Chitosan-Coated Liposomes of γ-Oryzanol for Oral Delivery

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***Abstract*—γ-Oryzanol is a mixture of ferulic acid esters of sterols and triterpene alcohols from rice bran, with antioxidant, anti-inflammatory and lipid-lowering effects in animal studies. Its poor water solubility and its tendency to oxidise limit oral use. Liposomes suit a lipophilic compound, but they are attacked by bile salts and lipases in the gut. Coating them with chitosan is a published way to add protection and mucoadhesion. This review draws on the abstracts of 42 papers on γ-oryzanol, oral liposome stability, chitosan-coated vesicles and Quality by Design (QbD). In single studies of other drugs, a chitosan-coated liposome retained more than 90% of its drug in simulated gastric and intestinal fluids and raised oral exposure about three-fold, while a thiolated chitosan coat gave almost two-fold mucoadhesion. A chitosan coating was also highly unstable in the presence of salt. We found no chitosan-coated γ-oryzanol liposome and no Box-Behnken study of an oral chitosan-coated liposome. We outline a QbD plan and a set of in-vitro tests. The design is conceptual and no experimental data are reported.**

***Index Terms*—γ-Oryzanol; chitosan-coated liposomes; gastrointestinal stability; dissolution; Quality by Design; Box-Behnken design; oral delivery**

I. INTRODUCTION

γ-Oryzanol is not a single compound. It is a complex mixture of ferulic acid esters of phytosterols and triterpene alcohols found mainly in rice bran, and its composition and concentration vary considerably among rice varieties [1]. Reported effects include antioxidant, anti-inflammatory and lipid-lowering activity [1,2]. The compound is poorly soluble in water and prone to oxidative degradation, and both problems limit its use in foods and medicines [3,4].

Liposomes are vesicles of one or more phospholipid bilayers that can carry both water-soluble and lipid-soluble substances [5,6]. A lipophilic compound such as γ-oryzanol would be expected to sit in the bilayer. The difficulty with oral use is that liposomes are not stable in the gut, where bile salts and pancreatic lipase can disrupt them [7,8]. Coating liposomes with chitosan, a cationic polymer, has been proposed to protect them and to prolong their contact with the intestinal mucosa [9,10].

The aims of this review are to summarise the evidence on γ-oryzanol and its delivery systems, to describe the stability problem of oral liposomes and the evidence for chitosan coating, and to outline a QbD approach to a chitosan-coated γ-oryzanol liposome for better gastrointestinal stability and dissolution. We searched online sources and read the abstracts of the papers cited. We report no experimental results of our own, and statements about a paper rest on its abstract only.

II. Γ-ORYZANOL: SOURCE, ACTIVITY AND ABSORPTION

Source and composition

Rice bran is the main source [1], and on a whole-grain basis rice is richer in γ-oryzanol than other cereals [11]. In 30 European brown rice samples γ-oryzanol ranged from 26 to 63 mg/100 g. The main steryl ferulates were cycloartenyl ferulate and 24-methylenecycloartanyl ferulate, and environmental conditions, not grain maturity, affected the content and composition [12]. The steryl ferulate profile also differs between varieties: in an HPLC survey of black-

purple, red, green and brown rice, four of nine black-purple varieties had a distinct profile, low in campesteryl ferulate and high in campestanyl and stigmastanyl ferulate [13]. Among the antioxidants measured in brown rice, cycloartenyl ferulate was the most abundant, at 89.00 ± 8.07 nmol/g [14]. In a survey of 50 agricultural products by UHPLC-MS/MS, rice bran had the highest content of the two main ferulates, 34.3 and 42.6 × 10^3 µg/kg [15]. Raman spectroscopy with partial least squares regression has been proposed as a rapid method for γ-oryzanol in rice bran oil [16]. For formulation, the point is that the starting material varies, so a defined source and a marker-based specification are needed.

Pharmacological activity

In hamsters fed cholesterol, 1% oryzanol in the diet for 7 weeks lowered total cholesterol by 28%, non-HDL cholesterol by 34% and cholesterol absorption by 25%. At 0.5% for 10 weeks it lowered total cholesterol by 44%, triglycerides by 46% and aortic fatty streaks by 67% [17]. A systematic review of nine rodent studies (394 animals) found better insulin secretion and sensitivity, lower fasting glucose and lower markers of oxidative stress and inflammation, but the evidence was preclinical only [18]. In mice, 100 mg/kg for 21 days improved cognition after an LPS challenge and raised HO-1 and NQO1 in the hippocampus [19], and 0.5% in the diet blocked anxiety-like behaviour induced by a high-fat diet and lowered amygdala TNF-α and IL-1β mRNA [20]. In human neuroblastoma cells, γ-oryzanol stimulated neurite outgrowth and raised BDNF and TrkB expression, and in zebrafish the BDNF response depended on Nrf2 [21]. Cycloartenyl ferulate protected cells from hydrogen peroxide from 10 µM and raised nuclear Nrf2 translocation and HO-1 mRNA [14]. A narrative review suggests that the diverse effects arise from a network of interactions triggered by its antioxidant properties [2]. None of these abstracts describes a special delivery system, so the activity is shown for the plain compound, and what a dosage form must add is better absorption and protection.

