Journal of Clinical Trials
ISSN: 2167-0870
OPEN ACCESS Freely available online
Research Article

Phosphatidylserine Matrix Enhances Lutein/Zeaxanthin Exposure *Versus* Phosphatidylcholine, Liposome, and MCT Oil in Rats: A 24-hour Pharmacokinetic Study

S. Mehkri[1], K.G. Dinesh[2], G. Ashok[2], Krathish Bopanna[3*]

[1]Bio-gen Extracts Pvt. Ltd., R&D Division, Bangalore, India; [2]Radiant Research, R&D Division, Bangalore, India; [3]Tejhana Consulting LLP, Bangalore, India

ABSTRACT

Background: Lutein and Zeaxanthin (LZ) support macular function, but oral absorption is limited by intestinal solubilization and uptake; phospholipid environments may improve micellization and enterocyte transport.

Objective: To evaluate whether an anionic Phosphatidylserine (PS) matrix enhances single-dose LZ exposure compared to Phosphatidylcholine (PC), a reconstituted liposomal powder, and Medium-Chain Triglyceride (MCT) oil in rats.

Methods: Male Sprague-Dawley rats (n=6 per group) received oral gavage of dose-matched lutein (10 mg/kg) in four matrices (MCT, MCT+PC, MCT+PS, liposome); serial plasma samples (0-24 h) were analysed using validated LC-MS/MS (calibration 5-250 ng/mL). Noncompartmental pharmacokinetics were calculated; group differences in Cmax and AUC$_{0-t}$ were evaluated *via* one-way ANOVA with Dunnett contrasts *versus* MCT.

Results: LZ exposure followed the order PS > PC ≈ liposome > MCT. Cmax (ng/mL, mean ± SD): 52.54 ± 0.70 (MCT), 60.45 ± 1.24 (PC), 69.63 ± 0.78 (PS), 62.39 ± 1.12 (liposome). AUC$_{0-t}$ (ng·h/mL): 494.51 ± 13.70 (MCT), 596.37 ± 30.29 (PC), 620.23 ± 16.41 (PS), 536.70 ± 18.42 (liposome). Dunnett *vs* MCT: PS $p<0.001$; PC and liposome $p<0.01$. T_max was approximately 2 hours for PS/PC *versus* about 3 hours for MCT/liposome; half-life was similar (~7.7-8.3 hours).

Conclusions: Anionic PS oils increase LZ exposure and bioavailability more effectively than PC or liposomal powder at equal doses, without altering elimination.

Keywords: Lutein; Zeaxanthin; Phosphatidylserine; Phosphatidylcholine; Liposome; LC-MS/MS; Pharmacokinetics; Rats

ABBREVIATIONS

AUC$_{0-t}$: Area Under the Concentration-time curve to the last measurable time; Cmax: Maximum plasma concentration; CV %: Coefficient of variation; JBHS: Journal target; LZ: Lutein/Zeaxanthin; MCT: Medium-Chain Triglyceride; NCA: Noncompartmental analysis; PC: Phosphatidylcholine; PK: Pharmacokinetics; PS: Phosphatidylserine; tmax: Time to Cmax; t½: Half-life; pAUC$_{0-4h}$: Partial AUC to 4h.

INTRODUCTION

Lutein and Zeaxanthin (LZ) are dietary xanthophyll carotenoids that concentrate in the macula lutea, where they help visual function by filtering short-wavelength light and quenching reactive oxygen species produced in photoreceptor outer segments. Epidemiologic, interventional, and mechanistic evidence collectively support a role for adequate LZ intake in maintaining macular pigment optical density and promoting

Correspondence to: Krathish Bopanna, Tejhana Consulting LLP, Bangalore, India, E-mail: krathishbopanna@tejhana.net

Received: 29-Oct-2025, Manuscript No. JCTR-25-38917; **Editor assigned:** 31-Oct-2025, PreQC No. JCTR-25-38917 (PQ); **Reviewed:** 14-Nov-2025, QC No. JCTR-25-38917; **Revised:** 21-Nov-2025, Manuscript No. JCTR-25-38917 (R); **Published:** 28-Nov-2025, DOI: 10.35248/2167-0870.25.15.607

