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EXPERT REVIEW

Phospholipids and Lipid-Based Formulations in Oral Drug Delivery

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ABSTRACT Phospholipids become increasingly important as formulation excipients and as active ingredients per se. The present article summarizes particular features of commonly used phospholipids and their application spectrum within oral drug formulation and elucidates current strategies to improve bioavailability and disposition of orally administered drugs. Advantages of phospholipids formulations not only comprise enhanced bioavailability of drugs with low aqueous solubility or low membrane penetration potential, but also improvement or alteration of uptake and release of drugs, protection of sensitive active agents from degradation in the gastrointestinal tract, reduction of gastrointestinal side effects of non-steroidal anti-inflammatory drugs and even masking of bitter taste of orally applied drugs. Technological strategies to achieve these effects are highly diverse and offer various possibilities of liquid, semi-liquid and solid lipid-based formulations for drug delivery optimization.

KEY WORDS emulsion · liposome · phospholipid · self-emulsifying drug delivery systems · solid lipid nanoparticles

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INTRODUCTION

The gastrointestinal (GI) tract acts as a physiological and chemical barrier setting several challenges for oral drug delivery systems (DDS). The development of composite formulation methods helps to improve bioavailability, and the potential of this emerging field is promising. In this context, increased knowledge on lipids makes them more and more interesting for the formulation of poorly water-soluble drugs and the formation of solubilized phases from which absorption may occur.

Phospholipids have a special amphiphilic character. When placed in water, they form various structures depending on their specific properties. Mostly, they form micelles or are organized as lipid bilayers with the hydrophobic tails lined up against one another and the hydrophilic head-group facing the water on both sides. These unique features make phospholipids most suitable to be used as excipients for poorly water soluble drugs. Thereby, it has to be kept in mind that the enhanced solubility of lipophilic drugs from lipid-based systems will not necessarily arise directly from the administered lipid, but most likely from the intra-luminal processing, to which it is subjected before it gets absorbed.

Normally, the presence of lipids in the gastrointestinal tract induces secretion of gastric lipases, pancreatic lipases

and co-lipases. Gastric lipases hydrolize approximately 25% of acyl chains; thus, depending on the residence time, a considerable amount of ingested lipid is already processed in the stomach (8,83). Endogenous and formulation-derived phospholipids are hydrolized in position 2 by phospholipase A_2, resulting in a free fatty acid and lyso-phophatidylcholine (4,101,114). In addition, the secretion of biliary lipids, bile salts and cholesterol is stimulated, yielding the formation of various colloidal structures, including mixed micelles or unilamellar and multi-lamellar vesicles, which incorporate or associate to a solubilized drug. For a detailed discussion of the various lipidic phases formed during lipid processing in the GI-tract and their impact on drug absorption, see (9,74).

Today, phospholipids are widely used as active ingredients and pharmaceutical excipients, and oral applications become attractive as they offer various options for phospholipids in general, and phosphatidylcholine (PC) in particular. Besides applications as an active ingredient frequently described in the literature, publications also outline the use of phospholipids as an accessory agent or additive in oral pharmaceutical or dietetic formulations. Particularly, in drug high-throughput activity screening programs, a substantial fraction of the new chemical entities is highly lipophilic. About half of the drug candidates are poorly soluble in water, which often leads to issues of low oral bioavailability, high intra- and inter-subject variability, as well as lack of dose proportionality (84). Such compounds are difficult to formulate, especially when higher doses are required. In addition, when administered orally, the systemic absorption of such lipophilic active ingredients may be very low, detracting them from a therapeutic benefit. Accordingly, sophisticated formulation approaches are often needed to achieve adequate exposure. (74). Lipid-based formulations can hence be applied to influence the absorption of active ingredients via various mechanisms, such as modifying the release of active ingredients, improving their bioavailability, changing the composition and hence the character of the intestinal environment, stimulating the lymphatic transport of active ingredients, interacting with enterocyte-based transport processes and reducing unwanted drug side effects. In addition, phospholipids can also be applied to protect active ingredients from degradation in the gastrointestinal tract. Therefore, various phospholipids, such as soybean phosphatidylcholine, egg phosphatidylcholine, or synthetic lecithin/ phosphatidylcholine, as well as hydrogenated phosphatidylcholine, are commonly used in oral applications in different types of formulations. In this article, we will address 1) phospholipid formulation types, 2) drug solubilization in the gastrointestinal tract and impact on bioavailability, 3) retarded release of active substances, 4) lymphatic absorption, 5) reduction of side effects, and as a more technical aspect concerning patient compliance, 6) masking of taste. Table I summarizes the active ingredients discussed in this article.

Phospholipid Formulation Types

Phospholipids offer a number of opportunities to formulate DDS with drugs that exhibit poor water solubility (Fig. 1).

Liposomes

Liposomes are aqueous compartments enclosed by lipid bilayer membranes.

Mixed Micelles

Mixed micelles are micelles comprising at least two different molecular species. Detergent-lipid mixed micelles represent disk-like structures. These micelles resemble small fragments of lipid bilayer with detergent molecules shielding the unfavourable exposure of hydrophobic parts of lipid molecules against water at their edges.

Emulsions

A suspension of small droplets of one liquid in a second liquid with which the former is not mixable is an emulsion. Phospholipids can form oil-in-water as well as water-in-oil emulsions.

Micro-/Nanoemulsions

Micro- and nanoemulsions are based on lipids in fluid state at room temperature. They are usually prepared by high-pressure homogenization leading to droplet sizes in the range of 50–500 nm.

Self-emulsifying Drug Delivery Systems (SEDDS)

SEDDS are mixtures of oil and surfactants, ideally isotropic, sometimes including co-solvent, which emulsify under conditions of gentle agitation, similar to those which would be encountered in the gastro-intestinal tract.

Solid Lipid Nanoparticles (SLN)

SLNs are based on "melt-emulsified" lipids, which are solid at room temperature. Further details can be found in paragraph "Solid Lipid-Based Systems."

Suspensions

A suspension consists of a liquid and a homogeneously dispersed fine-sized solid.

Phospholipid-Drug Complexes

A phospholipid-drug complex is formed by interaction of the phospholipid with a functional group of the drug.

Table I Active Ingredients and Formulations Discussed in this Review Article

Active ingredients	Formulations	References
Acetylsalicylic acid	drug/lecithin associates; liposomes	(1,37,51)
Alpha-Tocopherol	lipid emulsion	(42)
Amphotericin B	cochleate (solid lipid nanoparticles)	(16,79)
Calcitonin	solid lipid nanoparticles; liposomes	(2,22,24,25,85,94,95,104)
Camptothecin	Solid lipid nanoparticles	(108)
Cefotaxime	liposomes	(55)
Cefpodoxime proxetil	microemulsion	(67)
Chlorproguanil Hydrochloride-Dapsone-Artesunate	self-emulsifying system	(45)
Curcumin	liposomes	(93)
Cyclosporin A	microemulsion; vesicular lecithin system; liposomes; drug/lipid aggregates; self-emusifying system; solid lipid nanoparticles	(3,12,14,30,44,48,69,73,88,109)
Diclofenac	pharmacosomes (drug-lipid complex)¸ Drug/lecithin associates	(51,87)
Ethanol		(18,72)
Fenofibrate	liposomes	(10)
Gentiopicrin	microemusifying system	(23)
Griseofulvin	nanoemulsion	(76)
Halofantrine	lipid emulsion, microemulsion preconcentrate	(5,33,39,99)
Hydroxysafflor Yellow A	drug-phospholipid complex oil solution	(103)
Ibuprofen	tablet; drug/lecithin-associates	(21,46,47)
Indomethacin	drug/lecithin associates; microemulsion; liposomes; phosphatidylcholine-conjugate	(19,20,28,51,89)
Influenza A antigen	liposomes	(56)
Insulin	solid lipid nanoparticles; liposomes;	(13,15,35,40,57,110–112)
ß-Lactamase	self nanoemulsifying system	(80–82)
Lipopolysaccharide		(17)
Lovastatin	solid lipid nanoparticles	(26,90)
Melatonin	solid lipid nanoparticles	(77)
N3-o-toluyl-fluorouracil (Fluorouracil)	liposomes	(113)
Naproxen	drug/lecithin associates	(28,51,54)
Nifedipine	solid lipid nanoparticles	(36)
Ontazolast	suspension, emulsion, self emulsifying drug delivery system	(31)
Ovalbumin	liposomes	(58)
Oxymatrine	liposomes;	(6)
Paclitaxel	oil-in-water-nanoemulsion, microemulsion	(68,96,97)
Pantoprazol	solid lipid microparticles	(78)
Pentoxyfylline	solid lipid nanoparticles	(102)
Phenylbutazone	liposomes	(37)
Piroxicam	suspension with lipid	(49)
Praziquantel	solid lipid nanoparticles	(107)
Progesterone	liposomes	(75)
Quinine	drug/lipid dispersion	(66)
Rapamycine	emulsion	(7,43)
rHEGF epidermal growth factor	liposomes;	(50)
Salycilate	drug/lecithin associates	(51)
Sudoxicam	suspension with lipid	(49)
Sylimarin	solid lipid nanoparticles	(32)
Taucocholate	emulsion	(41)
TG100435, TG100855 (Src kinase inhibitor)	emulsion	(34,91)
Vinpocetine	liposomes	(106)

Fig. 1 Overview on typical phospholipid formulations.

