to provide compositions and methods to enhance the absorption and bioavailability of orally administered drugs, particularly oligonucleotides.

SUMMARY OF THE INVENTION

[0004] Because of the advantages of oral delivery of drugs, including antisense oligonucleotides, the compositions and methods of the invention can be used in therapeutic methods as explained in more detail herein. The compositions and methods herein provided may also be used to examine the function of various proteins and genes in an animal, including those that are essential to animal development. The methods of the invention can be used, for example, for the treatment of animals that are known or suspected to suffer from any disease treatable with an oral pharmaceutically active compound, such as ulcerative colitis, rheumatoid arthritis, Crohn’s disease, inflammatory bowel disease, or undue cellular proliferation.

[0005] One embodiment of the present invention is a delayed release oral formulation for enhanced intestinal drug absorption, comprising:

(b) a second population of carrier particles comprising a penetration enhancer and a delayed release coating or matrix, wherein the penetration enhancer is released at a second location in the intestine downstream from the first location, whereby absorption of the drug is enhanced when the drug reaches the second location. Preferably, the drug is a protein, peptide, nucleic acid, oligonucleotide, peptide hormone, antibacterial, antimicrobial agent, vasoconstrictor, cardiovascular drug, vasodilator, enzyme, bone metabolism controlling agent, steroid hormone, antihypertensive, non-steroidal anti-inflammatory agent, antithrombin, antithrombus, expectorant, chemotherapeutic agent, sedative, antidepressant, beta-blocker, analgesic and angiotensin converting enzyme (ACE) inhibitor. In one aspect of this preferred embodiment, the oligonucleotide is an antisense oligonucleotide. Preferably, the penetration enhancer in (a) and (b) is the same. Alternatively, the penetration enhancer in (a) and (b) is different. In one aspect of this preferred embodiment, the penetration enhancer is a fatty acid, bile acid, chelating agent or non-chelating non-surfactant. Advantageously, the fatty acid is arachidonic acid, oleic acid, laurie acid, capric acid, caprylic acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicarboxyl tricarboxyl, monoklein, dilaurin, glycerin 1-monocarboxyl, 1-dodecylacyclocloheptan-2-one, an acylamidone, an acylehohol, a monoglyceride or a pharmaceutically acceptable salt thereof. Preferably, the bile acid is cholic acid, dehydrocholic acid, deoxycholic acid, cholic acid, glycocholic acid, glycochenodeoxycholic acid, taurocholic acid, taurochenodeoxycholic acid, chenodeoxycholic acid, ursodeoxycholic acid, sodium tauro-24, 25-dihydroxisulfate, sodium glycodihydroxisulfolate, polyoxyethylene-9-lauryl ether or a pharmaceutically acceptable salt thereof. In one aspect of this preferred embodiment, the chelating agent is EDTA, citric acid, a salicylate, an N-acetyl derivative of collagen, laurate-9, an N-amino acyl derivative of a beta-diketone or a mixture thereof. Advantageously, the non-chelating non-surfactant is an unsaturated cyclic urea, 1-alkyl-alkanone, 1-alkenylacycloalkanone, steroid anti-inflammatory agent or mixtures thereof. Preferably, the formulation is a capsule, tablet, compression coated tablet or bilayer tablet. In one aspect of this preferred embodiment, the carrier particles are biodegradable. Advantageously, the carrier particles comprise poly-amino acids, polypeptides, polycarboxylates, polyalkylacrylates, polyoxethanes, polyalkylcyanoacrylates, cationized gelatins, albumins, starches, acrylates, polyethylene glycol, DEAE-derivatized polylamines, polysulphines, celluloses, chitosan, poly-L-lysine, polyhistidine, polyornithine, polylysines, protamine, polyvinylpyridine, polypropylenedimethylene-polyethyleneplamine-polyethylene glycol, poly(ethyleneoxide)ester, poly(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(alcoxyalkylcyanoacrylate), poly(isoalkylcyanoacrylate), polyamide, polyurethane, polycaprolactone, polyolefin and polyethylene. The oligonucleotide may be an antisense oligonucleotide, a catalytic oligonucleotide, a ribozyme, a pseudoribozyme, a ribozyme, or a ribozyme-antisense oligonucleotide hybrid.
DEAE-methacrylate, DEAE-ethylhexylacrylate, DEAE-acrylamide, DEAE-albumin, DEAE-dextran, poly(methylacrylate), poly(ethylene glycol) (PEG), poly(DL-lactic-co-glycolic acid) (PLGA) or polyethylene glycol (PEG). In one aspect of this preferred embodiment, the carrier particles are cationic. Advantageously, the carrier particles comprise a complex of poly-L-lysine and alginates, a complex of protamine and alginates, lysine, diolysine, triolysine, calcium, albumin, glucosamine, arginine, galactosamine, nicotinamide, creatine, lysine-ethyl ester or arginine ethyl-ester. Preferably, the delayed release coating or matrix is acetate phthalate, propylene glycol, sorbitan monololate, cellulose acetate phthalate (CAP), cellulose acetate trimellitiate, hydroxypropyl methyl cellulose phthalate (HPMCP), methacrylates, chitosan, guar gum or polyethylene glycol (PEG).

DETAILED DESCRIPTION OF THE DRAWINGS

[0008] FIG. 1 is a graph showing the plasma concentration of oligonucleotide over time after intrajejunal administration to monkeys. Saturation of oligonucleotide uptake pathways does not occur at the doses studied.

[0009] FIG. 2 is a graph showing the plasma concentration of oligonucleotide in monkeys after a 30 minute infusion into a region of intestine made permeable by a 50 mg/kg bolus of sodium caprate at t=5 minutes.

[0010] FIGS. 3A-B are schematic diagrams showing the concentration of penetration enhancer in the intestinal lumen after administration of an immediate release formulation (FIG. 3A) and the pulsatile release formulation of the present invention (FIG. 3B).

DETAILED DESCRIPTION OF THE INVENTION

[0011] The present invention provides oral pharmaceutical compositions that result in enhanced intestinal absorption of biologically active substances. In particular, the present invention provides compositions and methods for enhancing the intestinal absorption of drugs, preferably antisense oligonucleotides and other nucleic acids, thereby circumventing the complications and expense which may be associated with intravenous and other parenteral routes of administration. This enhancement is obtained by encapsulating at least two populations of carrier particles. The first population of carrier particles comprises a biologically active substance and a penetration enhancer, and the second (and optionally additional) population of carrier particles comprises a penetration enhancer and a delayed release coating or matrix.

[0012] Enhanced bioavailability of biologically active substances is also achieved via the oral administration of the compositions and methods of the present invention. The term “bioavailability” refers to a measurement of what portion of an administered drug reaches the circulatory system when a non-parenteral mode of administration is used to introduce the drug into an animal. The term is used for drugs whose efficacy is related to the blood concentration achieved, even if the drug’s ultimate site of action is intracellular (van Berge-Henegouwen et al., Gastroenterol., 1977, 73, 300). Traditionally, bioavailability studies determine the degree of intestinal absorption of a drug by measuring the change in peripheral blood levels of the drug after an oral dose (DiSanto, Chapter 76 In: Remington’s Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990, pages 1451-1458). The area under the curve (AUC<sub>0</sub>) is divided by the area under the curve after an intravenous (i.v.) dose (AUC<sub>i.v.</sub>) and the quotient is used to calculate the fraction of drug absorbed. This approach cannot be used, however, with compounds which have a large “first pass clearance,” i.e., compounds for which hepatic uptake is so rapid that only a fraction of the absorbed material enters the peripheral blood. For such compounds, other methods must be used to determine the absolute bioavailability (van Berge-Henegouwen et al., Gastroenterol., 1977, 73, 300). With regards to oligonucleotides, studies suggest that they are rapidly eliminated from plasma and accumulate mainly in the liver and kidney after i.v. administration (Miyao et al., Antisense Res. Dev., 1995, 5, 115; Takakura et al., Antisense & Nucl. Acid Drug Dev., 1996, 6, 177).

[0013] Another “first pass effect” that applies to orally administered drugs is degradation due to the action of gastric acid and various digestive enzymes. Furthermore, the entry of many high molecular weight active agents (such as peptides, proteins and oligonucleotides) and some conventional and/or low molecular weight drugs (e.g., insulin, vasopressin, leucine enkephalin, etc.) through mucusal routes (such as oral, pulmonary, buccal, rectal, subdermal, vaginal and ocular) to the bloodstream is frequently obstructed by poor transport across epithelial cells and concurrent metabolism during transport. This type of degenerative metabolism is known for oligonucleotides and nucleic acids. For example, phosphodiesterases are known to cleave the phosphodiester linkages of oligonucleotides and many other modified linkages present in synthetic oligonucleotides and nucleic acids.

[0014] One means of ameliorating first pass clearance effects is to increase the dose of administered drug, thereby compensating for proportion of drug lost to first pass clearance. Although this may be readily achieved with i.v. administration by, for example, simply providing more of the drug to an animal, other factors influence the bioavailability of drugs administered via non-parenteral means. For example, a drug may be enzymatically or chemically degraded in the alimentary canal or blood stream and/or may be impermeable or semipermeable to various mucosal membranes.

[0015] The delayed release pulsatile oral pharmaceutical formulations of the present invention comprise at least two populations of carrier particles. The first population of carrier particles comprises a biologically active substance of interest and a penetration enhancer, also known as an absorption enhancer. These are substances which facilitate the transport of a biologically active substance across mucosal surfaces and other epithelial cell membranes, particularly the intestinal mucosa. The first population of carrier particles is released from the formulation at a first location in the intestine and quickly release the biologically active substance and the penetration enhancer. The penetration enhancer promotes absorption of the biologically active substance; however, because the enhancer is quickly absorbed, there is often an insufficient amount of enhancer to promote absorption of the entire dose of biologically active substance. The present invention solves this problem.
by providing a second (and optionally additional) population of particles comprises a penetration enhancer and a delayed release coating or matrix. Because of the delayed release coating or matrix, the penetration enhancer in the second population of carrier particles is released in the intestine downstream from the first location where it promotes further absorption when the biologically active substance reaches this site. The penetration enhancer in the first population of carrier particles may be either the same or different from the penetration enhancer in the second set of carrier particles.

[0016] Biologically active substance refers to any molecule or mixture of complex of molecules that exerts a biological effect in vitro and/or in vivo, including pharmaceuticals, drugs, proteins, vitamins, steroids, polyanions, nucleosides, nucleotides, oligonucleotides, polynucleotides, etc.

[0017] Drugs refer to any therapeutic or prophylactic agent which is used in the prevention, diagnosis, alleviation, treatment, or cure of a disease in an animal, particularly a human. Therapeutically useful oligonucleotides and polypeptides are within the scope of this definition for drugs.

[0018] Penetration enhancers include, but are not limited to, members of molecular classes such as surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactant molecules. (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 92). Carriers are inert molecules that may be included in the compositions of the present invention to interfere with processes that lead to reduction in the levels of bioavailable drug.

[0019] In connection with the present invention, surfactants (or “surface-active agents”) are chemical entities which, when dissolved in an aqueous solution, reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the result that absorption of oligonucleotides through the alimentary mucosa and other epithelial membranes is enhanced. In addition to bile salts and fatty acids, surfactants include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-ethyl ether (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 92; and fluorocarbon emulsions, such as FC-43 (Takahashi et al., J. Pharm. Pharmacol., 1988, 40, 252).

[0020] Fatty acids and their derivatives which act as penetration enhancers and may be used in compositions of the present invention include, for example, oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolin (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glyceryl 1-monocaprate, 1-docosylazacycloheptan-2-one, acylcarbinol, acylethanes and mono- and di-glycerides thereof and/or physiologically acceptable salts thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linolate, etc.) (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 92; Muramishid, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1; El-Hariri et al., J. Pharm. Pharmacol., 1992, 44, 651).

[0021] A variety of bile salts also function as penetration enhancers to facilitate the uptake and bioavailability of drugs. The physiological roles of bile include the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (Brunton, Chapter 38 In: Goodman & Gilman’s The Pharmacological Basis of Therapeutics, 9th Ed., Hardman et al., eds., McGraw-Hill, New York, N.Y., 1996, pages 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus, the term “bile salt” includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. The bile salts of the invention include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), cholic acid (sodium cholate), glycocholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (CDCA, sodium chenodeoxycholate), ursocholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate (STDFH), sodium glycodeoxyfusidate and polyoxylethylene-9-lauryl ether (POE) (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 92; Swinyard, Chapter 39 In: Remington’s Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990, pages 782-783; Muramishid, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1; Yamamoto et al., J. Pharm. Exp. Ther., 1992, 263, 25; Yamashita et al., J. Pharm. Sci., 1990, 79, 579).

[0022] In a particular embodiment, penetration enhancers useful in the present invention are mixtures of penetration enhancing compounds. For example, a particularly preferred penetration enhancer is a mixture of UDCA (and/or CDCA) with capric and/or lauric acids or salts thereof, e.g. sodium. Such mixtures are useful for enhancing the delivery of biologically active substances across mucosal membranes, in particular the intestinal mucosa. Preferred penetration enhancer mixtures comprise about 5-95% of bile acid or salt(s) UDCA and/or CDCA with 5-95% capric and/or lauric acid. Particularly preferred are mixtures of the sodium salts of UDCA, capric acid and lauric acid in a ratio of about 1:2:2 respectively.