Citation: Mehkri S, Dinesh KG, Ashok G, Bopanna K (2025). Phosphatidylserine Matrix Enhances Lutein/Zeaxanthin Exposure *versus* Phosphatidylcholine, Liposome and MCT Oil in Rats: A 24-Hour Pharmacokinetic Study. J Clin Trials. 15:607.

retinal resilience, yet oral bioavailability of LZ remains modest and highly dependent on formulation [1-4]. From a biopharmaceutic perspective, LZ are amphipathic molecules with very low aqueous solubility, and their absorption in the intestine depends on their incorporation into bile salt-phospholipid mixed micelles. After gastric dispersion, dietary lipids, endogenous bile salts, and luminal phospholipids assemble into colloidal structures that solubilize lipophilic compounds and transport them across the unstirred water layer to enterocyte membranes. The quality of this colloidal phase-its size distribution, surface charge, and interfacial composition-significantly influencesthe rate and extent of absorption. Small differences in the formulation's interfacial chemistry can therefore cause notable variations in exposure [5-9]. Phosphatidylcholine (PC), a zwitterionic phospholipid, stabilizes emulsions and enhances the organization and stability of mixed micelles compared to neutral triglyceride oils alone. However, electrostatic interactions at the oil-water interface also affect colloidal behavior.

Phosphatidylserine (PS) contains an anionic headgroup that increases negative zeta potential during digestion. In model systems, a more negative surface charge correlates with smaller, more numerous, and more stable colloids, which can enhance early-phase solubilization and flux of amphipathic xanthophylls. These mechanistic considerations suggest that PS-rich interfaces may accelerate and amplify absorption compared to PC-rich or neutral interfaces [10-12]. Liposomal delivery offers a third, distinct approach. Reconstituted liposomal powders encapsulate actives within bilayer vesicles that can protect against oxidative and photo- degradation before ingestion. However, most liposomes undergo bile salt- mediated destabilization in the gastrointestinal environment, with lipids eventually entering the mixed- micelle pathway. Therefore, entropy- based gains in exposure depend on formulation specifics and may be limited if vesicles disassemble quickly without sustained interfacial advantages. Medium- Chain Triglyceride (MCT) oils are convenient dispersants with low viscosity but provide minimal interfacial structuring, often serving as a baseline comparator for for carotenoid delivery [13-15]. Despite the widespread use of PC and liposomal approaches, direct comparisons against an anionic PS matrix for LZ are limited. Prior studies have focused on total carotenoid dose, fed or fasted state, or antioxidant co-formulants, leaving unclear the specific impact of phospholipid headgroup charge on absorption kinetics and overall exposure. This gap is important because enhancements during the absorption phase (earlier tmax, higher Cmax) could be used to align dosing with physiological demand or co-administration of other nutrients. Furthermore, higher AUC at a fixed dose supports dose-sparing strategies and reduces variability among subjects [3,5,7,10]. This work aimed to isolate the effect of matrix chemistry under dose-matched, fasted conditions in a controlled preclinical model. We compared single- dose LZ pharmacokinetics in male Sprague–Dawley rats across four matrices: (i) neutral MCT oil, (ii) MCT with PC, (iii) MCT with PS, and (iv) reconstituted liposomal powder. By maintaining a consistent LZ dose and applying uniform bio-analytical and noncompartmental methods, we sought to attribute differences between groups to formulation- driven changes in absorption rather than to analytical or dosing confounders [16-18].

We hypothesized that the anionic PS matrix would produce (a) an earlier tmax and higher Cmax, indicating quicker and more efficient micellar delivery across the unstirred water layer, and (b) a higher AUC_{0-t} compared to MCT, with intermediate performance for PC and the reconstituted liposomal powder. We further expected similar terminal half-lives across groups, consistent with a primarily absorption-limited mechanism.