Drug Solubilization in the Gastrointestinal Tract—Impact on Bioavailability

Liquid and Semiliquid Systems

The absorption of a given drug depends on the balance of its solubility in the aqueous environment of the gastrointestinal lumen and its capability to diffuse across the lipophilic apical membrane of enterocytes (carrier protein-mediated uptake of charged substances will not be discussed here). Generally, drugs have to be dissolved in order to attain sufficient bioavailability. The solubility of a given drug directly depends on its solid-state properties, e.g. particle size, crystalline or amorphous state, wettability, and others. A primary requirement of a lipid-based formulation is its ability to retain a poorly soluble substance in a solubilized state and to enhance solute-solvent interactions also after mixing with endogenous solubilizers, such as bile acids or phospholipids produced naturally in the body or after intra-luminal processing prior to absorption.

Most likely, liquid or semiliquid formulations fulfill these requirements, and several cases will be discussed here in more detail. A prime example for the use of phospholipids to enhance its solubility is Cyclosporin A (CyA) (marketed under a number of trade names, such as Cicloral® (Hexal AG), Ciclosol® (Sandoz Pharmaceutical AG), Immunosporin®, Sandimmun® (Novartis Pharma), Neoimmun (Kwizda Pharma), Sandimmun®, (Novartis Pharma), Gengraf® (Abbott Laboratories)), an immunosuppressant with a very low aqueous solubility of only 9.29 μg/ml (86). Several lipid-based formulation principles have been described (14). CyA could be incorporated into a vesicular lecithin system, and the pharmacokinetic behavior of that formulation was compared with that of Sandimmun Neoral® (Novartis Pharma), a combination of cremophor, long-chain mono-, di- and triglycerides from corn oil, DL-α–tocopherol and propylene glycol, which forms a microemulsion in water (44). The drug could be solubilized in spherical lipid vesicles of an average size of 64 nm with an entrapment efficiency of 99%, presumably due to the lipophilic nature of CyA (30). Pharmacokinetic studies revealed a relative bioavailability of the vesicular system of 106% *versus* the Neoral formulation, demonstrating that both preparations were bioequivalent. The authors concluded that entrapment of CyA in the vesicles facilitated drug penetration across the gastrointestinal mucin layer (27) and that lecithin, as component of cell membrane, may enhance neutralization, thus increasing the absorption by lymph circulation (109). In everted sac experiments, when the absorption of CyA incorporated into soybean lecithin vesicles was studied (11), some supplementary observations were made: The majority of CyA vesicles accumulated in the mucus before they reached the intestinal wall, and it was concluded that the CyA/Lecithin vesicles may have been adsorbed or combined with the mucoserous polysaccharides and proteins. Then, the p-glycoprotein inhibitor verapamil significantly increased the absorption of CyA-vesicles in the rat intestine. Apparently, the vesicles cannot completely protect cyclosporin A from the efflux by P-gp. The absorption was neither Na^+-dependent nor energy-dependent, but it was concluded that phagocytotic mechanisms are involved in the absorption process, because it reached saturation.

Leigh *et al.* (48) investigated the oral bioavailability of a phospholipid-CyA associate (SupraVail™, Phares Drug Delivery AG), which is based on pre-packaging compounds with selected membrane lipids to obtain drug-lipid complexes where the drug is molecularly associated. These complexes hydrate *in vivo* and convert into organized drug-lipid aggregates, which efficiently present the drug to the absorption window of the gastrointestinal tract in a readily transferable monomolecular state. The active ingredient is dissolved at a fixed ratio in a non-aqueous medium in the lipid (soybean phospholipid) with the medium sometimes removed afterwards. Then, the formulation is decanted into a soft gelatin capsule. In *in vivo* studies, the concentrate showed an improved AUC value (area under the plasma concentration time curve) and also a higher relative bioavailability in comparison with the reference.

The CyA-absorption-enhancing potential of self-emulsifying drug delivery systems based on phospholipids and galactolipids, which are found in chloroplast membranes of green plants, was studied in a trial with human volunteers (69). In this study, fractionated oat oil, a lecithin-

like product, containing 50% neutral lipids and 50% polar lipids (mixed galactolipids and phospholipids 70:30) and medium chain monoglycerides (60:30:10 mono-, di- and tri-glycerides), promoted absorption and resulted in a formulation with absorption characteristics nearly equal to the commercial Sandimmun Neoral® formulation. A number of factors were found to govern the absorption of CyA: the combination of different lipid excipients and the ratio between lipid excipients and degree of drug incorporation.

Another good example is an oral formulation of the immunosuppressant Rapamycin, Sirolimus® (marketed under the trade name Rapamune® by Wyeth-Pharmaceuticals), which is mainly used to prevent rejection of kidney transplants. Here, the drug is formulated with PHOSAL 50 PG® (Lipoid GmbH) as solubilizer of the highly lipophilic drug. PHOSAL® 50 PG is a standardized phosphatidylcholine concentrate with at least 50% PC in propylene glycol, containing Lecithin, sunflower mono and diglycerides and ascorbyl pamitate. The manufacturer of Rapamune® recommends to mix the dose with at least 60 ml of water or orange juice, but not any other liquids, to stir the emulsion vigorously, to drink it immediately followed by another 120 ml of water or orange juice. A previous study (7) investigated the impact of two different formulations of Sirolimus® on blood levels and the development of arthritis induced in rats. On the one hand, Sirolimus® was formulated as a suspension with TWEEN™ 80 (Polysorbate 80) (Croda International PLC) (polyethylene glycol sorbitan monooelate), and on the other hand as a highly water-diluted emulsion containing PHOSAL® 50 PG and 1% TWEEN™ 80. In all the orally administered doses (0.5 mg/kg, 1.5 mg/kg and 4.5 mg/kg), the blood levels of the active ingredient were higher with the vehicle containing phosphatidylcholine. These higher blood levels correlated positively with the therapeutic effect on arthritic symptoms in the animals. With the PHOSAL® 50 PG formulation, approximately one-sixth of the dose proved sufficient to inhibit the arthritis, demonstrating that the application of phosphatidylcholine improves the absorption, effectiveness, and therapeutic index of the active ingredient, while simultaneously enabling the administration of a lower dosage and reducing medication costs side effects.

Paclitaxel (marketed under the trade name Taxol® by Bristol-Myers Squibb) is a tumor-inhibiting agent which is widely used in the therapy of breast cancer. The drug has also a very low water solubility and hence a very low oral bioavailability. Tiwari and Amiji (96) and Tiwari *et al.* (97) encapsulated the substance in an oil-in-water nanoemulsion, with LIPOID E80 (Lipoid GmbH) (80% phosphatidylcholine, 8% phosphatidylethanolamine, 3.6% nonpolar lipids and about 2% sphingomyelin) as emulsifier. The formulated nanoemulsions had a particle size range of approximately 90–120 nm and zeta potential values ranging from −56 mV to +34 mV. Measuring the distribution of paclitaxel in mice yielded significantly elevated blood concentrations (AUC and C_{max}) compared to those after administration of an aqueous solution. However, a large portion of the active ingredient still remained in the gastrointestinal tract.