[0023] Chelating agents, as used in connection with the present invention, can be defined to be compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of oligonucleotides through the alimentary and other mucosa is enhanced. With regards to their use as penetration enhancers in the present invention, chelating agents have the added advantage of also serving as D-glucose inhibitors, as most characterized D-glucose nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, J. Chromatogr., 1993, 618, 315). Chelating agents of the invention include, but are not limited to, disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (e.g., sodium salicylate, 5-methoxysalicylate and homovanilate), N-acyl derivatives of collagen, laurate-9 and N-amino acyl derivatives of beta-diketones (enamines) (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 92; Muramishid, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1; Buur et al., J. Control Rel., 1990, 14, 43).

[0024] As used herein, non-chelating non-surfactant penetration enhancers may be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of oli-
gonucleotides through the alimentary and other mucosal membranes (Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1). This class of penetration enhancers includes, but is not limited to, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacycloalkanone derivatives (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita et al., J. Pharm. Pharmacol., 1987, 39, 621).

[0025] Agents that enhance uptake of oligonucleotides at the cellular level may also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipectin (Junichi et al., U.S. Pat. No. 5,705,188), cationic glycoler derivatives, and polycationic molecules, such as polylysin (Lollo et al., PCT Application WO 97/30731), can be used.

[0026] The oral pharmaceutical formulation into which the populations of carrier particles are incorporated may be, for example, a capsule, tablet, compression coated tablet or bilayer tablet. In a preferred embodiment, these formulations comprise an enteric outer coating which resists degradation in the stomach and dissolves in the intestinal lumen. In a preferred embodiment, the formulation comprises an enteric material effective in protecting the nucleic acid from pH extremes of the stomach, or in releasing the nucleic acid over time to optimize the delivery thereof to a particular mucosal site. Enteric materials for acid-resistant tablets, capsules and caplets are known in the art and typically include acetate phthalate, propylene glycol, sorbitan monolet, cellulose acetate phthalate (CAP), cellulose acetate trimellitate, hydroxypropyl methyl cellulose phthalate (HPMC), methacrylates, chitosan, guar gum, pectin, locust bean gum and polyethylene glycol (PEG). One particularly useful type of methacrylate are the EUDRAGITSM. These are anionic polymers that are water-impermeable at low pH, but become ionized and dissolve at intestinal pH. EUDRAGITSM L100 and S100 are copolymers of methacrylic acid and methyl methacrylate.

[0027] Enteric materials may be incorporated within the dosage form or may be a coating substantially covering the entire surface of tablets, capsules or caplets. Enteric materials may also be accompanied by plasticizers that impart flexible resiliency to the material for resisting fracturing, for example during tablet curing or aging. Plasticizers are known in the art and typically include diethyl phthalate (DEP), tricetin, dibutyl sebacate (DBS), dibutyl phthalate (DBP) and triethyl citrate (TEC).

[0028] A "pharmacologically acceptable" component of a formulation of the invention is one which, when used together with excipients, diluents, stabilizers, preservatives and other ingredients are appropriate to the nature, composition and mode of administration of a formulation. Accordingly it is desired to select penetration enhancers which facilitate the uptake of drugs, particularly oligonucleotides, without interfering with the activity of the drug and in a manner such that the same can be introduced into the body of a mammal without unacceptable side-effects such as toxicity, irritation or allergic response.

[0029] A "carrier particle" is defined herein as a granule, bead, microparticle, miniparticle, nanoparticle or any other solid dosage form which can be incorporated into the oral pharmaceutical formulations described above.

[0030] Preferred carrier particle-forming substances include poly-amino acids, polyimines, polycrylates, den- trimers, polyalkylenoacylcarlates, cationized gelatins, albumins, starches, acrylates, polyethylene glycols (PEG), DEAE-derivatized polyimines, pollulans and celluloses.

[0031] In other preferred embodiments, the carrier particle-forming substance includes polycationic polymers such as chitosan, poly-L-lysine, polyhistidine, polyornithine, polylysines, protamine, polyvinylpyridine, polyiodoethylenimino-methylene P(TDPE), polyaminosteryne (e.g. para-amin), poly(methylenoacylate), poly (ethylene-anoacrylate), poly (butylenoacylate), poly(isobutyllyno-acrylate), poly(isoxylenoacylate), DEAE-meth- acrylate, DEAE-hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran. In another preferred embodiment, the particle-forming substance is poly-L-lysine complexed with alginate.

[0032] In an alternative embodiment, carrier particle-forming substances are non-polycationic, i.e., carry an overall neutral or negative charge, such as polycrylates, for example polycrylates (e.g., methyl, hexyl), polyox-ethanes, poly(DL-lactic-co-glycolic acid) (PLGA) and poly- ethelyngelycol.

[0033] In another embodiment, the pharmaceutical formulations of the invention may further comprise a biodegradable material that serves to adhere carrier particles to mucosal membranes. Carrier particles may themselves be biodegradable, as is the case with PCL-alginate carrier particles, or may be coated with a biodegradable material. Such materials are well known in the formulation art, examples of which are described in PCT WO85/02092, the contents of which are incorporated herein by reference. Preferred biodegradable materials include polyacrylic polymers (e.g. carboxamer and derivatives of carboxamer), tragacanth, polyethyleneoxide cellulose derivatives (e.g. methylcellulose, carboxymethylcel- lulose, hydroxypropylmethylcellulose (HPMC), hydroxyethylcellulose (HEC), hydroxypropylcellulose (HPC) and sodium carboxymethylcellulose (NaCPC)), karya gum, starch, gelatin and pectin.

[0034] The formulations of the invention may further comprise a mucolytic substance which serves to degrade or erode mucin, partially or completely, at the site of the mucosal membrane to be traversed. Mucolytic substances are well known in the formulation art and include N-acetylcysteine, dithiothreitol, pepsin, pilocarpine, guanifencine, glycerol guaiacolate, terpin hydrate, ammonium chloride, guattencin, ambroxol, bromhexine, carbocystine, domi- odol, leotostine, mescyctine, mesna, sobretol, stepronin, tiopronin and tyloxapol.

[0035] The drug may be associated with the carrier particles by electrostatic (e.g., ionic, polar, Van der Waals), covalent or mechanical (non-electrostatic, non-covalent) interactions depending on the drug and carrier particles, as well as the method of preparing the carrier particles. For example, an anionic drug such as an oligonucleotide can be bound to cationic carrier particles by ionic interaction.

[0036] The carrier particles may also comprise an excipient. Typical pharmaceutical excipients include, but are not limited to, binding agents (e.g., pregelatinized maize starch, polyvinylpyrolidone or hydroxypropyl methylcellulose, etc.); fillers (e.g., lactose and other sugars, microcrystalline
cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, etc.); lubricants (e.g., magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, etc.); disintegrants (e.g., starch, sodium starch glycolate, EXPLOTAB); and wetting agents (e.g., sodium lauryl sulphate, etc.).

In a preferred embodiment, the second population of carrier particles (comprising the penetration enhancer) further comprise an enteric delayed release coating or matrix to delay dissolution until reaching a location in the intestine downstream from where the drug and penetration enhancer are released from the first population of carrier particles which do not comprise a delayed release coating or matrix. This delayed release coating or matrix is different from, or has a different thickness than, the delayed release coating or matrix on the pharmaceutical formulation (e.g. capsule or tablet) described above which causes release of the penetration enhancer after the combination of drug and penetration enhancer is released from the first population of carrier particles. In a preferred embodiment, the coating on the second population of carrier particles is pH independent.

[0038] There are three practical mechanisms by which a pharmaceutical formulation can be targeted into the intestine (small intestine or colon) following oral administration: activation by colonic bacterial enzymes or reducing environment created by the microflora, pH-dependent coating and time-dependent coating (coating thickness).

[0039] To promote release of penetration enhancer from the second population of carrier particles after the coating on the formulation has been dissolved, one or more of these mechanisms may be used. For example, the pH of the intestine increases as material passes through. Thus, the coating on the formulation may be one which dissolves at a lower pH than the coating on the second population of carrier particles to promote release of first and second populations of carrier particles prior to release of penetration enhancer from the second population of carrier particles.

[0040] In an alternate embodiment, the thickness and/or nature of the biodegradable coating on the formulation and the second population of carrier particles are different. The dissolution time of a coating increases as the thickness increases. Thus, in one embodiment, the thickness of the coating on the formulation is greater than the thickness of the coating on the second population of carrier particles which promotes release of the carrier particles prior to release of penetration enhancer from the second population of carrier particles. The nature of the coating is also a consideration since different coatings dissolve at different rates.

[0041] Delayed release coatings, and the properties which influence their dissolution, are well known in the art and are described in, for example, Bauer et al., "Coated Pharmaceutical Dosage Forms," Medpharm Scientific Publishers, CRC Press, New York, 1998 and by Watts et al., Drug Devel., Industr. Pharm. 23:893-913, 1997, the entire contents of which are incorporated herein by reference.

[0042] The compositions of the present invention may additionally comprise other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritica, astrignents, local anesthetics or anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the composition of present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, do not unduly interfere with the biological activities of the components of the compositions of the present invention.

[0043] The pharmaceutical compositions of the invention are used to deliver drugs including peptides, proteins, monoclonal antibodies and fragments thereof, nucleic acids (DNA and RNA), oligonucleotides, antisense oligonucleotides, and small molecules. Types of drugs suitable for use in the pharmaceutical formulations of the invention include, but are not limited to, peptide hormones, antibiotics and anti-microbial agents, vasocostricators, cardiovascular drugs, vasodilators, enzymes, bone metabolism controlling agents, steroid hormones, angiotensin-converting enzyme (ACE) inhibitors.

[0044] In a preferred embodiment, the pharmaceutical formulations are used to deliver oligonucleotides for use in antisense modulation of the function of DNA or messenger RNA (mRNA) encoding a protein the modulation of which is desired, and ultimately to regulate the amount of such a protein. Hybridization of an antisense oligonucleotide with its mRNA target interferes with the normal role of mRNA and causes a modulation of its function in cells. The functions of mRNA to be interfered with include all vital functions such as translation of the RNA to the site for protein translation, actual translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, turnover or degradation of the mRNA and possibly even independent catalytic activity which may be engaged in by the RNA. The overall effect of such interference with mRNA function is modulation of the expression of a protein, wherein “modulation” means either an increase (stimulation) or a decrease (inhibition) in the expression of the protein. In the context of the present invention, inhibition is the preferred form of modulation of gene expression.

[0045] In the context of the present invention, the term “oligonucleotide” refers to an oligomer or polymer of ribonucleic acid or deoxyribonucleic acid. This term includes oligonucleotides composed of naturally occurring nucleobases, sugars and covalent intersugar (backbone) linkages as well as modified oligonucleotides having non-naturally-occurring portions that function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced binding to target and increased stability in the presence of nuclease.

[0046] Oligonucleotides of the present invention may be, but are not limited to, those nucleic acids bearing modified linkages, modified nucleobases, or modified sugars, and chimeric nucleic acids.

[0047] A number of bioequivalents of oligonucleotides and other nucleic acids may also be employed in accordance
with the present invention. The invention therefore, also
encompasses oligonucleotide and nucleic acid equivalents
such as, but not limited to, prodrugs of oligonucleotides and
nucleic acids, deletion derivatives, conjugates of oligonucle-
otides, aptamers, and ribozymes.

[0048] An oligonucleotide is a polymer of repeating units
generically known as a nucleotides. An unmodified (natu-
really occurring) nucleotide has three components: (1) a
nitrogenous base linked by one of its nitrogen atoms to (2)
a 5-carbon cyclic sugar and (3) a phosphate, esterified to
Carbon 5 of the sugar. When incorporated into an oligo-
nucleotide chain, the phosphate of a first nucleotide is also
esterified to carbon 3 of the sugar of a second, adjacent
nucleotide. The “backbone” of an unmodified oligonucle-
otide consists of (2) and (3), that is, sugars linked together
by phosphodiester linkages between the carbon 5 (5') posi-
tion of the sugar of a first nucleotide and the carbon 3 (3')
position of a second, adjacent nucleotide. A “nucleoside” is
the combination of (1) a nucleobase and (2) a sugar in the
absence of (3) a phosphate moiety (Kornberg, A., DNA
Replication, W. H. Freeman & Co., San Francisco, 1980,
Pages 4-7). The backbone of an oligonucleotide positions a
series of bases in a specific order; the written representation
of this series of bases, which is conventionally written in 5'
to 3' order, is known as a nucleotide sequence.