We present our findings with direct implications for nutraceutical formulation design: choosing the phospholipid headgroup and interfacial charge can be a primary factor in increasing xanthophyll exposure, which could enable dose reduction, faster effects, and more consistent results in both preclinical and clinical settings [10-12,16].

A concise mechanistic summary of the matrices and their expected pharmacokinetic impact is provided in Table 1 and Figures 1-2.

Table 1: Mechanistic context for matrices used in this study.

Matrix	Key features during digestion	Expected impact on LZ absorption
MCT (neutral TG oil)	Baseline emulsification; relies on bile salt–lecithin mixed micelles for solubilization.	Lowest exposure; reference condition.
PC (zwitterionic phospholipid)	Improves interfacial stability; forms mixed micelles with bile salts (neutral headgroup).	Moderate ↑ exposure *vs* MCT; earlier tmax than MCT.
PS (anionic phospholipid)	Adds negative interfacial charge; can increase mixed micelle capacity and favor earlier uptake.	Greatest ↑ exposure among tested oils; earlier tmax.
Reconstituted liposome	Vesicular delivery; may partially disassemble in gastric/intestinal phases and feed into micellar pathways.	Modest ↑ *vs* MCT; typically < PS, ≈ PC depending on product/reconstitution.

Notes for citations: Background on bile salt-phospholipid mixed micelles and carotenoid absorption; charge effects on micellization; liposome stability under lipolysis

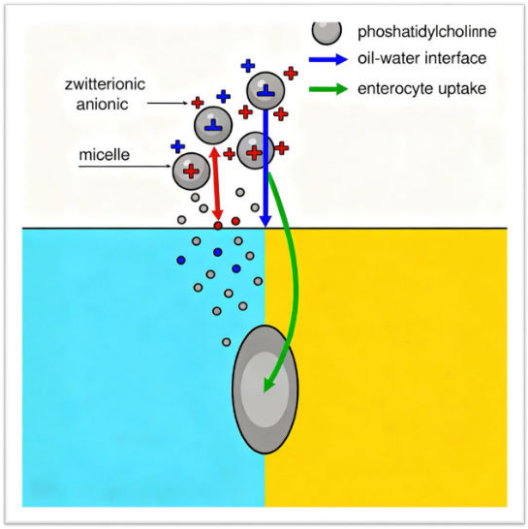

Figure 1: Mechanistic schematic of interfacial charge effects at the oil–water interface illustrating zwitterionic *versus* anionic phospholipid environments and their influence on micellization and enterocyte absorption.

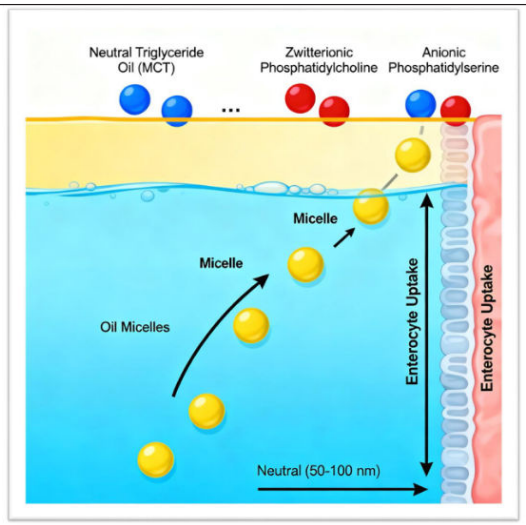

Figure 2: Conceptual diagram comparing neutral MCT, zwitterionic PC, and anionic PS at the interface, demonstrating predicted differences in micelle formation and absorption by enterocytes.

MATERIALS AND METHODS

Study design and endpoints

This was a randomized, parallel-group, single-dose pharmacokinetic (PK) study evaluating lutein/zeaxanthin (LZ) exposure from four oral matrices: (i) MCT oil (neutral), (ii) MCT + Phosphatidylcholine (PC), (iii) MCT +Phosphatidylserine (PS), and (iv) a reconstituted liposomal powder. The primary endpoints were Cmax and AUC_{0-t} for lutein; secondary endpoints were Tmax and apparent terminal half-life ($t_{½}$). The analysis set included all dosed animals with valid concentration-time profiles (no prespecified exclusions were triggered). Sample size rationale: with n=6 per group, the design has approximately 80% power (α=0.05, Dunnett adjustment for three comparisons) to detect about 20%-25% differences in geometric means of Cmax or AUC_{0-t} on the ln-scale, assuming within-group SD ≈ 0.15-0.20-consistent with prior rat PK studies of carotenoids under controlled conditions.