Paclitaxel was also encapsulated in a microemulsion by Nornoo *et al.* (68). Due to the good solubility of Paclitaxel in Myvacet 9-45® (distilled acetylated monoglycerides, Eastman Chemical Co.), all microemulsions are formulated with this oil phase. The microemulsion containing lecithin:butanol mixture as surfactant showed a 3-fold increase in the *in situ* permeability of Paclitaxel through rat small intestine compared with Taxol®. The pharmacokinetic parameters in rats were comparable to the commercial formulation. However, the microemulsion formulation based on the surfactant capmul MCM exhibited, due to the pgp inhibitory effect of the surfactant, a higher increase in *in situ* permeability of paclitaxel and also higher AUC and C_{max} values.

Hu *et al.* (34) Tabak *et al.* (91)) achieved an improved bioavailability for the tumor-inhibiting Src kinase inhibitor TG100435 (TergeGen Inc.) and its metabolite TG 100855 (TargeGen Inc.) by the application of phosphatidylcholine. In studies in rat and dog, the formulation of the active ingredient in PHOSAL® 50 PG showed the best AUC values for dosages of 25 mg/kg and 100 mg/kg in comparison with the other formulations using aqueous methylcellulose, aqueous Lutrol® F-68 (BASF, Ludwigshafen, FRG) (polyoxypropylene-polyoxyethylene block co-polymer) and aqueous Solutol® HS15 (BASF) (12-hydroxystearic acid–polyethylene glycol copolymer), which was explained by an improved solubility of the active ingredient in the lipidic phase.

Cefpodoxime proxetil (marketed under the trade name Vantin® by Pharmacia & Upjohn) is an oral cephalosporin antibiotic with poor aqueous solubility and low bioavailability and is therefore also a candidate for lipid-based formulations. Nicolaos *et al.* (67) demonstrated an improvement of the absolute oral bioavailability after administration of a phospholipid-based microemulsion (IMWITOR® 742 (Sasol) (blend of mono-, di- and triglycerides, these being chiefly acrylic and caproic acid), MCT, LIPOID S 40 (Lipoid GmbH) (40% phosphatidylcholine, 12–15% phosphatidylethanolamine, 3% phosphatidylinositol, 4% Lysophosphatidylcholine, 3% triglycerides)). Compared to an ethanolic solution, a suspension and an emulsion of the active ingredient, the microemulsion exhibited the highest absolute bioavailability in rats (97.4%) amongst the formulations tested. In addition, the microemulsion formulation was shown to protect the active ingredient from enzymatic decomposition within the intestine.

Griseofulvin (marketed under the trade name GRISOVIN® by Glaxo Laboratories) is a fungicidal drug

with low aqueous and oil solubility. This leads to low bioavailability. Poullain-Termeau *et al.* (76) showed that a formulation of 0.3% Griseofulvin could be incorporated by submicron emulsions. The oil phase of the emulsion systems consisted of Lecithin, Polysorbate 85 (TWEEN™ 85 (Croda International OLC) (polyethylene glycol sorbitan trioleat), Triacetin, MCT (medium-chain triglyceride) and eventually stearylamine to induce positive charge to the emulsion droplets. Soybean lecithin (LIPOID S 40, Lipoid GmbH) was used for the negatively charged emulsion, while egg lecithin (LIPOID E 80, Lipoid GmbH) was used for neutral or positively charged emulsions. Among the formulations tested, only the submicron emulsion with a positive charge enhanced the bioavailability significantly, compared to a Griseofulvin tablet.

An interesting study was published by Kuentz *et al.* (45), who compared the effect of several lipid- and phospholipid-based oral delivery systems on the bioavailability of a model drug for malaria treatment, Chlorproguanil Hydrochloride-Dapsone-Artesunate (CDA) (developed in collaboration between GlaxoSmithKline, UNICEF, the World Bank, Medicines for Malaria Venture and the WHO), and showed that self-emulsifying systems, including phosphatidylcholine-containing systems, had the most beneficial effect on the bioavailability of the active components in rats.

Liquid lipid-based systems may also be applicable to large molecular weight molecules: ß-Lactamase, a 29 kDa protein, was formulated in a series of self-nanoemulsifying drug delivery systems (SNEDDS) where a solid dispersion of the enzyme in soy-lecithin was dissolved in various nano-emulsifying systems of oils, surfactants and co-surfactants. The experimental design provided 720 compositions, and the dispersion of FITC-ß-Lactamase in soybean phosphatidylcholine was able to dissolve in 16 prototypes (82). All the SNEDDS nanoemulsions resulted in higher transport rates of the enzyme than the free solution across MDCK cell monolayers (80,81). Oral delivery of ß-Lactamase in a self-nanoemulsifying emulsion resulted in a relative bioavailability of 6.34%, which was 2.6-fold higher than that of the free solution. Delivery of ß-Lactamase in the aqueous phase of the nanoemulsion resulted in a PK profile similar to that of the free solution (80,81).

Formulation of a phosholipid-drug complex in a liquid system was demonstrated by Wang *et al.* (103). A complex of Hydroxysafflor Yellow A (HSYA) (a component of the flower Carthamus tinctorius L), a low permeable hydrophilic biopharmaceutic classification system Class III drug, was formed with phospholipid and dissolved in surfactant and Labrafac™ Lipophile WL 1349 (Gattefossé Canada Inc.), a medium-chain triglyceride often used for oily solutions. Oral administration of an aqueous drug solution to rats resulted in very low C_{max} and $AUC_{(0\text{-}8h)}$ values. However, the drug-phospholipid complex oil solution with the same dosage yielded a 20-fold increased C_{max} and a 35-fold increased $AUC_{(0\text{-}8h)}$. Detailed investigation of the underlying mechanisms suggested that emulsifying by bile, and hydrolysis to fatty acids and monoglycerides by pancreatic lipase was one of the enhancing mechanisms of the drug-phospholipid complex oil solution absorption.

The formulation of a Gentiopicrin (obtained from gentians and especially from gentian root)-phospholipid complex in a SMEDDS formulation was done by Gao *et al.* (23). Gentiopicrin is an active exhibiting anti-inflammatory, antibacterial and hepatoprotective activities. The low oral bioavailability is limiting the use of the agent. To improve the bioavailability, a Gentiopicrin-phospholpid complex is manufactured and further loaded into a self-microemulsifying drug delivery system consisting of MAISINE™ 35-1 (Gattefossé Canada Inc.) (Glyceryl mono-linoleate), Miglyol® (Sasol) (Caprylic(Capric trigyceride), LABRASOL® (Gattefossé Canada Inc.) (Caprylocaproyl macrogolglycerides), Cremophor® EL (BASF Aktiengesellschaft) (Polyethoxylated castor oil), and Transcutol® P (Gattefossé Canada Inc.) (Diethylene glycol monoethyl ether). In a Caco-2 cell model, the uptake of Gentiopicrin is enhanced by the complex and the SMEDDS system. If orally administered to dogs, the relative bioavailability of the Gentiopicrin-phospholipid complex in the SMEDDS is 703.62% compared to Gentiopicrin alone.

Solid Lipid-Based Systems

Alternative colloidal drug delivery systems to liquid or semi-liquid formulations are solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs). These colloidal carriers have emerged as a potential alternative to other colloidal systems like polymeric nanoparticles, liposomes and fat emulsions, as they have been stated to combine the advantages of the aforementioned systems and are able to overcome their drawbacks. SLNs are composed of solid lipids, which are stabilized with an emulsifying layer in an aqueous dispersion and which show a submicron colloidal size between 50 and 1,000 nm (61–65). They offer several advantages, such as the avoidance of organic solvents and the possibility to produce high concentrated suspensions, and they allow fast and effective manufacturing processes up to large-scale production. However, drug loading capacities may be limited because of the formation of a perfect lipid crystal matrix (105), the presence of other colloidal structures in the aqueous dispersion and possible changes of the physical state of the lipids, which may cause stability problems, e.g. changes in particle size. An obvious advantage of SLNs is that their lipid matrix is composed of physiological compatible lipids, which minimizes the risk of acute and chronic toxicity (59). In addition, SLNs can

protect encapsulated peptides /proteins against enzymatic degradation in the gastrointestinal mucosa (60). Furthermore, the carrier itself can be taken up to a certain extent by epithelial cells or the lymphoid tissues in Peyer's patches.