Absorption and delivery systems

Absorption is only partly understood. In rats given 14C-labelled γ-oryzanol at 50 mg/kg, 9.8% of the dose appeared in urine over 72 hours, none of it unchanged, and more than 80% of the absorbed radioactivity reached the mesenteric vein as intact material, so absorption was mainly through portal blood and not lymph [22]. After a single oral dose of 300 µmol/kg, intact oryzanol, ferulic acid and its sulfate and glucuronide conjugates were all found in blood, which suggests that the intact esters may contribute to effects and not only the hydrolysed acid [23]. Physical state in the gut may also matter: in mixtures with cholesterol, γ-oryzanol formed stable liquid-crystalline phases with a marked fall in melting temperature, and the authors suggest this could change cholesterol solubility and absorption [24].

Several carriers have been described (Table 1). Nanoemulsions made by ultrasonication gave droplets of about 30 nm, and high-pressure homogenisation gave the best stability [3]. Zein nanoparticles retained more γ-oryzanol than the free compound over 60 days [4]. Ethyl cellulose nanoparticles of 70 to 100 nm reduced its crystallinity [25]. In a comparison of lipid nanoparticles and a nanoemulsion, only the nanoemulsion was unstable at 45 °C, and release from solid lipid particles was biphasic [26]. Nanoprecipitated particles of 93.3 nm, PDI 0.149, released about 70% at 24 hours and, at 50 µg/mL, lowered iNOS and NO to about 65% of control in macrophages [27]. PLGA nanoparticles of 241.6 nm gave an entrapment efficiency of 86.22% and 3.2-fold higher cellular uptake than the free drug [28]. Plant-derived nanovesicles isolated from soaked rice water carried γ-oryzanol, and the brown-rice preparation with γ-oryzanol showed stronger antioxidant activity than the native compound, which the authors partly link to better uptake [29].

None of these carriers is a liposome with a polymer coat. One further record on liposomes based on γ-oryzanol lacked complete bibliographic data, so we do not cite it. Of the carrier abstracts cited here, only the zein study used simulated gastric and then intestinal fluids [4], and no study of a γ-oryzanol vesicle with a coating was found.

III. LIPOSOMES AND THE GASTROINTESTINAL BARRIER

Liposomes are described as the most common nanocarriers for water- and lipid-soluble substances because they are biocompatible, biodegradable and weakly immunogenic [6]. Lipid composition, size and

surface charge can be tailored to change their behaviour [5]. Size and polydispersity index (PDI) are key determinants of stability and efficacy and need to be controlled and validated [30]. Preparation methods that can be scaled up, with their strengths and limits, are reviewed in the same literature [5].

The oral route is demanding. In early work, 10 mM bile salts released more than 80% of the entrapped marker from every multilamellar composition tested except two, and distearoylphosphatidylcholine (DSPC)-cholesterol vesicles also resisted pancreatic lipase, while low or high pH alone had little effect on most compositions [7]. In another study two multilamellar formulations lost a substantial share of entrapped glucose and all entrapped carboxyfluorescein under acid, bile-salt or lipase conditions, and nearly all liposomes stayed in the intestine after 3 hours in situ [31]. Lipid choice mattered: lipids with a high transition temperature (DPPC, DSPC, sphingomyelin) retained significantly more marker than low-transition lipids, and equimolar cholesterol did not meaningfully improve retention of the low-transition lipids [32]. A review of oral liposomes lists instability in the gastrointestinal tract, difficulty crossing membranes and large-scale manufacture as the main obstacles and says that adding polymers or ligands to the bilayer can improve both stability and permeability [8].

IV. CHITOSAN-COATED LIPOSOMES

Chitosan is deacetylated chitin. It is biocompatible and biodegradable, can enhance mucoadhesion and permeation, and carries a positive charge, but its solubility depends on pH and it is sensitive to ions, which calls for specific strategies [33]. Figure 1 shows the rationale for the proposed system, and Table 1 lists the evidence we found.