[0049] Oligonucleotides may comprise nucleotide
sequences sufficient in identity and number to effect specific
hybridization with a particular nucleic acid. Such oligo-
nucleotides which specifically hybridize to a portion of the
sense strand of a gene are commonly described as “anti-
sense.” In the context of the invention, “hybridization”
means hydrogen bonding, which may be Watson-Crick,
Hooogsteen or reversed Hooogsteen hydrogen bonding,
between complementary nucleotides. For example, adenine
and thymine are complementary nucleobases that pair
through the formation of hydrogen bonds. “Complement-
ary,” as used herein, refers to the capacity for precise
pairing between two nucleotides. For example, if a nucle-
otide at a certain position of an oligonucleotide is capable of
hydrogen bonding with a nucleotide at the same position of
a DNA or RNA molecule, then the oligonucleotide and the
DNA or RNA are considered to be complementary to each
other at that position. The oligonucleotide and the DNA or
RNA are complementary to each other when a sufficient
number of corresponding positions in each molecule are
occupied by nucleotides which can hydrogen bond with each
other. Thus, “specifically hybridizable” and “complemen-
tary” are terms which are used to indicate a sufficient degree
of complementarity or precise pairing such that stable and
specific binding occurs between the oligonucleotide and the
DNA or RNA target. It is understood in the art that an
oligonucleotide need not be 100% complementary to its
target DNA sequence to be specifically hybridizable. An
oligonucleotide is specifically hybridizable when binding of
the oligonucleotide to the target DNA or RNA molecule
interferes with the normal function of the target DNA or
RNA to cause a decrease or loss of function, and there is a
sufficient degree of complementarity to avoid non-specific
binding of the oligonucleotide to non-target sequences under
conditions in which specific binding is desired, i.e., under
physiological conditions in the case of in vivo assays or
therapeutic treatment, or in the case of in vitro assays, under
conditions in which the assays are performed.

[0050] Antisense oligonucleotides are commonly used as research reagents, diagnostic aids, and therapeutic agents. For example, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used by those of ordinary skill to elucidate the function of particular genes, for example to distinguish between the functions of various members of a biological pathway. This specific inhibitory effect has, therefore, been harnessed by those skilled in the art for research uses. Antisense oligonucleotides have also been used as diagnostic aids based on their specific binding or hybridization to DNA or mRNA that are present in certain disease states and due to the high
degree of sensitivity that hybridization based assays and
amplified assays that utilize some of polymerase chain
reaction afford. The specificity and sensitivity of oligonucle-
otides is also harnessed by those of skill in the art for
therapeutic uses. For example, the following U.S. patents
demonstrate palliative, therapeutic and other methods uti-
izing antisense oligonucleotides. U.S. Pat. No. 5,135,917
provides antisense oligonucleotides that inhibit human inter-
leukin-1 receptor expression. U.S. Pat. No. 5,098,890 is
directed to antisense oligonucleotides complementary to the
c-myc on gene and antisense oligonucleotide therapies for
certain cancerous conditions. U.S. Pat. No. 5,087,617 pro-
vides methods for treating cancer patients with antisense
oligonucleotides. U.S. Pat. No. 5,166,195 provides oligo-
nucleotide inhibitors of Human Immunodeficiency Virus
(HIV). U.S. Pat. No. 5,004,810 provides oligomers capable
of hybridizing to herpes simplex virus Vsnw65 mRNA and
inhibiting replication. U.S. Pat. No. 5,194,428 provides
antisense oligonucleotides having antiviral activity against
influenzavirus. U.S. Pat. No. 4,806,463 provides antisense
oligonucleotides and methods using them to inhibit HTLV-
III replication. U.S. Pat. No. 5,286,717 provides oligonucle-
otide having a complementary base sequence to a portion of
5,264,423 are directed to phosphorothioate oligonucleotide
analogus used to prevent replication of foreign nucleic acids
in cells. U.S. Pat. No. 4,689,320 is directed to antisense
oligonucleotides as antiviral agents specific to cytome-
glovirus (CMV). U.S. Pat. No. 5,098,890 provides oligo-
nucleotides complementary to at least a portion of the
5,242,906 provides antisense oligonucleotides useful in the
utilization of latent Epstein-Barr virus (EBV) infections.
Other examples of antisense oligonucleotides are provided
herein.

[0051] Further, oligonucleotides used in the compositions of the present invention may be directed to modify the effects of mRNAs or DNAs involved in the synthesis of proteins that regulate adhesion of white blood cells and to other cell types. The adherence of white blood cells to vascular endothelium appears to be mediated in part if not in
toto by five cell adhesion molecules ICAM-1, ICAM-2,
ELAM-1, VCAM-1 and GMP-140. Dustin and Springer, J.
Cell. Biol. 1987, 107, 321. Such antisense oligonucleotides are
designed to hybridize either directly to the mRNA or to
a selected DNA portion encoding intercellular adhesion
molecule-1 (ICAM-1), endothelial leukocyte adhesion mol-
ecule-1 (ELAM-1), or E-selectin, and vascular cell adhesion
molecule-1 (VCAM-1) as disclosed in U.S. Pat. No. 5,514,
788 (Bennett et al., May 7, 1996) and U.S. Pat. No.
5,591,623 (Bennett et al., Jan. 7, 1997), and pending U.S.
patent applications Ser. Nos. 08/440,740 (filed May 12,
1995) and 09/062,416 (filed Apr. 17, 1998). These oligonucleotides have been found to modulate the activity of the targeted mRNA, leading to the modulation of the synthesis and metabolism of specific cell adhesion molecules, and thereby result in palliative and therapeutic effects. Inhibition of ICAM-1, VCAM-1 and/or ELAM-1 expression is expected to be useful for the treatment of inflammatory diseases, diseases with an inflammatory component, allograft rejection, psoriasis and other skin diseases, inflammatory bowel disease, cancers and their metastases, and viral infection. Methods of modulating cell adhesion comprising contacting the animal with an oligonucleotide composition of the present invention are provided.

[0052] Exemplary antisense compounds include the following:

[0053] ISIS 2302 is a 2'-deoxyoligonucleotide having a phosphorothioate backbone and the sequence 5'-GCC-CAA-GCT-GGC-ATC-CGT-CA-3' (SEQ ID NO:1). ISIS 2302 is targeted to the 3'-untranslated region (3'-UTR) of the human ICAM-1 gene. ISIS 2302 is described in U.S. Pat. Nos. 5,514,788 and 5,591,623, hereby incorporated by reference.

[0054] ISIS 15839 is a phosphorothioate 2'-oligodeoxynucleotide “hemimer” derivative of ISIS 2302 having the structure 5'-GCC-CAA-GCT-GGC-ATC-CGT-CA-3' (SEQ ID NO:1), wherein embedded “C” residues have 5-methylcytosine (m5C) bases and wherein the embedded, double-underlined residues further comprise a 2'-methoxyethoxy modification (other residues are 2'-deoxy). ISIS 15839 is described in co-pending U.S. patent application Ser. No. 09/062,416, filed Apr. 17, 1998, hereby incorporated by reference.

[0055] ISIS 1939 is a 2'-oligodeoxynucleotide having a phosphorothioate backbone and the sequence 5'-CCC-CCA-CCA-CCT-CCC-CTC-TC-3' (SEQ ID NO:2). ISIS 1939 is targeted to the 3'-untranslated region (3'-UTR) of the human ICAM-1 gene. ISIS 1939 is described in U.S. Pat. Nos. 5,514,788 and 5,591,623, hereby incorporated by reference.

[0056] ISIS 2302 (SEQ ID NO: 1) has been found to inhibit ICAM-1 expression in human umbilical vein cells, human lung carcinoma cells (A549), human epidermal carcinoma cells (A431), and human keratinocytes. ISIS 2302 has also demonstrated specificity for its target ICAM-1 over other potential nucleic acid targets such as HLA-A and HLA-B. ISIS 1939 (SEQ ID NO:2) and ISIS 2302 markedly reduced ICAM-1 expression, as detected by northern blot analysis to determine mRNA levels, in C8161 human melanoma cells. In an experimental metastasis assay, ISIS 2302 decreased the metastatic potential of C8161 cells, and eliminated the enhanced metastatic ability of C8161 cells resulting from TNF-α treatment. ISIS 2302 has also shown significant biological activity in animal models of inflammatory disease. The data from animal testing has revealed strong anti-inflammatory effects of ISIS 2302 in a number of inflammatory diseases including Crohn’s disease, rheumatoid arthritis, psoriasis, ulcerative colitis, and kidney transplant rejection. When tested on humans, ISIS 2302 has shown good safety and activity against Crohn’s disease. Further ISIS 2302 has demonstrated a statistically significant steroid-sparing effect on treated subjects such that even after five months post-treatment subjects have remained weaned from steroids and in disease remission. This is a surprising and significant finding of ISIS 2302’s effects.

[0057] The oligonucleotides used in the compositions of the present invention preferably comprise from about 8 to about 30 nucleotides. It is more preferred that such oligonucleotides comprise from about 10 to about 25 nucleotides.

[0058] Antisense oligonucleotides employed in the compositions of the present invention may also be used to determine the nature, function and potential relationship of various genetic components of the body to normal or abnormal body states of animals. Heretofore, the function of a gene has been chiefly examined by the construction of loss-of-function mutations in the gene (i.e., “knock-out” mutations) in an animal (e.g., a transgenic mouse). Such tasks are difficult, time-consuming and cannot be accomplished for genes essential to animal development since the “knock-out” mutation would produce a lethal phenotype. Moreover, the loss-of-function phenotype cannot be transiently introduced during a particular part of the animal’s life cycle or disease state; the “knock-out” mutation is always present. The use of “Antisense knockouts,” that is, the selective modulation of expression of a gene by antisense oligonucleotides, rather than by direct genetic manipulation, overcomes these limitations (see, for example, Albert et al., Trends in Pharmacological Sciences, 1994, 15, 250). In addition, some genes produce a variety of mRNA transcripts as a result of processes such as alternative splicing; a “knock-out” mutation typically removes all forms of mRNA transcripts produced from such genes and thus cannot be used to examine the biological role of a particular mRNA transcript. By providing compositions and methods for the simple oral delivery of drugs, including oligonucleotides and other nucleic acids, the present invention overcomes these and other shortcomings.

[0059] Specific examples of some preferred modified oligonucleotides envisioned for use in the compositions of the present invention include oligonucleotides containing modified backbones or non-natural intersugar linkages. As defined in this specification, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that have an atom (or group of atoms) other than a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their intersugar backbone, including peptide nucleic acids (PNAs) are also considered to be oligonucleotides.

[0060] Specific oligonucleotide chemical modifications are described in the following subsections. It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the following modifications may be incorporated in a single antisense compound or even in a single residue thereof, for example, at a single nucleoside within an oligonucleotide.

[0061] A. Modified Linkages: Preferred modified oligonucleotide backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminooxyphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphoramidates, phosphoramidates including 3-aminophosphoramidate and aminooxymethylphosphoramidates, thionophosphoramidates, thionooxymethylphosphonates, thionooxymethylphosphotriesters, and boronophosphates having normal 3'-5' linkages, 2'-5' linked analogs of
these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3’-5’ to 5’-3’ or 2’-5’ to 5’-2’. Various salts, mixed salts and free acid forms are also included.

[0062] Representative United States patents that teach the preparation of the above phosphorus atom containing linkages include, but are not limited to, U.S. Pat. Nos. 5,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,433,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; and 5,697,248, certain of which are commonly owned with this application, and each of which is herein incorporated by reference.

[0063] Preferred modified oligonucleotide backbones that do not include a phosphorus atom therein (i.e., oligonucleosides) have backbones that are formed by short chain aliphyl or cyloalkyl intersugar linkages, mixed heteroatom and aliphyl or cycloalkyl intersugar linkages, or one or more short chain heterohetero or heterocyclic intersugar linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); sialonucleosides; sulfone, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkane containing backbones; sulfamate backbones; methyleneimino and methylenedihyrazinobenzenes; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O and CH2 component parts.

[0064] Representative United States patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,907; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,663,360; 5,677,437; and 5,677,439, certain of which are commonly owned with this application, and each of which is herein incorporated by reference.

[0065] In other preferred oligonucleotide mimetics, both the sugar and the intersugar linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an oligomeric acid mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoalicyclic backbone. The nucleobases are retained and are bound directly or indirectly to az nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen et al., Science, 1991, 254, 1497.

[0066] Some preferred embodiments of the present invention may employ oligonucleotides with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular —CH2—NH—O—CH2—
additionally or alternatively comprise one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2′-position: O-β-D-ribofuranosyl, O-β-D-2-thiotoranofuranosyl, or N-alkynyl, wherein the alkyl, alkynyl, and alkyne may be substituted or unsubstituted C₂ to C₁₀ alkyl or C₂ to C₁₀ alkynyl. Particularly preferred are O(CH₂)₅O⁻CH₃, O(CH₂)₂O⁻NH₂, O(CH₂)₂O⁻CH₃, O(CH₂)₂ONH₂, and O(CH₂)₂ON(CH₂)₂CH₃, where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2′-position: C₂ to C₁₀ lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkyl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONOO⁻, NO₂⁻, N⁻, NH₂, heterocycloalkyl, heterocycloalkyalkyl, aminocarboxyalkyl, polyalkylamine, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification includes 2′-methoxyethoxy (2′-O—CH₂CH₂OCH₃, also known as 2′-O(—2-methoxyethyl) or 2′-MOE) [Martin et al., Helv. Chim. Acta, 1995, 78, 486], i.e., an alkoxymethylene group. A further preferred modification includes 2′-dimethylaminomethoxy, i.e., an O(CH₂)₂ON(CH₂)₂ group, also known as 2′-DMAOE, as described in co-owned U.S. patent application Ser. No. 09/016,520, filed on Jan. 30, 1998, the contents of which are herein incorporated by reference.