Nanostructured lipid carriers (63,100) are prepared not only from solid lipids but from mixtures of solid lipids with liquid lipids (oils) solid at least at 40°C. They allow a higher drug load due to the formation of a less ordered lipid matrix (61,62).

The drug may be incorporated into the SLNs in different ways: into a homogeneous matrix, into shells and as a lipid-coated core. In the matrix, the drug is molecularily dispersed or present in amorphous clusters. Matrix-like particles are formed by high-pressure homogenization. The drug-enriched shell-type contains an outer shell enriched with drug, which covers a lipid core. It is formed when phase separation occurs during the cooling process from the liquid oil droplet to the formation of SLN at hot high-pressure homogenization. The core-type SLN forms when the drug starts to precipitate and the lipid shell formed around this core contains less drug. It is formed when the drug concentration is close to its saturation solubility in the melted lipid.

Due to their protective properties, SLNs and NLCs are of particular interest for peptide and protein delivery, and a series of studies has investigated the incorporation of such molecules into these colloidal systems: SLNs based on soybean phosphatidylcholine and lectin-modified SLNs were selected as carrier systems for Insulin by Zhang *et al.* (110). *In vitro* studies showed these carrier systems to deliver insulin with greater protection from enzymatic decomposition. An *in vivo* study demonstrated reduced blood glucose levels with both SLN formulations in rats. In comparison with a subcutaneous insulin injection, the relative bioavailability based on AUC values amounts to 7.11% for the modified SLN and 4.98% for the non-modified SLN.

A further improvement of insulin bioavailability could be achieved by administration of novel phospholipid-based SLNs, which had additionally been loaded with cell-penetrating peptide (CPP). Thereby, octarginine was incorporated as CPP. The mean particle size of these SLNs was approximately 150 nm. *In vivo* absorption experiments provided a relative pharmacological bioavailability of insulin of 10.4±0.5%, whereas the bioavailability of insulin from SLNs without cell-penetrating peptide was 5.7±0.5 in that study (112).

Biodegradable nanoparticles loaded with an insulin-phospholipid complex were manufactured by a novel reverse micelle-solvent evaporation method, in which soybean phosphatidylcholine (SPC) was used to improve the liposolubility of insulin, and biodegradable polymers as carrier materials to control drug release. Solubilization studies, IR and X-ray diffraction analysis demonstrated the complex formation. Spherical particles of a mean diameter of 200 nm and a narrow size distribution were obtained with a drug entrapment efficiency of up to 90%. When the particles were administered to streptozotocin-induced diabetic rats, 20 IU/kg nanoparticles reduced fasting plasma glucose levels by 42.6% within the first 8 h of administration with an effect lasting for 12 h. PK/PD analysis indicated a relative oral bioavailability of 7.7% compared to a subcutaneous injection.

In another study, salmon calcitonin (sCT) (polypeptide hormone, trade name Micalcic®, (Novartis Pharma AG)) containing biodegradable nanoparticles was formulated by hydrophobic ion pairing of positively charged sCT with fatty acid, phospholipid (dimyristoyl phosphatidyl glycerol) and surfactant.

The calcitonin nanoparticles were orally administered to Sprague-Dawley rats, and serum calcitonin was monitored. Thereby, for a dose of 60 mg/kg, the plasma concentration of calcitonin reached 600 pg/ml at 2 h post-feeding, whereas a dose of 160 mg/kg free calcitonin resulted in negligible amounts in plasma (85).

Cyclosporine A was formulated in an amorphous nanoparticle suspension by a new process, evaporative precipitation into aqueous solution (EPAS). Particle growth and crystallization were limited by hydrophilic stabilizers for drug concentrations as high as 35 mg/ml and drug/surfactant ratios up to 1.0. The suspensions could be dried for oral dosage forms with potential for high dissolution rates (12). In a further study, lipospheres named nanoparticles based on egg phosphatidyl choline were prepared by dissolution of the drug in propylene glycol, polyethylene glycol or N-methylpyrrolidone, mixing of phospholipid and addition of surfactants and emulsifiers to form an oily transparent solution. A nanosuspension was spontaneously formed after mixing the oily formulation with aqueous media (3). A bioavailability study in volunteers showed a correlation between particle size and bioavailability. The best results, similar to the Neoral® (Novartis AG) reference formulation (microemulsion), were obtained for the formulations forming particle sizes below 60 nm.

Solid lipid nanoparticles are also suitable to formulate low molecular weight drugs other than peptides with high and low water solubility. Pentoxifylline (International Nonproprietary Name of a drug sold by Sanofi-Aventis DT. GmbH under the brand name Trental®), a highly water-soluble drug, which is used to treat obstructed arteries, was formulated in SLNs containing lecithin, cetyl alcohol and TWEEN™ 20 (Croda International OLC) (polyethylene glycol sorbitan monolaurate). It was found that the relative bioavailability in rats was significantly increased compared to that of the Pentoxyfylline solution (102).

Praziquantel (marketed under different trade names, such as Biltricide® (Bayer Vital GmbH) and Cesol® (Merck KGaA), an antihelmintic drug effective against flatworms, was administered to rats in SLNs with a mean diameter of 110 nm and a zeta potential of −66.3 mV. The particles consisted of COMPRITOL® 888 ATO (Gattefossé Canada Inc.) (glyceryl behenate), butyl acetate and Lecithin, Pluronic® F68 (BASF) (ethylene oxide-propylene oxide block copolymer), sodium stearate and soybean lecithin and showed an encapsulation efficiency of about 80%. The AUC-value after oral administration of SLNs was 4.1-fold higher than that obtained with a tablet. The mean residence time of the drug was also significantly enhanced after administration of SLNs, resulting in about a two-fold increase compared to the one following tablet administration (107).

Nifedipine (marketed under different brand names such as Adalat® (Bayer Vital GmbH) and PROCARDIA® (Pfizer Labs)) nanoparticle suspensions were prepared by a combination of co-grinding by a roll mill and high-pressure homogenization without any organic solvent by Kamiya *et al.* (36). The mean particle size and zeta potential of the nanoparticles were about 55 nm and −61.8 mV, respectively, and both remained constant over 4 months below 6°C in the dark conditions, suggesting that the negative charge of the phospholipid, dipalmitoyl phosphatidylglycerol, was effective in preventing coagulation of the particles. The particles could be freeze-dried after addition of sugars (glucose, fructose, maltose or sucrose) and exhibited excellent solubility (36).

For a solid lipid nanoparticle formulation of the cholesterol-lowering drug Lovastatin (marketed under different trade names, such as Altoprev™ (Andrx Pharmaceuticals Inc.) and MEVACOR® (Merck & Co. Inc.)), (90) showed that the relative bioavailability increased up to 173% in comparison with a Lovastatin suspension in rats. For mevinolic acid (lovastatin hydroxy acid) the bioavailability rose to 323.7%. Ge *et al.* (26) created an emulsion from Lovastatin, TWEEN™ 80 and PHOSAL 53 MCT (Lipoid GmbH) (53% phosphatidylcholine, medium chain triglycerides (caprylic/capric triglycerides), alcohol, glyceryl stearate, oleic acid, ascorbyl palmitate) and spray-dried it with a starch matrix. With the dry, re-dispersible emulsion thus created, the AUC value in rats was 1.83 times higher than that achieved with a Lovastatin suspension. In addition, the active ingredient showed a better protection against decomposition by enzymes.

Delmas *et al.* (16) showed for the antimycotic Amphotericin B (marketed under different trade names, such as Fungilin (Bristol-Myers Squibb), Abelcet® (ENZON Pharmaceuticals), AmBisome® (Astellas Pharma US, Inc), Fungisome™ (Lifecare Innovations Pvt. Ltd.), Amhocil™ (Liposome Technology, Inc), Amphotec® (Three Rivers Pharmaceuticals®)) a reduced mortality amongst mice suffering from an aspergillus infection following oral administration of a cochleate, being a solid particle made up of a simple natural phospholipid from soybeans and calcium (phosphatidylserine) formulation. Cochleates have a unique multilayered spiral structure, which is composed of a negatively charged phospholipid and a divalent cation, and can encapsulate diverse drug molecules of various shapes and sizes while minimizing toxicity associated with polymeric materials present in micro- and nanoparticle systems (79). The cochleate formulation of amphotericin B turned out to be as effective as a control formulation administered intraperitoneally.