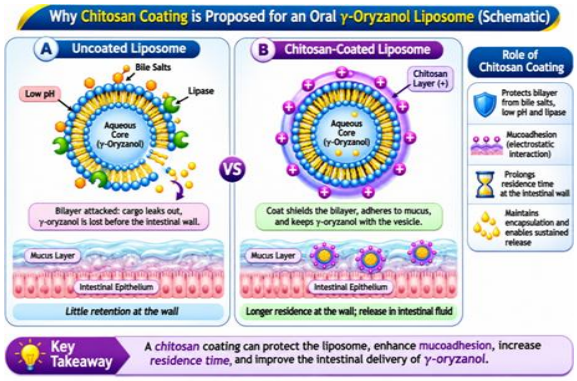


Figure 1. Schematic of the proposed rationale, not data. (A) An uncoated liposome can be attacked by low pH, bile salts and lipase, so the cargo is lost before it reaches the intestinal wall. (B) A chitosan layer is expected to shield the bilayer and to adhere to mucus.

In a rat study from 1996, chitosan-coated liposomes had the strongest mucoadhesion of three polymer coatings, and insulin in them lowered blood glucose for more than 12 hours [9]. Chemical coupling of thiolated chitosan to preformed liposomes raised size from about 167 nm to 439 nm and zeta potential from about -38 mV to +20 mV, and gave almost two-fold mucoadhesion on porcine intestine [34]. A chitosan-coated DSPC/DSPG liposome retained more than 90% of its drug after 24 hours in simulated gastric and intestinal fluids, showed 2.1- to 2.6-fold Caco-2 uptake and about three-fold higher oral exposure in rats [10]. Chitosan reversed the surface charge of curcumin liposomes without changing size or PDI and gave higher curcumin in the intestinal phase of an in-vitro digestion model, although the uncoated vesicles gave slightly more curcumin after the mouth and stomach phases [35].

Several results are cautionary. Chitosan-coated rhamnolipid liposomes (412.9 nm, +19.7 mV) were stable from pH 2 to 5 but highly unstable with added salt, although they delivered more bioavailable curcumin after in-vitro digestion than uncoated ones [36]. A chitosan and alginate coated nano-liposome of about 400 nm released less in simulated gastric fluid and more in simulated intestinal fluid, and its rat AUC was 109 times that of curcumin solution [37]. All of these use model compounds other than γ-oryzanol, so the values are indicative only.

Table 1. Evidence summary: delivery systems reported for γ-oryzanol and studies of coated oral vesicles with other compounds. Values are as given in the abstracts and are not directly comparable.

Compound and carrier	System	Finding	Ref.
γ-Oryzanol, nanoemulsion	Droplets by four high-energy methods	Ultrasonication gave about 30 nm; high-pressure gave best stability	[3]
γ-Oryzanol, zein nanoparticles	Storage and simulated fluids	More retained than free compound over 60 days; sustained release	[4]

Compound and carrier	System	Finding	Ref.
γ-Oryzanol, ethyl cellulose nanoparticles	Solvent evaporation	About 70-100 nm; reduced crystallinity; size stable for five weeks	[25]
γ-Oryzanol, lipid nanoparticles	Wax and glyceride SLN; Miglyol nanoemulsion	Only the nanoemulsion unstable at 45 °C; biphasic release from SLN	[26]
γ-Oryzanol, polymeric and PLGA nanoparticles	Nanoprecipitation; PLGA	93.3 nm, about 70% released at 24 h; PLGA 241.6 nm, entrapment 86.22%, uptake 3.2-fold	[27,28]
Insulin, chitosan-coated liposomes	Rat intestine; rats	Strongest mucoadhesion of three coats; glucose lowered for over 12 h	[9]
Calcitonin, thiolated-chitosan liposomes	Simulated fluids; porcine intestine	167 to 439 nm; zeta -38 to +20 mV; about two-fold mucoadhesion	[34]
Risedronate, chitosan-coated DSPC/DSPG liposomes	Simulated fluids; Caco-2; rats	Over 90% retained at 24 h; uptake 2.1-2.6-fold; exposure three-fold	[10]
Curcumin, chitosan-coated liposomes	In-vitro digestion	Size unchanged, charge reversed; more curcumin after intestinal phase	[35]
Curcumin, chitosan-coated rhamnolipid liposomes	pH and salt; in-vitro digestion	Stable at pH 2-5; highly unstable with salt; more bioavailable curcumin than uncoated	[36]
Curcumin, chitosan/alginate nano-liposome	Simulated fluids; rats	Less gastric, more intestinal release; AUC 109 times solution	[37]

V. QUALITY BY DESIGN FOR THE LIPOSOME

QbD examines input variables (materials, process and experimental design) and quality attributes through planned experiments, with risk assessment, in order to find the critical variables and the best formulation. It is useful for liposomes because trial-and-error optimisation is slow when many variables interact [38]. Its elements are a quality target product profile (QTPP), critical quality attributes (CQAs), critical material attributes (CMAs), critical process parameters (CPPs), a designed experiment and a design space with a control strategy. Table 2 shows how they could be filled in for the proposed system, and Figure 2 shows the workflow.