[0070] Other preferred modifications include 2′-methoxyethoxy (2′-O—CH₂CH₂OCH₃), 2′-aminopropyloxy (2′-OCH₂CH₂CH₂NH₂) and 2′-fluoro (2-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3′ position of the sugar on the 3′ terminal nucleotide or in 2′-5′ linked oligonucleotides and the 5′ position of 5′ terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. U.S. patent application Ser. No. 09/081,537 discloses a method of preparing oligonucleotides having modified sugar structures. However, these methods have limited utility for preparing oligonucleotides having desired modifications.

[0071] Other Modifications: Additional modifications may also be made at other positions on the oligonucleotide, particularly the 3′ position of the sugar on the 3′ terminal nucleotide and the 5′ position of 5′ terminal nucleotide. For example, one additional modification of the oligonucleotides employed in the compositions of the present invention involves chemically linking to the oligonucleotide one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Lentsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86, 6553), cholic acid (Manoharan et al., Bioorg. Med. Chem. Lett., 1993, 3, 2765), a thiocholsterol (Oberhauser et al., Nucl. Acids Res., 1992, 20, 533), an aliphatic chain, e.g., dodecanediol or undecylenyl residues (Sainon-Behmorras et al., EMBO J., 1991, 10, 111; Kabano et al., FEBS Lett., 1990, 259, 127; Svinaffhuk et al., Biochimica et Biophysica Acta, 1993, 75, 49), a phospholipid, e.g., di-hexadecyl-rac-glycerol or tri-ethylammonium 1,2-di-O-hexadecyl-rac-glycerol-3-H-phosphonate Manoharan et al., Tetrahedron Lett., 1995, 36, 3651; Shea et al., Nucl. Acids Res., 1990, 18, 3777), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14, 969), or adamantane acetamide (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264, 229), or an octadecylamine or hexylaminocarbonyloxylcholesterol moiety (Crocce et al., J. Pharmacol. Exp. Ther., 1996, 277, 923).

[0072] Representative United States patents that teach the preparation of such oligonucleotide conjugates include, but are not limited to, U.S. Pat. Nos. 4,822,979; 4,948,882; 5,218,105; 5,255,265; 5,541,313; 5,548,730; 5,552,538; 5,578,717; 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,460,063; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,355; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,808,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241; 5,371,923; 5,416,203; 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,598,726; 5,597,696; 5,597,902; 5,599,928 and 5,688,941, each of which is commonly owned, and each of which is herein incorporated by reference.

[0073] A preferred conjugate imparting improved absorption of oligonucleotides in the gut is folate conjugate. Accordingly, there is provided a composition for oral administration comprising an oligonucleotide and a carrier wherein said oligonucleotide is conjugated to a folate. The folate conjugate may be conjugated to the 3′ or 5′ termini of oligonucleotides, to a nucleobase or to a 2′ position of any of the sugar residues in the chain. Conjugation may be via any suitable chemical linker utilizing functional groups on the oligonucleotide and folate. Oligonucleotide-folate conjugates and methods in preparing are described in copending U.S. patent application Ser. No. 09/082,288 (filed Jun. 16, 1998) and U.S. patent application Ser. No. 09/275,505 (filed Mar. 24, 1999) both incorporated herein by reference.

[0074] E. Chimeric Oligonucleotides: The present invention also includes compositions employing antisense compounds which are chimeric compounds. "Chimeric" antisense compounds or "chimeras," in the context of this invention, are antisense compounds, particularly oligonucleotides, which contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of an oligonucleotide compound. These oligonucleotides typically contain at least one region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the oligonucleotide may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation
of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide inhibition of gene expression. Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used, compared to phosphorothioate oligodeoxynucleotides hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nuclease acid hybridization techniques known in the art. RNase H-mediated target cleavage is distinct from the use of ribozymes to cleave nucleic acids.

For example, such “chimeras” may be “gapmers,” i.e., oligonucleotides in which a central portion (the “gap”) of the oligonucleotide serves as a substrate for, e.g., RNase H, and the 5' and 3' portions (the “wings”) are modified in such a fashion so as to have greater affinity for, or stability when duplexed with, the target RNA molecule but are unable to support nuclease activity (e.g., 2'-fluoro- or 2'-methoxyethoxy-substituted). Other chimeras include “hemimers,” that is, oligonucleotides in which the 5' portion of the oligonucleotide serves as a substrate for, e.g., RNase H, whereas the 3' portion is modified in such a fashion so as to have greater affinity for, or stability when duplexed with, the target RNA molecule but is unable to support nuclease activity (e.g., 2'-fluoro- or 2'-methoxyethoxy-substituted), or vice-versa.

A number of chemical modifications to oligonucleotides that confer greater oligonucleotide-RNA duplex stability have been described by Freier et al. (Nucl. Acids Res., 1997, 25, 4429). Such modifications are preferred for the RNase H-refractory portions of chimeric oligonucleotides and may generally be used to enhance the affinity of an antisense compound for a target RNA.

Chimeric antisense compounds of the invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleo-

tides and/or oligonucleotide mimetics as described above. Such compounds have also been referred to in the art as hybrids or gapmers. Representative United States patents that teach the preparation of such hybrid structures include, but are not limited to, U.S. Pat. Nos. 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, certain of which are commonly owned, and each of which is herein incorporated by reference, and commonly owned and allowed U.S. patent application Ser. No. 08/465,880, filed on Jun. 6, 1995, also herein incorporated by reference.

The present invention also includes compositions employing oligonucleotides that are substantially chirally pure with regard to particular positions within the oligonucleotides. Examples of substantially chirally pure oligonucleotides include, but are not limited to, those having phosphorothioate linkages that are at least 75% Sp or Rp (Cook et al., U.S. Pat. No. 5,587,361) and those having substantially chirally pure (Sp or Rp) alkylphosphonate, phosphoromidate or phosphorothiester linkages (Cook, U.S. Pat. Nos. 5,212,295 and 5,521,302).

The present invention further encompasses compositions employing ribozymes. Synthetic RNA molecules and derivatives thereof that catalyze highly specific endoribo-

nuclease activities are known as ribozymes. (See, generally, U.S. Pat. Nos. 5,543,508 and 5,545,729) The cleavage reactions are catalyzed by the RNA molecules themselves. In naturally occurring RNA molecules, the sites of self-

catalyzed cleavage are located within highly conserved regions of RNA secondary structure (Buzayan et al., Proc. Natl. Acad. Sci. USA., 1986, 83, 8859; Forster et al., Cell, 1987, 50, 9). Naturally occurring autocatalytic RNA molecules have been modified to generate ribozymes which can be targeted to a particular cellular or pathogenic RNA molecule with a high degree of specificity. Thus, ribozymes serve the same general purpose as antisense oligonucleotides (i.e., modulation of expression of a specific gene) and, like oligonucleotides, are nucleic acids possessing significant portions of single-strandedness. That is, ribozymes have substantial chemical and functional identity with oligonucleotides and are thus considered to be equivalents for purposes of the present invention.

Other biologically active oligonucleotides may be formulated in the compositions of the invention and used for therapeutic, palliative or prophylactic purposes according to the methods of the invention. Such other biologically active oligonucleotides include, but are not limited to, antisense compounds including, inter alia, antisense oligonucleotides, antisense RNAs and ribozymes (described supra) and EGSS, as well as aptamers and molecular decoys (described infra).

Sequences that recruit RNase P are known as External Guide Sequences, hence the abbreviation “EGS.” EGSSs are antisense compounds that direct of an endogenous nuclease (RNase P) to a targeted nucleic acid (Forster et al., Science, 1990, 249, 783; Guerrier-Takada et al., Proc. Natl. Acad. Sci. USA, 1997, 94, 8468).

Antisense compounds may alternatively or additionally comprise a synthetic moiety having nuclease activity covalently linked to an oligonucleotide having an anti-

sense sequence instead of relying upon recruitment of an endogenous nuclease. Synthetic moieties having nuclease activity include, but are not limited to, enzymatic RNAs (as in ribozymes), lanthanide ion complexes, and the like (Haseloff et al., Nature, 1988, 334, 585; Baker et al., J. Am. Chem. Soc., 1997, 119, 8749).

Aptamers are single-stranded oligonucleotides that bind specific ligands via a mechanism other than Watson-Crick base pairing. Aptamers are typically targeted to, e.g., a protein and are not designed to bind to a nucleic acid (Ellington et al., Nature, 1990, 346, 818).

Molecular decoys are short double-stranded nucleic acids (including single-stranded nucleic acids designed to “fold back” on themselves) that mimic a site on a nucleic acid to which a factor, such as a protein, binds. Such decoys are expected to competitively inhibit the factor; that is, because the factor molecules are bound to an excess of the decoy, the concentration of factor bound to the cellular site corresponding to the decoy decreases, with resulting therapeutic, palliative or prophylactic effects. Methods of identifying and constructing nucleic acid decoy molecules are described in, e.g., U.S. Pat. No. 5,716,780.

Another type of bioactive oligonucleotide is an RNA-DNA hybrid molecule that can direct gene conversion of an endogenous nucleic acid (Cole-Strauss et al., Science, 1996, 273, 1386).
Examples of specific oligonucleotides and the target genes to which they inhibit, which may be employed in formulations of the present invention include:

- **SIS-2302**: GCCCA AGCTG GCATC CGTCA (SEQ ID NO: 1) ICAM-1
- **SIS-158.39**: GCCCA AGCTG GCATC CGTCA (SEQ ID NO: 1) ICAM-1
- **SIS-1939**: CCCC ACCAC TTCCC CTCTC (SEQ ID NO: 2) ICAM-1
- **SIS-2922**: GCTCT TCCTC TTCT CTCGC G (SEQ ID NO: 3) HCMV
- **SIS-3521**: GTCTG CCTGG GTGAG TTTCG (SEQ ID NO: 4) PKCo
- **SIS-9605**: GTCTG CCTGG GTGAG TTTCG (SEQ ID NO: 4) PKCo
- **SIS-9606**: GTCTG CCTGG GTGAG TTTCG (SEQ ID NO: 4) PKCo
- **SIS-14859**: AACTT GCTGT TCCTC (SEQ ID NO: 5) PKCo
- **SIS-2503**: TCCTG CATCC TCTCT CAGGG (SEQ ID NO: 6) Ha-ras
- **SIS-5132**: TCCTG CATCC TCTCT CAGGG (SEQ ID NO: 6) c-ref
- **SIS-14803**: GTCTG CATCG TCAC GCTCT (SEQ ID NO: 8) HCV
- **SIS-20889**: GTGCG AGAAG CACCC TACT (SEQ ID NO: 9) TNFα
- **SIS-104638**: GCTGA TGAAG GAGAGG TCCGC (SEQ ID NO: 10) TNFα
- **SIS-2105**: TTCTC TCATC TTCCC CTCTC (SEQ ID NO: 11) HPV

Wherein (i) each oligo backbone linkage is a phosphorothioate linkage (except ISIS-9605) and (ii) each sugar is 2-deoxy unless represented in bold font in which case it incorporates a 2'-O-methoxymethyl group and iii) underlined cytosine nucleotides incorporate a 5-methyl substituent on their nucleobase. ISIS-9605 incorporates natural phosphodiester bonds at the first five and last five linkages with the remainder being phosphorothioate linkages.

F. Synthesis: The oligonucleotides used in the compositions of the present invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, Calif.). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is also known to use similar techniques to prepare other oligonucleotides such as the phosphorothiolates and alkylated derivatives.

1. Synthesis of oligonucleotides: Teachings regarding the synthesis of particular modified oligonucleotides may be found in the following U.S. patents or pending patent applications, each of which is commonly assigned with this application: U.S. Pat. Nos. 5,138,045 and 5,218,105, drawn to polyamine conjugated oligonucleotides; U.S. Pat. No. 5,212,295, drawn to monomers for the preparation of oligonucleotides having chiral phosphonate linkages; U.S. Pat. Nos. 5,378,825 and 5,541,307, drawn to oligonucleotides having modified backbones; U.S. Pat. No. 5,386,023, drawn to backbone modified oligonucleotides and the preparation thereof through reduction coupling; U.S. Pat. No. 5,457,191, drawn to modified nucleobases based on the 3-deazapurine ring system and methods of synthesis thereof; U.S. Pat. No. 5,459,255, drawn to modified nucleobases based on N-2 substituted purines; U.S. Pat. No. 5,521,302, drawn to processes for preparing oligonucleotides having chiral phosphorus linkages; U.S. Pat. No. 5,539,082, drawn to peptide nucleic acids; U.S. Pat. No. 5,554,746, drawn to oligonucleotides having β-lactam backbones; U.S. Pat. No. 5,571,902, drawn to methods and materials for the synthesis of oligonucleotides; U.S. Pat. No. 5,578,718, drawn to nucleosides having alkylthio groups, wherein such groups may be used as linkers to other moieties attached at any of a variety of positions of the nucleoside; U.S. Pat. Nos. 5,587,361 and 5,599,797, drawn to oligonucleotides having phosphorothioate linkages of high chiral purity; U.S. Pat. No. 5,506,005, drawn to processes for the preparation of 2'-O-alkyl guanosine and related compounds, including 2,6-diamino purine compounds; U.S. Pat. No. 5,587,469, drawn to oligonucleotides having N-2 substituted purines; U.S. Pat. No. 5,587,470, drawn to oligonucleotides having 3-deazapurines; U.S. Pat. Nos. 5,223,168, issued Jun. 29, 1993, and 5,608,046, both drawn to conjugated 4'-desmethyl nucleoside analogs; U.S. Pat. Nos. 5,602,240, and 5,610,289, drawn to backbone modified oligonucleotide analogs; and U.S. patent application Ser. No. 08/383,666, filed Feb. 3, 1995, and U.S. Pat. No. 5,459,255, drawn to, inter alia, methods of synthesizing 2'-fluoro-oligonucleotides.