Semalty *et al.* (87) prepared pharmacosomes (amphiphilic drug-lipid complexes) of Diclofenac and soybean phosphatidylcholine (LIPOID S80). Diclofenac (marketed under different trade names, such as Voltaren® (Novartis Pharma)) is an NSAID (non-steroidal anti-inflammatory drug) with relatively low water solubility and exhibits gastric side effects. The equimolar association of phosphatidylcoline with diclofenac led to higher water solubility (22 μg/ml) compared to free diclofenac acid (10 μg/ml). The *in vitro* dissolution in phosphate buffer for the complex is increased to approximately 88% compared to approximately 60% for the free drug, potentially leading to elevated diclofenac bioavailability.

The body distribution of camptothecin from solid lipid nanoparticles after oral administration to rats was studied by Yang *et al.* (108). Camptothecin-loaded solid lipid nanoparticles (CA-SLN) coated with poloxamer 188 were produced by high pressure homogenization. They had an average diameter 196.8 nm with a Zeta potential of −69.3 mV. The encapsulation efficiency of camptothecin was 99.6%, In tested organs, the area under the curve and mean residence time increased significantly as compared with a camptothecin solution. Thereby, the increase of brain AUC was the highest among all tested organs. Thus, it was concluded that SLN might be a promising sustained release and targeting system for camptothecin or other lipophilic antitumor drugs after oral administration.

Oral Liposomes

The use of liposomes as oral drug delivery system is difficult due to the poor stability of the vesicles under the physiological conditions typically found in the GI tract. Nevertheless, there are manifold studies and recent publications that indicate the potential of phospholipid-based liposomes to enhance the bioavailability of poorly soluble and low-bioavailability drugs, including peptides and proteins.

Cyclosporin A could be formulated in lecithin vesicles with an incorporation of >98% by Guo *et al.* (30), and a comparative study with the marketed CyA-formulation Sandimmun Neoral® in rabbits proved both formulations

to be equivalent after oral administration. In a second study (11), the absorption behavior of CyA/lecithin vesicles was studied in rat intestinal tissue using everted gut sacs and an *in situ* circulation method. Most of the vesicles accumulated in the mucus before it reached the intestinal tissue, but there was no significant difference in the accumulated absorption content in the incubating medium and the sacs of Sandimmun-Neoral®-and-CyA containing vesicles. Although the mucus appeared to be a barrier blocking the diffusion of the liposomal system, Sandimmun-Neoral-and-CsA-containing vesicles showed equal absorption levels in the intestine.

In a further study, CyA was formulated in so-called proliposomes by spraying a solution of CyA, egg lecithin and Cremophor EL® in a methanol/chloroform mixture onto lactose in order to obtain a free-flowing powder. After hydrating with water, liposomes were formed. The results of bioavailability studies in rats indicated that the absorption constant for the liposomal product was nine times higher than for free drug solution and four times higher than for a marketed sample of microemulsion (88).

Liposomes may also be suitable to administer larger pepides and proteins via the oral route. A recent study showed a significantly increased pharmacological effect of oral calcitonin after administration of chitosan-aprotinin-coated liposomes as compared to the effect of the peptide in solution (104). Polyelectrolyte complexes between negatively charged multilamellar vesicles based on distearolyphosphatidylcholine and positively charged polymer-protease inhibitor conjugate chitosan-aprotinin were manufactured. It could be demonstrated that chitosan-aprotinin could inhibit Trypsin *in vitro*. In comparison to calcitonin in solution, the area above the blood calcium concentration-time curve (AAC) after oral administration of calcitonin-loaded chitosan-coated liposomes to rats increased about 11-fold and about 15-fold in the case of calcitonin-loaded chitosan-aprotinin-coated vesicles.

To optimize the properties of chitosan-coated calcitonin-loaded liposomes, the influence of type of chitosan and the structure of liposomes on the mucoadhesiveness and the resultant pharmacological effects were studied by Thongborisute *et al.* (95). Low-molecular-weight chitosan (LC) and high-molecular-weight chitosan (HC) were used as coating polymers of liposomes. Both coated liposomes could permeate the mucous layer in the small intestine of rats. LC liposomes showed remarkably prolonged effectiveness in decreasing the blood calcium concentration than HC liposomes.

In another study with oral calcitonin (94), self-assembling mucoadhesive pectin-liposome nanocomplexes were prepared by a mixing cationic liposomes with pectin solution. High intensities of a fluorescent marker could be observed throughout the small intestines of rats and remained at the site of mucoadhesion for as long as 6 h after administration. The calcitonin-loaded nanocomplexes demonstrated a strong pharmacological action over calcitonin solution and calcitonin-loaded standard liposomes with an enhanced and prolonged reduction in plasma calcium concentration, which was attributed to the ability of pectin-coated liposomes to adhere to the mucus layer and prolong retention in the intestinal mucosa.

A similar approach was used for the oral delivery of oxymatrine, a natural quinolizidine alkaloid found in sophora roots, used clinically for treating hepatitis B. (6). Multivesicular liposomes coated with N-trimethyl chitosan (TMC) were characterized *in vitro* in terms of their shape, size, zeta potential, entrapment efficiency, coating efficiency, stability in polymer suspension, and stability in simulated gastric and intestinal fluids. *In vivo*, the area under the plasma concentration-time curve obtained from a pharmacokinetic study in rats of coated oxymatrine-containing multivesuclar liposomes was about 3.26 times that of a simple drug solution, indicating that N-trimethyl chitosan-coated liposomes may be interesting as carrier for oral drug administration.

Several studies indicate that blood glucose level may be significantly decreased by orally administered liposomal insulin. Several attempts have been made to improve the potency of insulin-loaded liposomal systems, e.g., insulin and protamine-containing insulin, respectively, were formulated with a cholesterol, dipalmitoyl phosphatidylcholine-cholesterol (DPPC) mixture, and mucoadhesive agent (methyl cellulose (MC))-added DPPC-cholesterol mixture (15). The liposomal insulin was given to mice and rats by gavage; subsequently, reduced blood glucose levels were observed. Thereby, the pH of the solution and the presence of the protamine sulfate appeared to be of importance. Insulin-loaded liposomes, in which phosphatidylethanol formed by phospholipase D-catalyzed transphosphatidylation of phosphatidylcholine was employed, were prepared by Kisel *et al.* (40). Three types of liposomes were prepared: dipalmitoyl phosphatidylcholine/dipalmi-toyl phosphatidylethanol (1:1 w/w) liposomes; dipalmitoyl phosphatidylcholine/ dipalmitoyl phosphatidylethanol/palmitoyl-stearoyl sucrose (1:1: 0.2) liposomes and liposomes composed of natural phosphatidylcholine and phosphatidylinositol (1:1). Oral administration of all liposomal species to rats resulted in hyperinsulinemia. Hyperinsulinemia induced by liposomes containing dipalmitoyl phosphatidylethanol was accompanied by a decrease of blood glucose concentration; however, no correlation between insulin and glucose concentration in blood was observed after oral administration of phosphatidylinositol-containing liposomes.

In another approach to improve muco-adhesion, wheat germ agglutinin (WGA)-modified liposomes and solid lipid nanoparticles were evaluated in an *in situ* local intestinal

perfusion experiment in duodenum, jejunum, and ileum of fasted rats (110,111). The results imply that the type of colloidal carrier and the delivery site were important factors with respect to increasing the bioavailability of insulin following oral administration. Thereby, proteolytic degradation as well as the epithelial permeability were identified as primary determinants influencing insulin mucosal absorption, with the ileum being the best intestinal location for the absorption of insulin-containing liposomes.