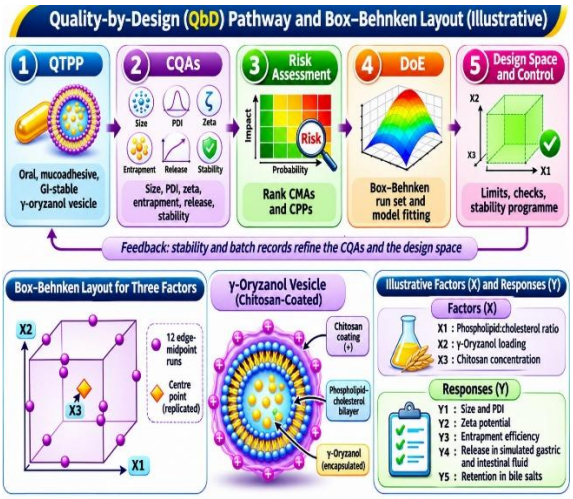


Figure 2. Schematic of the proposed QbD pathway (top) and a three-factor Box-Behnken layout (bottom). The factors and responses are illustrative choices from the literature reviewed and are not experimental results. QTPP, quality target product profile; CQA, critical quality attribute; CMA, critical material attribute; CPP, critical process parameter; DoE, design of experiments.

The QTPP would be an oral dispersion of γ-oryzanol liposomes that keeps its cargo in simulated gastric and intestinal fluids, is mucoadhesive, and is stable on storage. The CQAs follow from that: size and PDI [30], zeta potential to confirm that the surface charge has reversed [35], entrapment efficiency, release in simulated fluids and retention in bile salts [7,32]. Material attributes include the specification of the γ-oryzanol itself, since the content and steryl ferulate

profile vary with the source [1,12], the phospholipid and its transition temperature [32], and the chitosan. The abstracts do not report the effect of chitosan molecular weight or degree of deacetylation, so these would have to be screened. Chitosan amount is likely to be a critical factor, because in one study vesicle size rose from about 167 nm to 439 nm at the highest level of thiolated chitosan [34].

Table 2. Illustrative mapping of QbD elements onto the proposed chitosan-coated γ-oryzanol liposome. The entries are design proposals, not reported results.

QbD element	Proposed content	Basis	Ref.
QTPP	Oral liposome dispersion; cargo kept in simulated gastric and intestinal fluids; mucoadhesive; stable on storage	Obstacles to oral liposomes	[8,38]
CQAs	Size, PDI, zeta potential, entrapment efficiency, release, retention in bile salts and lipase	Size and PDI control; charge reversal; GI damage to vesicles	[7,30,35]
CMAs	γ-Oryzanol source and marker content; lipid type and transition temperature; chitosan grade and charge	Variable composition; lipid effect on retention; ion sensitivity of chitosan	[1,12,32,33]
CPPs	Method of coating (electrostatic or chemical); order and rate of mixing; hydration and sizing step	Coating by attraction or coupling; scalable preparation	[5,34,36]
Risk assessment and DoE	Rank factors; Box-Behnken design with three factors (12 runs plus replicated centre points); response-surface models	Designs used in liposome studies	[38,39]
Design space and control	Region where all CQAs meet limits, including in salt-containing media; raw-material specifications; stability programme	Salt sensitivity of the coat; ion sensitivity of chitosan	[33,36]

Liposome studies with experimental design exist, but they involve other drugs and other designs. A 3^2 factorial design on DPPC:cholesterol and DOPE:DPPC ratios showed that the DPPC:cholesterol ratio was the main factor for size and entrapment, with average prediction errors of 3.59% and 4.09% [39]. A Box-Behnken design with lipid, cholesterol and polymer as factors was used for a nasal venlafaxine liposome that included a chitosan-coated formulation, but the results sentence in the abstract is unclear, so we quote no values [40]. We found no Box-Behnken or central composite study of an oral chitosan-coated liposome.