2. Bioequivalents: The compositions of the present invention encompass any pharmaceutically acceptable compound that, upon administration to an animal including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to “prodrugs” and “pharmacologically acceptable salts” of the antisense compounds of the invention and other bioequivalents.

A. Oligonucleotide Prodrugs: The oligonucleotide and nucleic acid compounds employed in the compositions of the present invention may additionally or alternatively be prepared to be delivered in a “prodrug” form. The term “prodrug” indicates a therapeutic agent that is prepared in an inactive form that is converted to an active form (i.e., drug) within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or conditions. In particular, prodrug versions of the antisense compounds may be prepared as SATE [S-acetyl-2-thioethyl phosphate] derivatives according to the methods disclosed in WO 93/24510 (Gosselin et al., published Dec. 9, 1993).

B. Pharmacologically Acceptable Salts: The term “pharmacologically acceptable salts” refers to physiologically and pharmacologically acceptable salts of the oligonucleotide and nucleic acid compounds employed in the compositions of the present invention (i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto).

Pharmacologically acceptable base addition salts are formed with metals or amines, such as alkali and alkaline
earth metals or organic amines. Examples of metals used as cations are sodium, potassium, magnesium, calcium, ammonium, polyamines such as spermine and spermidine, and the like. Examples of suitable amines are chloroprocaine, choline, N,N-dibenzylethylenediamine, diethanolamine, dicyclohexylamine, ethylenediamine, N-methylglucamine, and procaine (see, for example, Berge et al., "Pharmaceutical Salts," *J. of Pharma Sci.*, 1977, 66:1). The base addition salts of said acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid in the conventional manner. The free acid forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid for purposes of the present invention.

**[0094]** During the process of oligonucleotide synthesis, nucleoside monomers are attached to the chain one at a time in a repeated series of chemical reactions such as nucleoside monomer coupling, oxidation, capping and detritylation. The stepwise yield for each nucleoside addition is above 99%. That means that less than 1% of the sequence chain failed to be generated from the nucleoside monomer addition in each step as the total results of the incomplete coupling followed by the incomplete capping, detritylation and oxidation (Smith, *Anal. Chem.*, 1988, 50:381A). All the shorter oligonucleotides, ranging from (n-1), (n-2), etc., to 1-mers (nucleotides), are present as impurities in the n-mer oligonucleotide product. Among the impurities, (n-2)-mer and shorter oligonucleotide impurities are present in very small amounts and can be easily removed by chromatographic purification (Warren et al., Chapter 9 In: *Methods in Molecular Biology*, Vol. 26: *Protocols for Oligonucleotide Conjugates*, Agrawal, S., Ed., 1994, Humana Press Inc., Totowa, N.J., pages 232-264). However, due to the lack of chromatographic selectivity and product yield, some (n-1) mer impurities are still present in the full-length (i.e., n-mer) oligonucleotide product after the purification process. The (n-1) portion consists of the mixture of all possible single base deletion sequences relative to the n-mer parent oligonucleotide. Such (n-1) impurities can be classified as terminal deletion or internal deletion sequences, depending upon the position of the missing base (i.e., either at the 5’ or 3’ terminus or internally). When an oligonucleotide containing single base deletion sequence impurities is used as a drug (Crooke, *Hematologic Pathology*, 1995, 9, 59), the terminal deletion sequence impurities will bind to the same target mRNA as the full length sequence but with a slightly lower affinity. Thus, to some extent, such impurities can be considered as part of the active drug component, and are thus considered to be bioequivalents for purposes of the present invention.

**[0095]** Pharmaceutically acceptable organic or inorganic carrier substances suitable for oral administration which do not deleteriously react with nucleic acids can also be used to formulate the compositions of the present invention. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxyethylcellulose, polyvinylpyrrolidone and the like. The formulations can be sterilized, and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

**[0096]** The present invention provides compositions and methods for oral delivery of a drug to an animal. For purposes of the invention, the term “animal” is meant to encompass humans as well as other mammals, as well as reptiles, fish, amphibians, and birds. The compositions of the present invention may be prepared and formulated as emulsions. Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter. (Idson, in *Pharmaceutical Dosage Forms: Disperse Systems*, Vol. 1, Lieberman, Rieger, and Banker, Eds., Marcel Dekker, Inc., New York, N.Y., 1988, p. 199; Rosoff, in *Pharmaceutical Dosage Forms: Disperse Systems*, Vol. 1, Lieberman, Rieger and Banker, Eds., Marcel Dekker, Inc., New York, N.Y., 1988, p. 245; Block, in *Pharmaceutical Dosage Forms: Disperse Systems*, Vol. 2, Lieberman, Rieger and Banker, Eds., Marcel Dekker, Inc., New York, N.Y., 1988, p. 335; Higuchi et al., in “Remington’s Pharmaceutical Sciences,” Mack Publishing Co., Easton, Pa., 1985, p. 301). Emulsions are often biphasic systems comprising of two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions may be either water in oil (w/o) or of the oil in water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase the resulting composition is called a water in oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase the resulting composition is called an oil in water (o/w) emulsion.

**[0097]** Emulsions may contain additional components in addition to the dispersed phases and the active drug that may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants may also be present in emulsions as needed. Pharmaceutical emulsions may also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil in water in oil (w/o/w) and water in oil in water (o/w/o) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclosed small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous provides an o/w/o emulsion.

**[0098]** Emulsions are characterized by little or no thermo-dynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Either of the phases of the emulsion may be a semisolid or a solid, as is the case of emulsion-style ointment bases and creams. Other means of stabilizing emulsions entail the use of emulsifiers that may be incorporated into either phase of the emulsion. Emulsifiers may broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (Idson, in *Pharmaceutical Dosage Forms: Disperse Systems*, Vol. 1, 2003/0124196 A1, Jul. 3, 2003)
Synthetic surfactants, also known as surface active agents, have found wide applicability in the formulation of emulsions and have been reviewed in the literature (Rieger, in *Pharmaceutical Dosage Forms: Disperse Systems*, Vol. 1, Lieberman, Rieger and Banker, Eds., Marcel Dekker, Inc., New York, N.Y., 1988, p. 285; Idson, in *Pharmaceutical Dosage Forms: Disperse Systems*, Vol. 1, Lieberman, Rieger and Banker, Eds., Marcel Dekker, Inc., New York, N.Y., 1988, p. 199). Surfactants are typically amphiphilic and comprise a hydrophilic and a hydrophobic portion. The ratio of the hydrophilic to the hydrophobic nature of the surfactant has been termed the hydrophilic/lipophilic balance (HLB) and is a valuable tool in categorizing and selecting surfactants in the preparation of formulations. Surfactants may be classified into different classes based on the nature of the hydrophilic group into: nonionic, anionic, cationic and amphoteric (Rieger, in *Pharmaceutical Dosage Forms: Disperse Systems*, Vol. 1, Lieberman, Rieger and Banker, Eds., Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

Naturally occurring emulsifiers used in emulsion formulations include lanolin, beeswax, phospholipids, lecithin and acacia. Absorption bases possess hydrophilic properties such that they can soak up water to form w/o emulsions yet retain their semisolid consistencies, such as anhydrous lanolin and hydrophilic petrolatum. Finely divided solids have also been used as good emulsifiers especially in combination with surfactants and in viscous preparations. These include polar inorganic solids, such as heavy metal hydroxides, nonswelling clays such as bentonite, attapulgite, hectorite, kaolin, montmorillonite, colloidal aluminum silicate and colloidal magnesium aluminum silicate, pigments and nonpolar solids such as carbon or glyceryl tristearate.

A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives and antioxidants (Block, in *Pharmaceutical Dosage Forms: Disperse Systems*, Vol. 1, Lieberman, Rieger and Banker, Eds., Marcel Dekker, Inc., New York, N.Y., 1988, p. 335; Idson, Id., p. 199).

Hydrophilic colloids or hydrocolloids include naturally occurring gums and synthetic polymers such as polysaccharides (for example, acacia, agar, alginic acid, carrageenan, guar gum, karaya gum, and tragacanth), cellulose derivatives (for example, carboxymethyl cellulose and carboxypropyl cellulose), and synthetic polymers (for example, carbomers, cellulose ethers, and carboxyvinyl polymers). These disperse or swell in water to form colloidal solutions that stabilize emulsions by forming strong interfacial films around the dispersed-phase droplets and by increasing the viscosity of the external phase.

Since emulsions often contain a number of ingredients such as carbohydrates, proteins, sterols and phospholipids that may readily support the growth of microbes, these formulations often incorporate preservatives. Commonly used preservatives included in emulsion formulations include methyl paraben, propyl paraben, quaternary ammonium salts, benzalkonium chloride, esters of p-hydroxybenzoic acid, and boric acid. Antioxidants are also commonly added to emulsion formulations to prevent deterioration of the formulation. Antioxidants used may be free radical scavengers such as tocopherols, alkyl gallates, butylated hydroxyanisole, butylated hydroxytoluene, or reducing agents such as ascorbic acid and sodium metabisulfite, and antioxidant synergists such as citric acid, tartaric acid, and lecithin.

The application of emulsion formulations via dermatological, oral and parenteral routes and methods for their manufacture have been reviewed in the literature (Idson, in *Pharmaceutical Dosage Forms: Disperse Systems*, Vol. 1, Lieberman, Rieger and Banker, Eds., Marcel Dekker, Inc., New York, N.Y., 1988, p. 199). Emulsion formulations for oral delivery have been very widely used because of reasons of ease of formulation, efficacy from an absorption and bioavailability standpoint. (Rosoff, in *Pharmaceutical Dosage Forms: Disperse Systems*, Vol. 1, Lieberman, Rieger and Banker, Eds., Marcel Dekker, Inc., New York, N.Y., 1988, p. 245; Idson, Id., p. 199). Mineral-oil base laxatives, oil-soluble vitamins and high fat nutritive preparations are among the materials that have commonly been administered orally as o/w emulsions.

In one embodiment of the present invention, the compositions of oligonucleotides and nucleic acids are formulated as microemulsions. A microemulsion may be defined as a system of water, oil and amphoteric which is a single optically isotropic and thermodynamically stable liquid solution (Rosoff, in *Pharmaceutical Dosage Forms: Disperse Systems*, Vol. 1, Lieberman, Rieger and Banker, Eds., Marcel Dekker, Inc., New York, N.Y., 1988, p. 245). Typically microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: *Controlled Release of Drugs: Polymers and Aggregate Systems*, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type depends on the properties of the oil and surfactant used and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in “Remington’s Pharmaceutical Sciences,” Mack Publishing Co., Easton, Pa., 1985, p. 271).

The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (Rosoff, in *Pharmaceutical Dosage Forms: Disperse Systems*, Vol. 1, Lieberman, Rieger and Banker, Eds., Marcel Dekker, Inc., New York, N.Y., 1988, p. 245; Block, Id., p. 335). Compared to conventional emulsions, microemulsions offer the advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets that are formed spontaneously.

Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants,
non-ionic surfactants, Brij 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetracyclol monolaurate (MI310), tetracyclol monolate (MO310), hexacyclol monoate (PO310), hexacyclol pentaate (PO500), decacyclol fatty acids (MCAT750), decacyclol monoate (MO750), decacyclol sequestrate (SO750), and decacyclol decaolate (DAO750), alone or in combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, and 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film because of the void space generated among surfactant molecules. Microemulsions may, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase may typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of ethylene glycol. The oil phase may include, but is not limited to, materials such as Captox 300, Captox 355, Capmul MCM, fatty acid esters, medium chain (C8-C12) mono, di, and tri-glycerides, polyoxyethylated glycerol fatty acid esters, fatty alcohols, polyglycerolated glycerides, saturated polyglycerolated C8-C10 glycerides, vegetable oils and silicone oil.