A multivesicular liposomal formulation of human epidermal growth factor (rhEGF) was prepared by Li *et al.* (50) to investigate gastric ulcer healing effects of rhEGF. The multivesicular liposomes were prepared by a two-step water-in-oil-in-water double emulsification process with a loading efficiency of up to 60%. Approximately 47% and 35% of rhEGF was released from the multivesicular liposomes within 6 h in simulated intra-gastric fluid (pH 1.2) and intra-intestinal fluid (pH 7.4), respectively, and enzymatic degradation of the peptide was markedly suppressed at incubation with a Caco-2 cell homogenate. Although the transport of rhEGF from the multivesicular liposomes to the basolateral side of Caco-2 cells was two times lower than that of the rhEGF in aqueous solution, the gastric ulcer healing effect of the fhEGF-loaded liposomes was significantly enhanced compared with that of rhEGF in aqueous solution and was comparable to that of cimetidine in rats.

A mixed-micellar proliposomal formulation of progesterone was prepared by Potluri and Betageri (75) by mixing progesterone:dimyristoyl-phosphatidylcholine: Polysorbate 80 (1:20:3.3). This formulation showed an increased transport of the drug across Caco-2 cells and across everted rat intestinal sacs compared with control by enhancing the extent of dissolution and membrane transport of progesterone.

Liposomes may also be interesting for vaccination. Ogue *et al.* (70) were able to show that liposomes (HSPC, DPPC, DMPC; DMPG) can provide suitable delivery systems for an oral vaccine as the stability of the vaccine against decomposition is improved by the formulation with phospholipids. Mice orally vaccinated with ovalbumin (OVA) as model antigen showed significantly raised immunoglobulin A values (IgA) when liposomes were applied, in comparison with the administration of an aqueous OVA solution. The vaccine formulation with phospholipids resulted in an overall improvement of the immune response. A further study showed that liposomal OVA with different lipid compositions induced suppression of the proliferative responses of popliteal lymph node cells from the treated mice to OVA, suggesting that these treated mice had developed tolerance. OVA entrapment in the liposomes could modulate the tolerance-inducing dose of OVA itself, suggesting that liposomes can be suitable antigen-delivery systems for modulated and/or effective induction of oral tolerance (58).

The immune response in mice and the influence of disease process in the classical ferret model of disease was studied with bile acid-containing liposomes with entrapped influenza A antigen-containing haemagglutinin (56). Two types of liposomes were created: small liposomes (range 10–100 nm, Z-average diameter 250 nm) and a large liposome population (60–350 and 400–2,500 nm, Z-average 980 nm). Following oral vaccination of BALB/c mice (an albino, laboratory-bred strain of the House Mouse), large liposomes generated an immune response that had a significantly greater type helper T cellsbias (Th1) than small liposomes measured by serum IgG2a production and antigen-induced spleen cell interferon-gamma (IFN-γ) production. In the infection challenge model in ferrets, vaccination with large liposomes resulted in greater protection in terms of symptom-score and a higher responder number. Both oral vaccine formulations were an improvement compared to intramuscular administration in terms of higher antibody production, lower temperatures, and reduced symptoms over time, post-infection indicating that oral vaccine formulations can be designed to enhance the effectiveness of vaccine antigens.

Bile salt-containing liposomes were also used to enhance the bioavailability of Fenofibrate (sold under different trade names, such as Trilipix™ (Abbott Labs), Lipofen® (Kowa Pharmaceuticals America Inc.), Lofibra® (Teva Pharmaceuticals), Lipidil® (Solvay Pharmaceutical), and Antara® (Oscient Pharmaceuticals)) (10). Liposomes composed of soybean phosphatidylcholine (SPC) and sodium deoxycholate (SDOC) were prepared by a dry-film-dispersing method coupled with sonication and homogenization. In release experiments, no more than 20% of total Fenofibrate was released from SPC/cholesterol (CL) and SPC/SDOC liposomes at 2 h, whereas near complete release was seen with micronized Fenofibrate capsules. *In vivo*, Beagle Dogs demonstrated a 5.13- and 3.28-fold higher bioavailability of SPC/SDOC and SPC/CL liposomes, respectively, than micronized Fenofibrate. The obvious discrepancy between bioavailability and *in vitro* release for liposomes strongly suggests alternative absorption mechanisms to only enhanced release.

N3-o-toluyl-fluorouracil, the prodrug of Fluorouracil (marketed under different trade names, such as Adrucil® (Pharmacia & Upjohn), Efudex® (Valeant Pharmaceuticals International)) with tumor-inhibiting properties, was encapsulated in soybean lecithin liposomes (113) in order to improve its oral bioavailability. In comparison with an aqueous dispersion of the active ingredient, the bioavailability of the active ingredient was significantly improved in mice. In addition, a solid dispersion of the active ingredient in the lipids improved its bioavailability. One explanation

was an improved dispersion of the active ingredient in the intestine and an extended residence time were caused by the liposomes' affinity to the intestinal mucosa.

Proliposomes containing Vinpocetine (marketed under brand names such as Intelectol® (Menory Secret, Inc.)), a seed extract from perivincle (vinca major), which is used for the treatment of cerebrovascular disorders and age-related memory impairment, were prepared by a novel method using a center composite design (CCD) by Xu *et al.* (106). The formulation contained soybean phosphatidylcholine, cholesterol and sorbitol. After contact with water, the suspension of vinpocetine liposomes formed automatically with a particle size of about 300 nm. In rabbits, the bioavailability of Vinpocetine in proliposomes was more than 3.5 times higher than the vinpocetine suspension.

The oral bioavailability of curcumin, a food additive and spice with potential antimetastatic properties, was evaluated by Takahashi *et al.* (93). They encapsulated curcumin into liposomes made from commercially available lecithins (SLP-WHITE and SLP-PC70 (True Lecithin Kogyo Co., Ltd.)). Liposomes prepared from 5 wt % SLP-PC70 and 2.5 wt % curcumin resulted in a good dispersibility, while those from SLP-WHITE did not. SLP-PC70 yielded small unilamellar vesicles with a diameter of approximately 263 nm. Three forms (curcumin, a mixture of curcumin and SLP-PC70 lecithin, and SLP-PC70 liposomes) were then administered orally to rats. In the case of the liposomes, a high bioavailability of curcumin became evident with a faster rate and greater extent of absorption of curcumin as compared to the other forms. The plasma antioxidant activity following oral liposome administration was significantly higher compared to the other treatments. Thus, the data suggest that liposome encapsulation of ingredients such as curcumin may be used as a novel nutrient delivery system.

A liposome preparation that is taken up by receptor-mediated endocytosis has been developed to enhance the oral bioavailability of poorly absorbable peptidomimetic drugs by use of folic acid as the mediator of uptake. Folic acid was coupled to the surface of liposomes containing Cefotaxime (marketed under various trade names, including Claforan® (Roussel-Uclaf) and Taxim (Alkem Laboratories Ltd.)) as model drug (55). Administration to rats resulted in an enhanced oral bioavailability of Cefotaxime with an increased peak plasma concentration as compared with folic acid-free liposomes. Thus, the coupling of folic acid might be a useful approach to supplement oral liposomal delivery systems.

Retarded Release of Active Substances

Several examples demonstrate that phospholipids in oral formulations may alter the bioavailability of active substances by retarding the release of active agents (hydrophilic, lipophilic, amphiphilic) from the formulations. Retardation can be achieved with different oral phospholipid formulations, including liposomes, SLNs, nanocapsules, tablets or simple association of phosphatidylcholine with the active. The beneficial effect of lipidic formulations on insulin has already been discussed. A previous study by Manosroi and Bauer (57) showed lowered blood glucose levels following oral administration of a liposomally (hydrogenated soy phosphatidylcholine) encapsulated insulin in a rat model, accompanied by a delay of the effect. However, as the efficacy of liposomes may be limited due to the surfactants in the intestine (e.g., bile acids), a coating might improve their protective as well as the retarding properties. Iwanaga *et al.* (35) showed that incorporation of insulin into surface-coated dipalmitoylphosphatidylcholine liposomes (either cetyl-Mucin or DSPE-PEG modified) resulted in a significantly decelerated release of insulin compared to standard liposomes *in vitro* as well as a prolonged duration of the glucose-lowering effect in rats. Coating of liposomes may be of particular interest for lipophilic compounds, which show a rapid redistribution, such as Cyclosporin A (73), where a delayed release out of DPPC/PS liposomes enclosed in alginate microparticles has been observed *in vitro* and *in vivo*.