The approximate formula used was $n/group \approx 2\cdot(z_{1-\alpha} + z_{1-\beta})^2\cdot\sigma^2/\Delta^2$ on the log scale.

Animals, housing and ethics

Male Sprague-Dawley rats (8-10 weeks; target body weight 170-190 g at dosing) were obtained from a CCSEA-registered breeder. Animals were acclimated for at least 7 days in SPF rooms (22 ± 2 °C; 45%-65% RH; 12:12 h light/dark cycle; approximately 150-200 lux during the day). They had access to standard pelleted diet and RO water ad libitum, except during the overnight fast before dosing. Environmental enrichment (nesting material) was provided. Maximum cage density was 3-4 rats per cage in polycarbonate cages with corncob bedding, changed twice weekly. All procedures were approved by the Institutional Animal Ethics Committee (Radiant Research Services, Bangalore; protocol ID: RR250056-65/PC/PK/01-2025) and adhered to CCSEA guidelines for care, anesthesia, and humane endpoints.

Randomization and blinding

Animals were randomly assigned to treatment groups based on baseline body weight strata (block size=4) generated in R (set.seed 2025). Dosing personnel, who were aware of group assignments, prepared matrices accurately; bio-analytical staff and PK/statistical analysts remained blinded to group codes until database lock. Cage placement was rotated daily to reduce positional bias. Sample tubes were labelled with anonymised barcodes to preserve analytical blinding.

Test articles and formulations

LZ active (Bio-gen Extracts Pvt. Ltd.) was administered across all groups at a target lutein dose of 10 mg/kg. Excipients used were food or analytical grade: MCT oil (caprylic/capric triglyceride), PC (≥70% phosphatidylcholine), and PS (≥ 50% phosphatidylserine), sourced from reputable suppliers (lot numbers recorded in the raw data).

Preparation

MCT (neutral: LZ suspended in MCT with vortexing (2 × 60 s) and brief probe sonication (≤ 30 s; ice bath) to improve dispersion.

MCT+PC: As above, with PC at 10% w/w of the oil phase (pre-dissolved in a small aliquot of MCT before combining).

MCT+PS: As above, with PS at 10% w/w of the oil phase.

Reconstituted liposome: Liposomal powder containing LZ reconstituted in sterile water immediately before dosing (1:10 w/v) according to supplier instructions; gentle inversion avoided foam.

All preparations were performed under low light using amber lab ware to minimize photo-degradation. Short-term

homogeneity and bench stability (4 h) were verified by measuring absorbance at 445 nm on aliquots drawn at the start and end of dosing (acceptance: ≤ 5% difference). A small pilot aliquot from each matrix was diluted 1:1000 in isopropanol to visually check for undissolved particulates.

Optional pre-dose colloidal characterization

To support mechanistic interpretation (non-inferential), a subset of the MCT+PC and MCT+PS dispersions was gently emulsified in simulated intestinal fluid (bile salt/lecithin) and assessed by Dynamic Light Scattering (DLS) for z-average diameter and polydispersity; zeta potential was measured by electrophoretic light scattering. These exploratory data were used qualitatively and not subjected to formal statistics [16].