Microemulsions are particularly of interest from the standpoint of drug solubilization and the enhanced absorption of drugs. Lipid based microemulsions (both o/w and w/o) have been proposed to enhance the oral bioavailability of drugs, including peptides (Constantinides et al., Pharmaceutical Research, 1994, 11, 1385; Ritschel, Meth. Find. Exp. Clin. Pharmacol., 1993, 13, 205). Microemulsions afford advantages of improved drug solubilization, protection of drug from enzymatic hydrolysis, possible enhancement of drug absorption due to surfactant-induced alterations in membrane fluidity and permeability, ease of preparation, ease of oral administration over solid dosage forms, improved clinical potency, and decreased toxicity (Constantinides et al., Pharmaceutical Research, 1994, 11, 1385; Ho et al., J. Pharm. Sci., 1996, 85, 138). Often microemulsions may form spontaneously when their components are brought together at ambient temperature. This may be particularly advantageous when formulating thermolabile drugs, peptides or oligonucleotides. Microemulsions have also been effective in the transdermal delivery of active components in both cosmetic and pharmaceutical applications. It is expected that the microemulsion compositions and formulations of the present invention will facilitate the increased systemic absorption of oligonucleotides and nucleic acids from the gastrointestinal tract, as well as improve the local cellular uptake of oligonucleotides and nucleic acids within the gastrointestinal tract.

There are many organized surfactant structures besides microemulsions that have been studied and used for the formulation of drugs. These include monolayers, micelles, bilayers and vesicles. Vesicles, such as liposomes, have attracted great interest because of their specificity and the duration of action they offer from the standpoint of drug delivery. Further advantages are that liposomes obtained from natural phospholipids are biocompatible and biodegradable, liposomes can incorporate a wide range of water and lipid soluble drugs, liposomes can protect encapsulated drugs in their internal compartments from metabolism and degradation (Rosoff, in Pharmaceutical Dosage Forms: Disperse Systems, Vol. 1, Lieberman, Rieger and Banker, Eds., Marcel Dekker, Inc., New York, N.Y., 1988, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes. Liposomes can be administered orally and in aerosols and topical applications.

Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophilic/lipophilic balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in Pharmaceutical Dosage Forms: Disperse Systems, Vol. 1, Lieberman, Rieger and Banker, Eds., Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkanolamides, N-alkylbetaines and phosphatides.

In a preferred embodiment of the invention, one or more nucleic acids are administered via oral delivery.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, troches, tablets or SECs (soft elastic capsules or “caplets”). Thickener, flavoring agents, diluents, emulsifiers, dispersing aids, carrier substances or binders may be desirably added to such formulations. A tablet may be made by compression or molding, optionally with one or more accessory ingredients.

Compressed tablets may be prepared by compressing in a suitable machine, the active ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder (PVP or gums such as tragacanth, acacia, carrageenan), lubricant (e.g. stearates such as magnesium stearate), glidant (talc, colloidal silica dioxide), inert diluent, preservative, surface active or dispersing agent. Preferred binders/dissintegrants include EMDEX (dextrate), PECRIOL (triglyceride), PEG, and AVICEL (cellulose). Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredients therein.

Various methods for producing formulations for alimentary delivery are well known in the art. See, generally, Nairn, Chapter 83; Block, Chapter 87; Rudnic et al., Chapter 89; Porter, Chapter 90; and Longer et al., Chapter 91 In: *Remington’s Pharmaceutical Sciences*, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990. The compositions of this invention can be converted in a known manner into the customary formulations, such as tablets, coated tablets, pills, granules, capsules, aerosols, syrups, emulsions, suspensions and solutions, using inert, non-toxic, pharmaceutically suitable excipients or solvents. The therapeutically active compound is present in a concentration of about 0.5% to about 95% by weight of the total mixture, that is to say in amounts which are sufficient to achieve the stated dosage range. Compositions may be formulated in a conventional manner using additional pharmaceutically acceptable carriers or excipients as appropriate. Thus, the composition may be prepared by conventional means with carriers or excipients such as binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., starch or sodium starch glycinate); or wetting agents (e.g., sodium lauryl sulfate). Tablets may be coated by methods well known in the art. The preparations may also contain flavoring, coloring and/or sweetening agents as appropriate.

Capsules used for oral delivery may include formulations that are well known in the art. Furthermore, multilayer hard capsules with control release properties as described by Digenis et al., U.S. Pat. No. 5,672,359, and water permeable capsules with a multi-stage drug delivery system as described by Amidon et al., U.S. Pat. No. 5,674,530 may also be used to formulate the compositions of the present invention.

The formulation of pharmaceutical compositions and their subsequent administration is believed to be within the skill of those in the art. Specific comments regarding the present invention are presented below.

In general, for therapeutic applications, a patient (i.e., an animal, including a human) having or predisposed to a disease or disorder is administered one or more drugs, preferably nucleic acids, including oligonucleotides, in accordance with the invention in a pharmaceutically acceptable carrier in doses ranging from 0.01 μg to 100 g per kg of body weight depending on the age of the patient and the severity of the disorder or disease state being treated. Further, the treatment regimen may be for a period of time which will vary depending upon the nature of the particular disease or disorder, its severity and the overall condition of the patient, and may extend from once daily to once every 20 years. In the context of the invention, the term “treatment regimen” is meant to encompass therapeutic, palliative and prophylactic modalities. Following treatment, the patient is monitored for changes in his/her condition and for alleviation of the symptoms of the disorder or disease state. The dosage of the drug may either be increased if the patient does not respond significantly to current dosage levels, or the dose may be decreased if an alleviation of the symptoms of the disorder or disease state is observed, or if the disorder or disease state has been abated.

Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual drugs, and can generally be estimated based on E<sub>50</sub> values found to be effective in vitro and in vivo animal models. In general, dosage is from 0.01 μg to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years. An optional dosing schedule is used to deliver a therapeutically effective amount of the drug being administered via a particular mode of administration.

The term “therapeutically effective amount,” for the purposes of the invention, refers to the amount of drug-containing formulation that is effective to achieve an intended purpose without undesirable side effects (such as toxicity, irritation or allergic response). Although individual needs may vary, optimal ranges for effective amounts of formulations can be readily determined by one of ordinary skill in the art. Human doses can be extrapolated from animal studies (Kato et al., Chapter 27 In: *Remington’s Pharmaceutical Sciences*, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990). Generally, the dosage required to provide an effective amount of a formulation, which can be adjusted by one skilled in the art, will vary depending on the age, health, physical condition, weight, type and extent of the disease or disorder of the recipient, frequency of treatment, the nature of concurrent therapy (if
any) and the nature and scope of the desired effect(s) (Nies et al., Chapter 3 In: Goodman & Gilman’s The Pharmacological Basis of Therapeutics, 9th Ed., Hardman et al., eds., McGraw-Hill, New York, N.Y., 1996).

[0126] Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the nucleic acid is administered in maintenance doses, ranging from 0.01 ug to 100 g per kg of body weight, once or more daily, to once every 20 years. For example, in the case of in individual known or suspected of being prone to an autoimmune or inflammatory condition, prophylactic effects may be achieved by administration of preventative doses, ranging from 0.01 ug to 100 g per kg of body weight, once or more daily, to once every 20 years. In like fashion, an individual may be made less susceptible to an inflammatory condition that is expected to occur as a result of some medical treatment, e.g., graft versus host disease resulting from the transplantation of cells, tissue or an organ into the individual.

[0127] Formulations for oral administration may include sterile and non-sterile aqueous solutions, non-aqueous solutions in common solvents such as alcohols, or solutions of the nucleic acids in liquid or solid oil bases. The solutions may also contain buffers, diluents and other suitable additives. Aqueous suspensions may contain substances that increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

[0128] The pharmaceutical formulations, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

[0129] In a preferred embodiment, the invention is directed to the oral administration of a nucleic acid, such as an oligonucleotide, having biological activity, to an animal. By “having biological activity,” it is meant that the nucleic acid functions to modulate the expression of one or more genes in an animal as reflected in either absolute function of the gene (such as ribozyme activity) or by production of proteins coded by such genes. In the context of this invention, “to modulate” means to either effect an increase (stimulate) or a decrease (inhibit) in the expression of a gene. Such modulation can be achieved by, for example, an antisense oligonucleotide by a variety of mechanisms known in the art, including but not limited to transcriptional arrest; effects on RNA processing (capping, polyadenylation and splicing) and transport; enhancement or reduction of cellular degradation of the target nucleic acid; and translational arrest (Crooke et al., Exp. Opin. Ther. Patents, 1996, 6, 1).

[0130] In an animal other than a human, the compositions and methods of the invention can be used to study the function of one or more genes in the animal. For example, antisense oligonucleotides have been systematically administered to rats in order to study the role of the N-methyl-D-aspartate receptor in neuronal death, to mice in order to investigate the biological role of protein kinase C-a, and to rats in order to examine the role of the neuropeptide Y1 receptor in anxiety (Wahlstedt et al., Nature, 1993, 363, 260; Dean et al., Proc. Natl. Acad. Sci. U.S.A., 1994, 91, 11762; and Wahlstedt et al., Science, 1993, 259, 528, respectively). In instances where complex families of related proteins are being investigated, “antisense knockouts” (i.e., inhibition of a gene by systemic administration of antisense oligonucleotides) may represent the most accurate means for examining a specific member of the family (see, generally, Albert et al., Trends Pharmacol. Sci., 1994, 15, 250).

[0131] As stated, the compositions and methods of the invention are useful therapeutically, i.e., to provide therapeutic, palliative or prophylactic relief to an animal, including a human, having or suspected of having or of being susceptible to, a disease or disorder that is treatable in whole or in part with one or more nucleic acids. The term “disease or disorder” (1) includes any abnormal condition of an organism or part, especially as a consequence of infection, inherent weakness, environmental stress, that impairs normal physiological functioning; (2) excludes pregnancy per se but not autoimmune and other diseases associated with pregnancy; and (3) includes cancers and tumors. The term “having or suspected of having or of being susceptible to” indicates that the subject animal has been determined to be, or is suspected of being, at increased risk, relative to the general population of such animals, of developing a particular disease or disorder as herein defined. For example, a subject animal could have a personal and/or family medical history that includes frequent occurrences of a particular disease or disorder. As another example, a subject animal could have had such a susceptibility determined by genetic screening according to techniques known in the art (see, e.g., U.S. Congress, Office of Technology Assessment, Chapter 5 In: Genetic Monitoring and Screening in the Workplace, OTA-BA-455, U.S. Government Printing Office, Washington, D.C., 1990, pages 75-59). The term “a disease or disorder that is treatable in whole or in part with one or more nucleic acids” refers to a disease or disorder, as herein defined, (1) the management, modulation or treatment thereof, and/or (2) therapeutic, palliative and/or prophylactic relief therefrom, can be provided via the administration of more nucleic acids. In a preferred embodiment, such a disease or disorder is treatable in whole or in part with an antisense oligonucleotide.

EXAMPLES

[0132] The following examples illustrate the invention and are not intended to limit the same. Those skilled in the art will recognize, or be able to ascertain through routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of the present invention.

Example 1
Preparation of Oligonucleotides

[0133] A. General Synthetic Techniques: Oligonucleotides were synthesized on an automated DNA synthesizer using standard phosphoramidite chemistry with oxidation using iodine. Beta-cyanoethylisopropyl phosphoramidites were purchased from Applied Biosystems (Foster City,
Calif.). For phosphorothioate oligonucleotides, the standard oxidation bottle was replaced by a 0.2 M solution of 3H-1,2-benzodithiole-3-one-1,1-dioxide in acetonitrile for the stepwise thiation of the phosphate linkages.

[0134] The synthesis of 2′-O-methyl(2′-methoxy-) phosphorothioate oligonucleotides is according to the procedures set forth above substituting 2′-O-methyl b-cyanoethylidihydroporphoramidites (Chemgenes, Needham, Mass.) for standard phosphoramidites and increasing the wait cycle after the pulse delivery of tetrazole and base to 360 seconds.

[0135] Similarly, 2′-O-propyl-(a.k.a 2′-propoxy-) phosphorothioate oligonucleotides are prepared by slight modifications of this procedure and essentially according to procedures disclosed in U.S. patent application Ser. No. 08/383,666, filed Feb. 3, 1995, which is assigned to the same assignee as the instant application and which is incorporated by reference herein.

[0136] The 2′-fluoro-phosphorothioate oligonucleotides of the invention are synthesized using 5′-dimethoxytrityl-3′-phosphoramidites and prepared as disclosed in U.S. patent application Ser. No. 08/383,666, filed Feb. 3, 1995, and U.S. Pat. No. 5,459,255, which issued Oct. 8, 1996, both of which are assigned to the same assignee as the instant application and which are incorporated by reference herein. The 2′-fluoro-oligonucleotides are prepared using phosphoramidite chemistry and a slight modification of the standard DNA synthesis protocol (i.e., deprotection was effected using methanolic ammonia at room temperature).

[0137] PNA antisense analogs are prepared essentially as described in U.S. Pat. Nos. 5,539,082 and 5,539,083, both of which (1) issued Jul. 23, 1996, (2) are assigned to the same assignee as the instant application and (3) are incorporated by reference herein.


[0139] 2′-Methoxyethoxy oligonucleotides of the invention are synthesized essentially according to the methods of Martin et al. (Helv Chim Acta, 1995, 78, 486).