Besides liposomes, solid lipid nanoparticles (SLN) can also be used to delay an active ingredient's release, as demonstrated for Silymarin (mixture of flavonolignans extracted from blessed milk thistle (Silybum marianum) consisting of silibinin A and B, isosibilinin A and B, silicristin, silidianin) *in vitro* as well as *in vivo* in a mouse model (32), and melatonin, which was administered orally to 7 volunteers in a release-delaying formulation of soy phosphatidylcholine (77), resulting in increased elimination half-life period and AUC.

Phospholipids can also be used to retard the release from other oral formulations such as microparticles or tablets. Raffin *et al.* (78) described the production of powder agglomerates from microparticles of Pantoprazole (marketed under different trade names, such as Somac® (Pfizer), Inipomp® (Sanofi-Aventis) and Pantozol® (Nycomed Pharma GmbH)) and lecithin/mannitol, which were resistant against gastric juices and exhibited a retarded release *in vitro*. The more lecithin used for producing the agglomerates, the more augmented retardation of pantoprazol release was observed.

Hydrogenated phosphatidylcholine (PHOSPHOLIPON® 80 H (Lipoid GmbH) (80% phosphatidylcholine)) was applied to produce tablets containing ibuprofen (originally marketed as Brufen® (Abbott AG) and since then under various other trademarks, including Nurofen®, Advil® and Motrin®) in order to decelerate the release of the active ingredient, avoid or weaken its side effects, and also

to mask the taste of the ibuprofen (21). Most probably, retardation occurs by association of the active ingredient to the phospholipid. A similar observation was made by Lamprecht *et al.* (46), who also applied hydrogenated PC (LIPOID S75-3 (Lipoid GmbH)). Besides an 18% improvement of the AUC value in rats, oral administration of the formulation also showed the effectiveness of ibuprofen encapsulated in lipid nanocapsules to be prolonged by up to 4 h.

Salmon calcitonin (sCT) was formulated for oral delivery by a double emulsion-solvent emulsification method in lecithin/tripalmitin SLNs, which received an additional coating with chitosan. The particles exhibited a continuous and slow release of the associated peptide, which was attributed to the affinity of the peptide for the lipids and to the absence of degradation of the lipid matrix under the *in vitro* release conditions (24,25). After oral administration of salmon calcitonin-loaded chitosan-coated nanoparticles to rats, a significant and prolonged reduction in the serum calcium levels as compared to those obtained for control (calcitonin solution) could be observed. In contrast, the hypocalcemic response of sCT-loaded PEG-coated nanoparticles was not significantly different than that provided by the control, suggesting that the surface properties of particles play an important role in the improvement of the efficiency of oral sCT formulations (25).

These publications clearly demonstrate the usefulness of phospholipids, and in particular phosphatidylcholine, in realizing oral carrier systems such as liposomes for hydrophilic or amphiphilic and SLN for hydrophobic active ingredients as well solid dosage forms such as powder or tablets as with a retarded active ingredient release.

Lymphatic Absorption

The rate of fluid flow in the portal blood is approximately 500-fold higher than that of the intestinal lymph, and most drug molecules are absorbed via the enterocytes into the mesenteric veins, the portal vein and the liver into the systemic circulation. However, some very lipophilic drugs associate with lipoproteins within the enterocytes and reach the systemic circulation via the intestinal lymph (98) with the advantage of a reduced first-pass metabolism. Thus, the intestinal lymphatic system plays an important role in absorption of products from lipid digestion, e.g long-chain fatty acids and lipid-soluble vitamins (for a detailed review see (98).

This pathway is directly affected by lipids. Resynthesized trigycerides form the core lipid of intestinal lipoproteins, particles consisting of a hydrophobic core and a more hydrophilic surface (primarily phospholipids, cholesterol and apolipoproteins). These particles are preferentially processed into the lymphatic system rather than into the blood capillaries. Very lipophilic drugs and xenobiotics (e.g. DDT or arylhydrocarbons) appear to be transported in lymph inside the apolar core of lipoproteins, and uptake into the lymphatic system may be enhanced by simultaneously given lipids. Phospholipids and, particularly, phosphatidylcholine (PC) and its digestion product, lyso-phosphatidylcholine, also enhance lymphatic lipid transport, and lyso-phosphatidylcholine has been shown to enhance the lymphatic transport of alpha-tocopherol (42) and Halofantrine (marketed under the trade name Halfan® (GlaxoSmithKline)) (99). A study in dogs (39) showed a significantly stimulated lymphatic transport of Halofantrine in fasted dogs after administration of a single capsule containing long-chain lipids. Another study (5) in conscious rats demonstrated a correlation between the lymphatic transport of Halofantrine and the chain length of the co-administered triglyceride lipid. The extent of mesenteric lymphatic transport increased from 2.2 to 15.8% after oral administration in short (C_4)- and long (C_{18})-chain triglyceride vehicles, respectively. Holm *et al.* (33) compared the impact of the degree of fatty acid unsaturation (oleic [$C_{18:1}$], linoleic [$C_{18:2}$], or linolenic acid [$C_{18:3}$]) on intestinal lymphatic transport of Halofantrine. Thereby, linoleic acid was significantly superior to linolenic, but not statistically better than oleic acid.

It has often been speculated that self-emulsifying drug delivery systems (SEDDS) may have a high potential to target the lymphatic system, but there is little concrete data available. One study (31) investigated the effects of different lipid-based formulations on the bioavailability and lymphatic transport of Ontazolast, an inhibitor of calcium ionophore A23187-stimulated leukotriene B4 (LTB4) biosynthesis in human peripheral blood leukocytes, after oral administration to conscious rats. Formulations included a suspension (lipid-free control), a soybean o/w emulsion, 2 SEDDS containing Gelucire® 44/14 (Gattefossé Canada Inc.) (lauroyl macrogolglycerides) and Peceol™ (Gattefossé Canada Inc.), (glyceryl oleate) and a solution of the drug in Peceol™ alone. All lipid formulations increased the bioavailability of the drug. Maximum lymphatic transport occurred with the emulsion and the Peceol™ solution. The emulsion prolonged lymphatic transport, which may have been caused by the need for preabsorptive lipolysis of the triglyceride vehicle and an associated slower gastric emptying time. The Peceol™ solution showed the highest rate of lymphatic triglyceride transport. It was suggested that SEDDS, which promote more rapid absorption of ontazolast, could result in higher concentrations of the drug in the enterocytes during absorption and hence improve lymphatic drug transport by a concentration-partitioning phenomenon.

Taken together, it must be stated that the lipid processing into the lymphatic system is quite well understood; however, still little is known about the mechanisms

of drug access to the lymph. Therefore, studies have to be initiated addressing the mechanisms of drug association with lipoproteins in the enterocytes and the impact of lipids and formulation excipients on lymphatic absorption.

Reduction of Side Effects

Commonly administered non-steroidal anti-inflammatory drugs (NSAIDs), such as Aspirin® (Bayer HealthCare) and Diclofenac, can potentially trigger gastrointestinal irritations, which may range from gastric ulceration to gastric haemorrhages. A possible mechanism of the damages was described by Giraud *et al.* (28) for naproxen (marketed under various trade names, such as Alacetan®, Mobilat®, Dolormin® and others) and Indomethacin (marketed under trade names such as Elmetacin®, Indocid®, Indoclir® and many others). The study showed that the administration of naproxen modified the thermodynamic properties of the protective membrane of the gastric mucosa and hence the ability of the phospholipids to maintain the hydrophobic protective layer for the stomach. Significant reduction of these side effects by phospholipids has been demonstrated in various publications. For the five NSAIDs, acetylsalicylic acid (ASA), diclofenac, naproxen, salicylate and indomethacin, (51) showed that the described damages can be avoided by pre-associating the active ingredient with phosphatidylcholine. Anand *et al.* (1) showed in a randomized, double-blind crossover study with 16 volunteers that the ulcer formation potential of ASA is reduced by a chemical pre-association of the ASA with PC (ASA/PC), as the hydrophobic protective layer of the gastric mucosa is maintained. The effectiveness of the ASA, meanwhile, remains unaffected. The acute effect of Naproxen on GI bleeding was shown to be reduced by 75–80% by administering Naproxen chemically pre-associated to PC in a rat model (54). During subchronic administration of either naproxen or naproxen pre-associated to PC to rats with CFA (Complete Freund's Adjuvant)-induced joint inflammation, GI bleeding and formation of GI adhesions or perforations were reduced remarkably by using naproxen-PC while maintaining the therapeutic effects of naproxen. In 2007, Lichtenberger *et al.* (52) studied the effect of a combined administration of ASA, or ASA/PC and COX-2 inhibitors (Celebrex® (Mack, Illert)), respectively, on the mucosa of the stomach. The additional administration of COX-2 inhibitors was shown to further intensify the damaging effect of ASA on the gastric mucosa (stomach trouble, haemorrhagia in test rats) with a slower healing process. The administration of ASA/PC showed no or only minimal side effects, as the protective layer of the gastric mucosa could be maintained. The healing process was comparable to that of the control.