For γ-oryzanol we suggest a three-factor Box-Behnken design with phospholipid:cholesterol ratio, γ-oryzanol loading and chitosan concentration as factors. It needs 12 edge-midpoint runs plus replicated centre points and avoids extreme corner combinations. Responses would be size and PDI, zeta potential, entrapment efficiency, release in simulated fluids and retention in bile salts. High-transition-temperature lipids should be considered before the factor ranges are set [32], and the ionic strength of the test media should be chosen deliberately given the salt sensitivity reported [33,36]. The factor ranges would come from preliminary screening work.

VI. EVALUATION AND CHARACTERISATION

Size, PDI and zeta potential would be measured by dynamic light scattering and electrophoretic methods, with the zeta potential used to confirm the coating [30,35]. For release, no official pharmacopeial test exists for liposomal products, and the methods in use include dialysis variants, sample-and-separate, in situ detection and modified USP apparatus [41]. The main difficulty is separating released from encapsulated drug, and standard protocols for colloidal carriers are still lacking [42]. Release and dissolution results should therefore be reported with the method described in full.

Stability should be tested in simulated gastric fluid, in intestinal fluid with bile salts, and with lipase, since these damage uncoated liposomes [7,32]. Because the coating is salt-sensitive [36], stability should also be checked at the ionic strength of intestinal fluid. Mucoadhesion can be measured on excised intestine [34], and Caco-2 uptake gives a first permeability estimate [10]. An in-vitro digestion model shows the fate of the vesicle through the gastric and intestinal phases [35]. The physical state of γ-oryzanol in the vesicle can be examined by DSC and X-ray diffraction, as was done for ethyl cellulose particles and cholesterol mixtures [24,25]. Quantification would use a validated method, and UHPLC-MS/MS after solid-phase extraction and Raman spectroscopy with HPLC comparison have been reported for rice products, not for liposomes [15,16]. Table 3 lists the proposed tests.

Table 3. Proposed tests for the chitosan-coated γ-oryzanol liposome and the reasons for including them. The tests follow the literature reviewed; no results are reported.

Test	Purpose	Note	Ref.
Size, PDI, zeta potential	Vesicle quality; confirm coating	Charge reversal expected after coating	[30,35]
Entrapment efficiency	Cargo loading	Reported as a percentage for oryzanol carriers, for example 86.22% for PLGA particles	[28]
Release (dialysis, sample-and-separate, USP apparatus)	Dissolution of γ-oryzanol from vesicle	No pharmacopeial method; describe method fully	[41,42]
Stability in simulated gastric and intestinal fluid, bile salts, lipase	Protection against GI damage	High-transition lipids resist better	[7,10,32]
Stability in salt-containing media	Check coat integrity	Coat highly unstable with salt in one study	[33,36]
Mucoadhesion on excised intestine	Retention at the wall	About two-fold gain with thiolated chitosan	[34]
Caco-2 uptake; in-vitro digestion	Permeability and fate in digestion	Other compounds only	[10,35]
DSC and X-ray diffraction	Physical state of γ-oryzanol	Crystallinity reduced in other carriers	[24,25]
γ-Oryzanol assay (UHPLC-MS/MS, Raman)	Drug content	Methods described for rice products, not liposomes	[15,16]

VII. GAPS AND CONCLUSION

Six gaps stand out. First, we found no chitosan-coated γ-oryzanol liposome. No γ-oryzanol vesicle of any kind has been tested for stability against bile salts and lipase in the abstracts we could cite. Second, we found no Box-Behnken or central composite study of an oral chitosan-coated liposome. The nearest examples are a nasal Box-Behnken study with unclear reporting and factorial designs for other liposomes [39,40]. Third, chitosan coatings can be highly unstable in salt [36], which is the condition of intestinal fluid, and this has not been tested for γ-oryzanol. Fourth, release testing for liposomes has no standard method [41,42]. Fifth, γ-oryzanol is a variable mixture and its absorption involves both intact esters and ferulic acid metabolites [12,23], so the choice of markers and assay needs thought. Sixth, the supporting results come from other

compounds and mainly from abstracts, and none of them shows that the effect will carry over to γ-oryzanol.

A chitosan-coated γ-oryzanol liposome is a reasonable development target. The compound is poorly soluble and variable in source, liposomes suit lipophilic cargo but are damaged in the gut, chitosan coating improved stability, mucoadhesion or exposure in other studies, and QbD tools are well described. The design presented here is conceptual. The next steps would be raw-material specifications, a lipid and chitosan screening study, a Box-Behnken optimisation with salt-containing media, simulated gastrointestinal stability tests and a fully described release method.

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