[0140] B. Oligonucleotide Purification: After cleavage from the controlled pore glass (CPG) column (Applied Biosystems) and deblocking in concentrated ammonium hydroxide, at 55° C. for 18 hours, the oligonucleotides were purified by precipitation 2x from 0.5 M NaCl with 2.5 volumes of ethanol followed by further purification by reverse phase high liquid pressure chromatography (HPLC). Analytical gel electrophoresis was accomplished in 20% acrylamide, 8 M urea and 45 mM Tris-borate buffer (pH 7).

[0141] C. Oligonucleotide Labeling: Antisense oligonucleotides were labeled in order to detect the presence of and/or measure the quantity thereof in samples taken during the course of the in vivo pharmacokinetic studies described herein. Although radiolabeling by tritium exchange is one preferred means of labeling antisense oligonucleotides for such in vivo studies, a variety of other means are available for incorporating a variety of radiological, chemical or enzymatic labels into oligonucleotides and other nucleic acids.

[0142] 1. Tritium Exchange: Essentially, the procedure of Graham et al. (Nucleic Acids Research, 1993, 21, 3737) was used to label oligonucleotides by tritium exchange. Specifically, about 24 mg of oligonucleotide was dissolved in a mixture of 200 μl of sodium phosphate buffer (pH 7.8), 400 μl of 0.1 mM EDTA (pH 8.3) and 200 μl of deionized water. The pH of the resulting mixture was measured and adjusted to pH 7.8 using 0.095 N NaOH. The mixture was lyophilized overnight in a 1.25 ml gasketed polypropylene vial. The oligonucleotide was dissolved in 8.25 μl of [3H]-mercaptoethanol, which acts as a free radical scavenger (Graham et al., Nucleic Acids Research, 1993, 21, 3737), and 400 μl of tritiated H2O (5 Ci/mmol). The tube was capped, placed in a 90° C. oil bath for 9 hours without stirring, and then briefly centrifuged to remove any condensate from the inside lid of the tube. (As an optional analytical step, two 10 μl aliquots (one for HPLC analysis, one for PAGE analysis) were removed from the reaction tube; each aliquot was added to a separate 1.5 ml standard microfuge tube containing 490 μl of 50 μM sodium phosphate buffer (pH 7.8)). The oligonucleotide mixture is then frozen in liquid nitrogen and transferred to a lyophilization apparatus wherein lyophilization was carried out under high vacuum, typically for 3 hours. The material was then resuspended in mL of double-distilled water and allowed to exchange for 1 hour at room temperature. After incubation, the mixture was again quick frozen and lyophilized overnight. (As an optional analytical step, about 1 mg of the oligonucleotide material is removed for HPLC analysis.) Three further lyophilizations were carried out, each with approximately 1 mL of double-distilled water, to ensure the removal of any residual, unincorporated tritium. The final resuspended oligonucleotide solution is transferred to a clean polypropylene vial and assayed. The tritium labeled oligonucleotide is stored at about −70° C.

[0143] 2. Other Means of Labeling Nucleic Acids: As is well known in the art, a variety of means are available to label oligonucleotides and other nucleic acids and to separate unincorporated label from the labeled nucleic acid. For example, double-stranded nucleic acids can be radiolabeled by nick translation and primer extension, and a variety of nucleic acids, including oligonucleotides, can be terminally radiolabeled by the use of enzymes such as T4 polynucleotide kinase or terminal deoxynucleotidyl transferase (see, generally, Chapter 3 in: Short Protocols in Molecular Biology, 2nd Ed., Ausubel et al., eds., John Wiley & Sons, New York, N.Y., pages 3-11 to 3-38; and Chapter 10 in: Molecular Cloning: A Laboratory Manual, 2nd Ed., Sambrook et al., eds., pages 10.1 to 10.70). It is also well known in the art to label oligonucleotides and other nucleic acids with nonradioactive labels such as, for example, enzymes, fluorescent moieties and the like (see, for example, Beck, Methods in Enzymology, 1992, 216, 143; and Ruth, Chapter 6 in: Protocols for Oligonucleotide Conjugates (Methods in Molecular Biology, Volume 26) Agrawal, S., ed., Humana Press, Totowa, N.J., 1994, pages 167-185).
Example 2

Oligonucleotide Targets

[0144] The present invention is drawn to compositions and formulations comprising oligonucleotides or nucleic acids and one or more mucosal penetration enhancers, and methods of using such formulations. In one embodiment, such formulations are used to study the function of one or more genes in an animal other than a human. In a preferred embodiment, oligonucleotides are formulated into a pharmaceutical composition intended for therapeutic delivery to an animal, including a human. Oligonucleotides intended for local or systemic therapeutic delivery, as desired, that may be orally administered according to the compositions and methods of the invention. Such desired oligonucleotides include, but are not limited to, those which modulate the expression of cellular adhesion proteins (e.g., ICAM-1, VCAM-1, ELAM-1), the rate of cellular proliferation (e.g., c-myc, v-EGF, c-raf kinase), or have biological or therapeutic activity against miscellaneous disorders (e.g., Alzheimer's, β-thalassemia) and diseases resulting from eukaryotic pathogens (e.g., malaria), retroviruses including HIV and non-retroviral viruses (e.g., Epstein-Barr, CMV).

[0145] Additional oligonucleotides that may be formulated in the compositions of the invention include, for example, ribozymes, aptamers, molecular decoys, External Guide Sequences (EGSs) and peptide nucleic acids (PNAs).

[0146] Various fatty acids, their salts and their derivatives act as penetration enhancers. These include, for example, oleic acid, a.k.a. cis-9-octadecenoic acid (or a pharmaceutically acceptable salt thereof, e.g., sodium oleate or potassium oleate); caprylic acid, a.k.a. n-octanoic acid (caprylate); capric acid, a.k.a. n-decanoic acid (caprate); lauric acid (laurate); acylcarinines; acyclic alcohols; and mono- and diglycerides (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92). Various natural bile salts, and their synthetic derivatives act as penetration enhancers. The physiological roles of bile include the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (Brunton, Chapter 38 in: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th Ed., Goodman et al., McGraw-Hill, New York, N.Y., 1996, pages 934-935). Bile salt derived penetration enhancers include, for example, cholic acid, cholic acid or 3a,7a,12a-trihydroxy-5b-cholan-24-oic acid (or its pharmaceutically acceptable sodium salt); deoxycholic acid, deoxycholic acid, 5b-cholan-24-oic acid-3a,12a-diol, 7-deoxycholic acid or 3a,12a-dihydroxy-5b-cholan-24-oic acid (sodium deoxycholate); glycocholic acid, (N-[3a,7a,12a-trihydroxy-24-oxocholan-24-yl]glycine or 3a,7a,12a-trihydroxy-5b-cholan-24-oic acid N-[carboxymethyl]amide or sodium glycocholate); glycocydoxycholic acid, (5b-cholan-24-oic acid N-[carboxymethyl]amide-3a,12a-diol), 3a,12a-dihydroxy-5b-cholan-24-oic acid N-[carboxymethyl]amide, N[3a,12a-dihydroxy-24-oxocholan-24-yl]glycine or glycodesoxycholic acid (sodium glycodeoxycholate); taurocholic acid, (5b-cholan-24-oic acid N-[sulfoethyl]amide-3a,7a,12a-triolo), 3a,7a,12a-trihydroxy-5b-cholan-24-oic acid N-[sulfoethyl]amide or 2-[3a,7a,12a-trihydroxy-24-oxo-5b-cholan-24-yl]amine)-ethanesulfonic acid (sodium taurocholate); tauroglycocholic acid, (3a,12a-dihydroxy-5b-cholan-2-0ic acid N[2-sulfoethyl]amide or 2-[3a,12a-dihydroxy-24-oxo-5b-cholan-24-yl]amine)-ethanesulfonic acid, or sodium taurodeoxycholate, or sodium taurodesoxycholate; chenoxycholic acid (chenodesoxycholic acid, chenoxycholic acid, 5b-cholan-3a,7a-diol, 3a,7a-dihydroxy-5b-cholan-3a,7a-diol, or sodium chenoxycholate, or CDCA); ursodeoxycholic acid, (5b-cholan-24-oic acid-3a,7b-diol, 7b-hydroxylicolcholic acid or 3a,7b-dihydroxy-5b-cholan-24-oic acid, or UDCA); sodium taurohydrofusidate (STHDF); and sodium glycodehydrofusidate (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Swinyard, Chapter 39 In: Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990, pages 782-783).

[0147] Unsubstituted and substituted phosphodiester oligonucleotides are alternately synthesized on an automated DNA synthesizer (Applied Biosystems model 380B) using standard phosphoramidite chemistry with oxidation by iodine.

[0148] Phosphorothioates are synthesized as per the phosphodiester oligonucleotides except the standard oxidation bottle was replaced by 0.2 M solution of 3H-1,2-benzodithiole-3-one 1,1-dioxide in acetonitrile for the stepwise thiation of the phosphite linkages. The thiation wait step was increased to 68 sec and was followed by the capping step. After cleavage from the CPG column and deblocking in concentrated ammonium hydroxide at 55° C. (18 hr), the oligonucleotides were purified by precipitating twice with 2.5 volumes of ethanol from a 0.5 M NaCl solution.

[0149] Phosphinate oligonucleotides are prepared as described in U.S. Pat. No. 5,508,270, herein incorporated by reference.

[0150] Alkyl phosphonate oligonucleotides are prepared as described in U.S. Pat. No. 4,469,863, herein incorporated by reference.

[0151] 3'-Deoxy-3'-methylene phosphonate oligonucleotides are prepared as described in U.S. Pat. Nos. 5,610,289 or 5,625,050, herein incorporated by reference.

[0152] Phosphoramide oligonucleotides are prepared as described in U.S. Pat. No. 5,256,775 or U.S. Pat. No. 5,366,878, hereby incorporated by reference.


[0154] 3'-Deoxy-3'-amino phosphoramic acid oligonucleotides are prepared as described in U.S. Pat. No. 5,476,925, herein incorporated by reference.

[0155] Phosphorister oligonucleotides are prepared as described in U.S. Pat. No. 5,023,243, herein incorporated by reference.

[0156] Boronophosphate oligonucleotides are prepared as described in U.S. Pat. Nos. 5,130,302 and 5,177,198, both herein incorporated by reference.

[0157] Methyleneemethylaminolino linked oligonucleosides, also identified as MMI linked oligonucleosides, methylene-dimethylhydrazo linked oligonucleosides, also identified as MDD linked oligonucleosides, and methylencarbonylamino linked oligonucleosides, also identified as amide-3 linked oligonucleosides, and methyleneaminocarbonyl linked oligonucleosides, also identified as amide-4 linked
oligonucleosides, as well as mixed backbone compounds having, for instance, alternating MMI and PO or PS linkages are prepared as described in U.S. Pat. Nos. 5,378,825; 5,386,023; 5,489,677; 5,602,240 and 5,610,289, all of which are herein incorporated by reference.

[0158] Formacetal and thioformacetal linked oligonucleosides are prepared as described in U.S. Pat. Nos. 5,264,562 and 5,264,564, herein incorporated by reference.

[0159] Ethylene oxide linked oligonucleosides are prepared as described in U.S. Pat. No. 5,223,618, herein incorporated by reference.


[0161] With regard to oligonucleotide absorption kinetics, it is important to determine if the oligonucleotide absorption rate function behaves in accordance with diffusion principles or if a zero order (i.e., saturable) process is predominant. This information is useful for formulation design since a zero order process would limit the absolute amount of oligonucleotide absorbed in a given amount of time. This would direct formulation efforts to release oligonucleotide upstream from the permeable region of the intestine at a rate \( \leq k_c \) (amount absorbed per unit time) to maximize uptake. However, should the absorption process be independent of concentration, then this formulation approach would not be used since a first-order absorption process would lead to a consistent percentage uptake.

[0162] Two studies were designed to address this issue. The first involved the intrajejunal (IJ) administration of increasing doses of ISIS 104838, holding the C10 dose constant at 50 mg/kg. The oligonucleotide doses were chosen to bracket the range of oligonucleotide dose considered feasible for human dosage form development—2.5, 10 and 50 mg/kg. Should saturation occur, the higher doses of oligonucleotide would demonstrate a diminished absolute bioavailability (BAV). The second study tested the hypothesis in a slightly different way by slowly presenting a lower concentration of a high dose of oligonucleotide to an activated segment of intestine (activated by bolus penetration enhancer administration). If a saturable process is present, the slower presentation of oligonucleotide should result in a higher BAV compared to bolus co-administration.

[0163] As can be seen in Table 1 and FIG. 1, saturation of oligonucleotide uptake pathways did not occur at the doses studied. The initial responses resulted in higher BAV values due to the inappropriate use of a low dose IV AUC for BAV calculations. After recalculation of the BAVs, it is clear that they are equivalent and therefore the absorption process appears to be linear and not saturable at the doses studied.