Dosing and serial sampling

After a 12-hour fast, rats received a single oral gavage (≤10 mL/kg) using rounded-tip feeding needles (16-18G, 75 mm long); the volume was adjusted based on weight. Food was returned 4 hours after dosing, and water remained available. Blood (~200 µL per time point) was collected from the retro-orbital plexus under isoflurane anaesthesia at pre-dose and at 0.5, 1, 2, 3, 4, 8, 12, and 24 hours. Samples were transferred to K_2-EDTA tubes, kept on wet ice, and centrifuged within 30 minutes (≈2000 g for 10 minutes at 4 °C). Plasma was aliquoted into amber tubes and stored at -70 °C; hemolyzed samples were flagged and evaluated for interference during validation [18]

Bioanalysis (LC-MS/MS)

Plasma lutein concentrations were measured using a validated LC–MS/MS method. Proteins were precipitated with chilled acetonitrile containing 0.2% formic acid and an internal standard (e.g., meso-zeaxanthin); supernatants were injected onto an ACQUITY HSS C18 column (≈2.1 × 100 mm, 1.8 µm) with gradient elution at ~0.4 mL/min and 40 °C. Detection used positive-mode electrospray with multiple-reaction monitoring (typical MRM transitions for lutein shown in the validation report) [18].

Calibration and validation: The calibration range was 5-250 ng/mL ($r^2 \geq 0.995$), with back-calculated standards within ± 15% (± 20% at LLOQ). Intra- and inter-day accuracy and precision for QC levels (low, mid, high) met bio-analytical acceptance criteria (± 15%; CV ≤ 15%). Recovery was high and consistent; matrix effect and carryover were negligible (carryover response ≤ 20% of LLOQ after upper-calibrator injection). Dilution integrity (up to 5- fold) met acceptance criteria. Stability was demonstrated for bench-top (2h, light-protected), processed samples (auto sampler, 12h at 10° C), three freeze-thaw cycles, and long-term storage (-70 °C), with deviations ≤ 15% from nominal. Incurred Sample Reanalysis (ISR) was performed on ≥ 10% of study samples, with ≥ 67% within ± 20% difference [18].

Pharmacokinetic analysis

PK parameters were estimated in Phoenix WinNonlin (v8.3) using non-compartmental analysis of individual concentration–time data. Cmax and tmax were obtained directly. AUC_{0-t} was computed by linear-up/log-down trapezoids; $AUC_{0-\infty}$ was calculated where feasible as $AUC_{0-t}+C_{last}/\lambda z$, with λz determined from the terminal log-linear portion (≥ 3 points; adjusted R^2 inspection). Apparent terminal half-life was $\ln(2)/\lambda z$. BLQ values prior to tmax were treated as zero; BLQ after tmax were excluded from λz estimation. Summary statistics are mean± SD for continuous variables and median [range] for tmax [17].

Statistical methods

Primary inference compared ln(Cmax) and $\ln(AUC_{0-t})$ among groups using one-way ANOVA with Dunnett's multiple comparisons *versus* the MCT reference at α=0.05. Model assumptions were evaluated by residual diagnostics (Q-Q plots) and Levene's test, if needed, heteroscedastic-robust ANOVA; was pre-specified as a sensitivity analysis. Exponentiating least-squares derived Geometric Mean Ratios (GMRs) and 95% CIs reflects mean differences. tmax was compared descriptively. All analyses were performed in validated statistical software (WinNonlin and R, v4.3+) [17].

Quality assurance and data integrity

Instruments were qualified and maintained under SOPs; analytical runs required ≥ 67% of QCs to pass with at least one at each level. Chain-of-custody was documented from sampling to storage and analysis. Deviations, if any, were logged contemporaneously and judged for impact on data integrity and primary endpoints by QA.

Risk mitigation and bias control

To minimise photodegradation and oxidation of carotenoids, low-light handling, amber containers, and nitrogen overlays were used where applicable. The order of dosing and sampling was randomized across groups within each time point. Analysts were shielded from group labels by barcode-based sample IDs.

Data management and traceability

Raw instrument files, processed chromatograms, calibration/QC worksheets, and PK exports were archived in the study e-folder with immutable timestamps. An audit trail captured data creation and modification. Any re-integrations require supervisory sign-off.

Additional planned sensitivity analyses

Sensitivity summaries (reported qualitatively): (i) re-computing AUC_{0-t} with linear-linear trapezoids; (ii) excluding any flagged hemolyzed specimens; (iii) repeating ANOVA on rank-transformed endpoints if residuals deviated from normality.