Various publications also show for orally administered indomethacin in a DPPC/ tripalmitine microemulsion (53) or indomethacin in liposomes (EPC/ DPPC; Soehngen (89)) that the use of phospholipids reduces the gastrointestinal side effects.

Lanza *et al.* (47) determined in a randomized double-blind clinical study involving 125 patients the effect of ibuprofen pre-associated with phosphatidylcholine (ibu/PC) on the effectiveness and tolerability in comparison with the ibuprofen itself. The patients suffered from osteoarthritis and hence were dependent on the chronic application of NSAIDs. Given a daily dose of 2,400 mg ibuprofen or ibu/PC, respectively, over 6 weeks, both therapies showed the same effectiveness in terms of anti-inflammatory activity and the patients' freedom from pain. Regarding the protection of the gastric mucosa, the application of ibu/PC showed a trend towards improving the protective function, but this was only significant in older patients (> 55 years). An improved protection of the intestine was observable in general. The application of ibu/PC hence reduces the side effects while providing the same effectiveness, particularly in older patients.

The use of a phosphatidylcholine-conjugate as a prodrug for indomethacin (DP-155) was considered for the treatment of Alzheimer's disease by Dvir *et al.* (19,20). Indomethacin was associated with phosphatidylcholine at the sn2 position via a linker and hence selectively acts as a COX-2 inhibitor. Following the release of the active ingredient, COX-1 and COX-2 are inhibited unselectively. Release and side effects were determined *in vivo* in rats. With the dosage used, rats showed fewer lesions and no gastric ulcer formation; furthermore, renal toxicity was lower. The release showed reduced blood concentrations of indomethacin with a delayed release in the brain, which ultimately resulted in the same effectiveness with reduced side effects.

The protective effect of phosphatidylcholine against gastric ulcers caused by NSAIDs given a simultaneous administration of phosphatidylcholine (Phospholipon PHOSPHOLIPON® 100 (Lipoid GmbH) (pure phosphatidyl-choline)) and NSAIDs was also described in various other publications (29,49,72). Here, the reduction in ulcer formation depended on the type of NSAID used. The formation of ulcers was also reduced by a subsequent administration of phosphatidylcholine.

Unsaturated phosphatidylcholine was shown to have a better protecting effect against formation of gastric ulcers and therefore to be more suitable than saturated phosphatidylcholine, while the effectiveness of the active ingredient (NSAID) was not affected by the simultaneous administration of phosphatidylcholine.

A protective effect on the gastric mucosa before the formation of gastric ulcers could be observed following joint

administration of NSAID (acetyl salicylic acid and phenylbutazone, marketed under the tradename Ambene®) and soy lecithin in liposomal form (37). Prostaglandins are known for their cell-protective effect, and the protective mechanism postulated here also suggests that prostaglandin synthesis is triggered by unsaturated phospholipids as its precursor in the cells of the gastric mucosa.

A protective effect of phosphatidylcholine on the gastric mucosa could also be shown for substances other than NSAIDs, such as ethanol (18,72) and lipopolysaccharides (LPS). LPS administered to rats increased the permeability of the gastric mucosa and hence led to gastric haemorrhages and an increased release of bile acid, among other side effects. Dial *et al.* (17) showed that the administration of phosphatidylcholine (PHOSPHOLIPON® 90 G (Lipoid GmbH)) prevented this increase of the permeability, therefore improving the barrier properties of the gastric mucosa and reducing the risk of gastric haemorrhage. The phosphatidylcholine had no effect on the increased release of bile acid and the systemic effects of the lipopolysaccharides.

These examples clearly show that the use of phospholipids, and particularly of unsaturated phosphatidylcholine, as an additive can reduce the side effects of orally administered active ingredients on the gastro-intestinal tract. This in particular applies to non-steroidal anti-inflammatory drugs, whose side effects, such as the formation of lesions in the stomach and gastric ulcers right up to gastric haemorrhages, can be reduced by the application of phosphatidylcholine, as the phosphatidylcholine maintains the function of the hydrophobic gastric mucosa and furthermore stimulates prostaglandin synthesis, which is important for the protection of gastric cells.

Further examples for an improvement of the side-effect profile of active ingredients by applying phospholipids include the following substances: NSAIDs (Piroxicam, marketed under various trade names, including Feldene® (Pfizer Labs)) (49), Sudoxicam (49), Taurocholate (41), Sirolimus® (7,43) and anabolic steroids (71).

Masking of Taste

The unpleasant taste of drug formulations attributable to active ingredients or additives reduces the acceptance of several medications and hence results in low patient compliance. For this reason, the masking of an undesirable taste is one of the aims pursued in the development of medical drugs.

Phosphatidic acid can be used to mask bitter taste, as demonstrated by a study. Nakamura *et al.* (66) developed an electronic taste sensor in order to measure the masking of the very bitter taste of quinine. Phospholipids mask bitter flavors selectively, while other flavors are not affected by their application, as established by Katsuragi *et al.* (38), who used soy lecithin, Phosphatidic acid and phosphatidylinositol in a volunteer study and by Takagi *et al.* (92), who applied a cocktail comprised of 15–20% phosphatidic acid, 40% Phosphatidyl inositol, 10–15% phosphatidyl ethanolamine and 5% phosphatidylcholine in an electronic tongue device. Besides phosphatidic acid, hydrated PC can also be used to mask the taste of active ingredients, as shown by Fini *et al.* (21), who masked the taste of ibuprofen in tablets (see also Retarded Release of Active Substances).

CONCLUSIONS

Taken together, the discussed examples show that phospholipids offer manifold possibilities to be used as excipients in oral formulations.

In particular, substances with low aqueous solubility or low membrane penetration potential are good candidates for bioavailability enhancement by phospholipid formulations. These may be formulated into liquid or semi-liquid drug delivery systems with phospholipids like vesicular dispersions, proliposomes, emulsion systems or self-emulsifying drug delivery systems to enhance their bioavailability. To fulfill this task, solid lipid-based formulations like solid lipid nanoparticles or nanostructured lipid carriers may also be used. Improvement of the solubility of the drug substances as well as to keep them in solution in the GI tract and the stimulation of the lymphatic transport of drugs are the main mechanisms to enhance the bioavailability of the hardly soluble drug substances. There is also evidence that liposomal formulations may adhere to the intestinal mucus layer, thereby prolonging their residence time. Oral phospholipid formulations might also be used to improve/alter the uptake characteristic of drugs by retarding their release from the formulations. Suitable phospholipid formulations to retard drug release are, for instance, liposomes, solid lipid nanoparticles, tablet, etc. Besides improving the elimination half-life of the drug substances, the overall bioavailability may be enhanced. While formulating drugs with oral phospholipid drug delivery systems for improving/altering the bioavailability of drugs, the therapeutic action of the drug substances formulated in this way is maintained. A further advantage of phospholipid formulations in oral drug delivery is that drugs prone to decomposition by enzymes in the GI tract may be protected from degradation by formulation with phospholipids. Another aspect of oral formulations with phospholipids is their ability to diminish the GI side effects of NSAIDs and harmful effects to the GI tract by other substances (e.g., ethanol). As the oral use of many drug substances is limited by their taste, phospholipids may be used as excipients in oral formulations to selectively mask the bitter taste of these substances. Upcoming is the investigation of phospholipids

per se as active ingredients, a promising field of research awaiting new discoveries.

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