**TABLE 1**

<table>
<thead>
<tr>
<th>ISIS 104838</th>
<th>C10 dose (mg/kg)</th>
<th>Ratio</th>
<th>% BAV (%)</th>
<th>% BAV (corrected to relevant IV AUC - extrapolated from other monkey exp.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LI dose (mg/kg)</td>
<td>Oligo:PE (1 mg/kg, IV)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>50</td>
<td>1:20</td>
<td>2.1 ± 1.1 *</td>
<td>0.125</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>1:5</td>
<td>3.0 ± 2.2</td>
<td>0.5</td>
</tr>
<tr>
<td>30</td>
<td>50</td>
<td>1:1.7</td>
<td>10.3 ± 6.1</td>
<td>2</td>
</tr>
</tbody>
</table>

average ± standard deviation

* AUCs calculated out to terminal time-point data from sensitive binding plate assay.

[0164] In the second study, oligonucleotide was slowly presented (15 mg/mL @ 30 mg/kg dose) by way of a 30 minute infusion into a region of intestine made permeable by a 50 mg/kg bolus of C10 at t=5 minutes. As previously postulated, if the absorption process is zero order (i.e., saturable) then this study design would result in increased oligonucleotide uptake over the corresponding (30 mg/kg) oligonucleotide bolus study described above. The resulting comparative bioavailability is presented in Table 2 and the plasma concentrations are shown in FIG. 2. There was no significant difference in oligonucleotide BAV. This further supports the conclusion that the oligonucleotide absorption process is first order, at least in the range of oligonucleotide dose-concentrations studied.

**TABLE 2**

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Bolus</th>
<th>Slow infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>2484M</td>
<td>4.2</td>
<td>3.8</td>
</tr>
<tr>
<td>2565M</td>
<td>9.7</td>
<td>10.1</td>
</tr>
<tr>
<td>2565M</td>
<td>11.2</td>
<td>4.4</td>
</tr>
<tr>
<td>2462M</td>
<td>3.5</td>
<td>4.0</td>
</tr>
<tr>
<td>2530M</td>
<td>10.3</td>
<td>4.8</td>
</tr>
<tr>
<td>2593M</td>
<td>1.9</td>
<td>8.3</td>
</tr>
<tr>
<td>Average ± SD</td>
<td>1.9</td>
<td>8.3</td>
</tr>
</tbody>
</table>

The data presented above strongly suggest that the approach for formulation design changes to improve BAV should focus on the C10 presentation rather than the oligonucleotide. This is supported by the fact that oligonucleotide is equivalently absorbed by dramatically different presentations (i.e., bolus vs. slow infusion in the face of equivalent C10 presentation). The rapid absorption and loss of C10 from the intestine may limit the area of permeabilization following single bolus C10 administration.

[0166] The central idea of this hypothesis is shown in FIGS. 3A-B, which demonstrates the manner in which current solution dosage forms are believed to present excess PE in the intestinal lumen (FIG. 3A). The excess PE is that amount represented by the area of the PE curve above the so-called maximal effect level required for the maximal mucosal permeability enhancement. An example of this
occurred during a human intubation experiment when a doubling of the PE level (to 3.3 g) failed to bring about an increased response.

Solid Dosage Formulations for Clinical Evaluation

[0167] The purpose of this study is to clinically evaluate PEG-based immediate releasing and pulsatile formulations for enhanced oral oligonucleotide absorption by way of rapidly producing and further extending the dynamic action of sodium caprate (C10) by releasing an additional amount of C10 after the initial amount (FIG. 3B). Three types of dosage forms, representing four formulations, will be evaluated in humans:

[0168] enteric coated (EC) capsules comprising a single population of immediate releasing (IR) 2 mm minitablets with the fall doses of oligonucleotide and C10
[0169] EC monolithic tablets comprising the full doses of oligonucleotide and C10
[0170] EC pulsed-release capsules comprising both a mixture of IR 2 mm minitablets with the full dose of oligonucleotide and partial dose of C10, and delayed release 2 mm minitablets having the remainder of the C10 dose and lacking oligonucleotide.

[0171] The immediate releasing components of the above three dosage forms (4 formulated batches) are made from, for example, hot-melt granulations of PEG-3350, ISIS 104838 and sodium caprate in a high shear mixer, preferably with a controlled temperature of about 70°C. The granules may be compressed into tablets or minitablets without the use of additional excipients.

[0172] Two approaches are intended for the delayed release (pulsed C10) minitablets. It is believed that a matrixed polymer will have a typical burst release of C10 followed by a sustained release over a designated time. A coated polymer approach is characterized by a lag time with more of a delayed (bolus release) profile rather than that expected from a sustained release (FIG. 1). Both of these will be pursued in order to effectively bracket the two parameters mentioned in dosage form iii) above, that is: the delay time and fractional amount of C10 to be released. The C10 released from the matrix burst should actually be construed as part of the initially released C10 pulse—from the other population of minitablets in the capsule (the IR formulation). This consideration of additional initial C10 is important in view of the perceived minimum threshold of dissolved C10 required for permeability enhancement. Accordingly, the appropriate populations of minitablets are filled into Size 00 capsules and then banded prior to enteric coating with HPMC-50.

[0173] Tables 3 and 4 detail four target (sample) formulations.

### TABLE 3

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Formulation 1</th>
<th>Formulation 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target dose per capsule</td>
<td>100:500 mg</td>
<td>100:500 mg</td>
</tr>
<tr>
<td>ISIS104838 (F.L. ODN): C10</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>PEG3350</td>
<td>tbd</td>
<td>tbd</td>
</tr>
<tr>
<td>Physical and Analytical Testing</td>
<td>Assay, Content Uniformity, Dissolution (acid, neutral), Dissolution</td>
<td>Assay, Content Uniformity, Dissolution (acid, neutral), Dissolution</td>
</tr>
<tr>
<td>Manufacture Process</td>
<td>Hot-melt gran</td>
<td>Hot-melt gran</td>
</tr>
<tr>
<td>Encapsulation (Size 00) &amp; EC Enteral Coating</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*One granule batch will be used for formulations 1 and 2

### TABLE 4

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Formulation 3 - matrix pulse</th>
<th>Formulation 4 - coated pulse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target dose per capsule</td>
<td>100:225 mg</td>
<td>100:225 mg</td>
</tr>
<tr>
<td>Composition (max wt ~700 mg)</td>
<td>mg</td>
<td>mg</td>
</tr>
<tr>
<td>C10 - Sodium Caprate</td>
<td>225</td>
<td>225</td>
</tr>
<tr>
<td>ISIS104838 Full Length Purity</td>
<td>tbd</td>
<td>tbd</td>
</tr>
<tr>
<td>Polymer (tbd)</td>
<td>tbd</td>
<td>tbd</td>
</tr>
</tbody>
</table>

Jul. 3, 2003
TABLE 4-continued

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Formulation 3 - matrix pulse</th>
<th>Formulation 4 - coated pulse</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Followed by encapsulation into size 90 caps and enteric coating)</td>
<td>Hot-melt gran Compression</td>
<td>Hot-melt gran Compression</td>
</tr>
<tr>
<td>Physical and Analytical Testing</td>
<td>Disolution</td>
<td>Disolution</td>
</tr>
<tr>
<td></td>
<td>Assay</td>
<td>Assay</td>
</tr>
<tr>
<td></td>
<td>Assay, Content Uniformity, Disintegration (acid, neutral), Dissolution</td>
<td>Assay, Content Uniformity, Disintegration (acid, neutral), Dissolution</td>
</tr>
</tbody>
</table>

IR minibatch details
Pulsed minibatch details

*The IR minibatch details for the two types of formulations are identical and will be made as a single batch.

[0175] The pharmaceutical formulations described above may be administered as a single (e.g., 200 mg oligonucleotide in a single tablet) or divided (e.g., 2×100 mg oligonucleotide tablets taken at the same time) oral dose once per day in an amount comprising between about 50 mg and 1,000 mg oligonucleotide, preferably between about 100 mg and 500 mg oligonucleotide, and more preferably between about 100 and 200 mg oligonucleotide. Alternatively, the total dosage may be divided and administered as separate dosages two, three or more times per day (i.e., one 100 mg tablet twice per day).

[0176] Those skilled in the art will appreciate that numerous changes and modifications may be made to the preferred embodiments of the invention and that such changes and modifications may be made without departing from the spirit of the invention. It is therefore intended that the appended claims cover all such equivalent variations as fall within the true spirit and scope of the invention.

[0177] It is intended that each of the patents, applications, printed publications, and other published documents mentioned or referred to in this specification be herein incorporated by reference in their entirety.

SEQUENCE LISTING

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<223> OTHER INFORMATION: Artificial Sequence

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<210> SEQ ID NO 2
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Artificial Sequence

<400> SEQUENCE: 2
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<210> SEQ ID NO 3
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 3

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<210> SEQ ID NO 4
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 4

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What is claimed is:

1. A delayed release oral formulation for enhanced intestinal drug absorption, comprising:

   (a) a first population of carrier particles comprising said drug and a penetration enhancer, wherein said drug and said penetration enhancer are released at a first location in the intestine; and

   (b) a second population of carrier particles comprising a penetration enhancer and a delayed release coating or matrix, wherein said penetration enhancer is released at a second location in said intestine downstream from said first location, whereby absorption of said drug is enhanced when said drug reaches said second location.

2. The formulation of claim 1, wherein the drug is selected from the group consisting of a protein, peptide, nucleic acid, oligonucleotide, peptide hormone, antibiotic, antimicrobial agent, vasoconstrictor, cardiovascular drug, vasodilator, enzyme, bone metabolism controlling agent, steroid hormone, antihypertensive, non-steroidal antiinflammatory agent, antihistamine, antitussive, expectorant, chemothapeutic agent, sedative, antidepressant, beta-blocker, analgesic and angiotensin converting enzyme (ACE) inhibitor.

3. The formulation of claim 2, wherein said oligonucleotide is an antisense oligonucleotide.

4. The formulation of claim 2, wherein the penetration enhancer in (a) and (b) is the same.

5. The formulation of claim 2, wherein the penetration enhancer in (a) and (b) is different.

6. The formulation of claim 2, wherein the penetration enhancer is selected from the group consisting of a fatty acid, bile acid, chelating agent and non-chelating non-surfactant.

7. The formulation of claim 6, wherein said fatty acid is selected from the group consisting of arachidonic acid, oleic acid, lauric acid, capric acid, caprylic acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcamitine, an acyleholine, a monoglyceride and a pharmaceutically acceptable salt thereof.

8. The formulation of claim 6, wherein said bile acid is selected from the group consisting of cholic acid, dehydrocholic acid, choxycholic acid, deoxycholic acid, glycodeoxycholic acid, chenodeoxycholic acid, ursodeoxycholic acid, sodium tauro-24,25-dihydrofusidate, sodium glycodihydrofusidate, polyoxyethylene-9-lauryl ether and a pharmaceutically acceptable salt thereof.

9. The formulation of claim 6, wherein said chelating agent is selected from the group consisting of EDTA, citric acid, a salicylate, an N-acyl derivative of collagen, laureth-9, an N-amino acyl derivative of a beta-diketone and a mixture thereof.

10. The formulation of claim 6, wherein said non-chelating non-surfactant is selected from the group consisting of an unsaturated cyclic urea, 1-alkyl-alkanone, 1-alkenylaza-cyloalkanone, steroid anti-inflammatory agent and mixtures thereof.

11. The formulation of claim 1, wherein said formulation is a capsule, tablet, compression coated tablet or bilayer tablet.

12. The formulation of claim 1, wherein said carrier particles are bioadhesive.

13. The formulation of claim 1, wherein said carrier particles comprise a substance selected from the group consisting of poly-amino acids, polylamines, polycarboxylates, polycarboxylic acids, carboxylated gelatins, albumins, starches, acrylates, polyethylene glycol, DEAE-derivatized polylamines, polylulans and celluloses.

14. The formulation of claim 1, wherein said carrier particles comprise a material selected from the group consisting of chitosan, poly-L-lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, poly-thiodiethylamino-methylene P(TDAE), polyaminostyrene, poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly-
(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(iso-
hexylcyanoacrylate), DEAE-methacylate, DEAE-ethyl-
hexylacrylate, DEAE-acrylamide, DEAE-albumin, DEAE-
dextran, polymethylacrylate, polyhexylacrylate, poly (D,L-
lactic acid), poly (DL-lactic-coglycolic acid) (PLGA) and
polyethylene glycol (PEG).

15. The formulation of claim 1, wherein said carrier
particles are cationic.

16. The formulation of claim 15, wherein said carrier
particles comprise a complex of poly-L-lysine and alginate,
a complex of protamine and alginate, lysine, dlysine, tril-
ysine, calcium, albumin, glucosamine, arginine, galactosar-
nine, nicotinamide, creatine, lysine-ethyl ester and arginine
ethyl-ester.

17. The formulation of claim 1 wherein said delayed
release coating or matrix is selected from the group con-
sisting of acetate phthalate, propylene glycol, sorbitan
monoleate, cellulose acetate phthalate (CAP), cellulose
acetate trimellitate, hydroxypropyl methyl cellulose phtha-
late (HPMCP), methacrylates, chitosan, guar gum and poly-
ethylene glycol (PEG).

18. A method for enhancing the absorption of a drug in an
animal, comprising administering the pharmaceutical for-
mulation of claim 1 to said animal.

19. The method of claim 18, wherein said animal is a
mammal.

20. The method of claim 19, wherein said mammal is a
human.

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