Reporting conventions

Unless stated otherwise, statistics are reported with two decimals; p-values are two-sided; CIs are 95%. Times are relative to dose administration (t=0).

RESULTS

Pharmacokinetic overview

All treatments produced quantifiable lutein concentrations from 0.5 h through 24 h with mono-exponential terminal decline and no anomalous profiles. No samples were excluded; no BLQ values occurred before Tmax. Mean concentration-time curves showed clear separation during the absorption phase (0.5-4 h), with PS>PC ≈ Liposome >MCT, and near-parallel slopes in the terminal phase, indicating similar elimination kinetics (Figure 3, Figure 4).

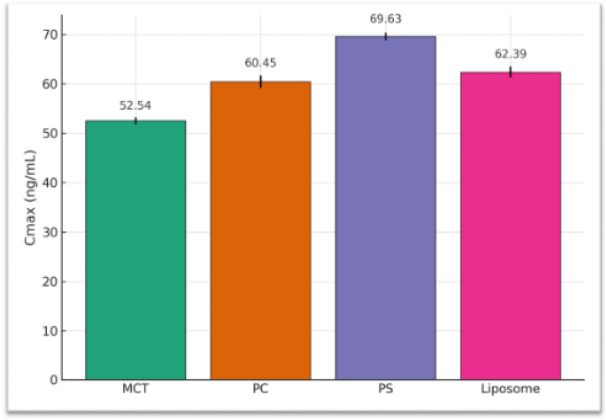

Figure 3: Plasma lutein Cmax by matrix (mean ± SD; n=6 per group).

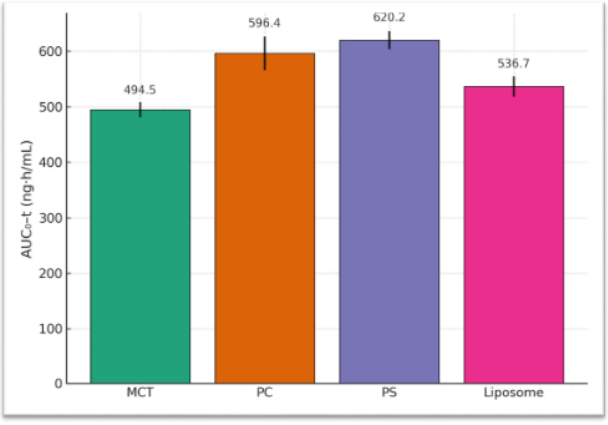

Figure 4: AUC0–t by matrix (mean ± SD; n=6 per group).

Primary exposure endpoints (Cmax, AUC0–t)

Group summaries are reported in Table 2 (mean ± SD). On ln-scale ANOVA with Dunnett contrasts *versus* MCT, PS yielded the most significant increases in Cmax and AUC0–t (both $p < 0.001$), with substantial but more minor gains for PC and Liposome ($p < 0.01$). Back-transformed Geometric Mean Ratios (GMR (95% CI)) *versus* MCT are visualized in Figure 5 and tabulated in Table 3.

Key pharmacokinetic endpoints by matrix are summarized in Table 2 below.

Table 2: Primary pharmacokinetic outcomes (dose-matched; n=6 per group). Values are mean ± SD unless stated otherwise; tmax is median (range).

Parameter	MCT	PC	PS	Liposome
Cmax (ng/mL)	52.54 ± 0.70	60.45 ± 1.24	69.63 ± 0.78	62.39 ± 1.12
AUC0–t (ng·h/mL)	494.51 ± 13.70	596.37 ± 30.29	620.23 ± 16.41	536.70 ± 18.42
tmax (h)	~3.0	~2.0	~2.0	~3.0
t½ (h)	7.7-8.3	7.7-8.3	7.7-8.3	7.7-8.3

Footnotes: ANOVA on ln-scale with Dunnett contrasts *vs* MCT at α=0.05. Exact p-values provided in text.

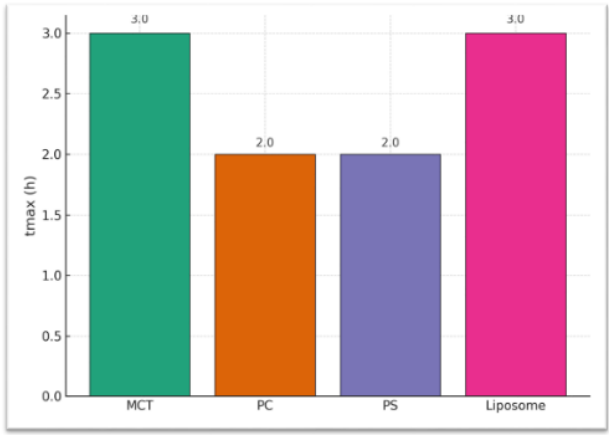

Figure 5: Time to Cmax (tmax; median) by matrix.

Absorption rate and early-phase exposure (tmax, pAUC$_{0-4h}$)

Median tmax was ~2 h for PS/PC and ~3 h for MCT/Liposome (Table 2). Partial AUC to 4 h (pAUC$_{0-4h}$) followed the same ranking (PS>PC ≈ Liposome>MCT), supporting a genuine absorption-phase advantage with phospholipid-containing matrices-particularly anionic PS.

Elimination phase (λz, t½)

The apparent terminal half-life (t½) did not differ meaningfully among groups (≈7.7-8.3 h); λz estimation used ≥ 3 terminal points (adjusted R^2 > 0.95 for all profiles), indicating matrix effects are localized to absorption rather than elimination.

Back-transformed geometric mean ratios (GMRs) *versus* MCT are presented in Table 3 below.

Table 3: Effect sizes *versus* MCT (Geometric Mean Ratio (GMR) and % change).

Endpoint	PC/MCT (GMR; %)	PS/MCT (GMR; %)	Lipo/MCT (GMR; %)
Cmax	1.151 (+15.1%)	1.325 (+32.5%)	1.187 (+18.7%)
AUC$_{0-t}$	1.206 (+20.6%)	1.254 (+25.4%)	1.085 (+8.5%)

Precision, robustness, and sensitivity checks

Within-group variability was low (Cmax CV% 1.1%-2.1%; AUC CV% 2.6%-5.1%; Table 4). Sensitivity analyses linear-linear trapezoids for AUC, exclusion of any hemolysis-flagged samples, and heteroscedastic-robust ANOVA did not alter inferences. Incurred Sample Reanalysis (ISR) acceptance was met (≥ 67% within ± 20%).

Assay-level precision (CV%) for key endpoints is shown in Table 4 below.

Table 4: Assay-level dispersion (CV%).

Endpoint	MCT	PC	PS	Liposome
Cmax CV%	1.33%	2.05%	1.12%	1.80%
AUC$_{0-t}$ CV%	2.77%	5.08%	2.65%	3.43%

DISCUSSION

Principal findings in context

This preclinical study shows that the interfacial chemistry of the lipid matrix significantly affects single-dose lutein exposure in rats under fasting conditions. Among four commonly used carriers — neutral MCT oil, MCT+PC, MCT+PS, and liposome — powder the anionic PS matrix achieved the highest Cmax and AUC$_{0-t}$, with an earlier Tmax compared to the other groups (Table 2 and Figures 1-4). PC and liposomes offered intermediate benefits over the neutral reference, while terminal slopes and apparent half-lives remained consistent across groups. Overall, these results indicates that the benefit of PS related to absorption rather than elimination differences.

The observed effect sizes are significant: the PS matrix increased peak and total exposure by approximately 33% and 25%, respectively, compared to MCT at an equivalent dose, with PC and liposome producing smaller but still positive shifts (Table 2). The pattern remains consistent across related endpoints (Cmax, AUC$_{0-t}$, Tmax, pAUC$_{0-4h}$). It is supported by low within-group variability (Table 3), indicating that the results are reliable despite analytical noise and sampling differences.

Mechanistic interpretation: Why anionic PS helps

Carotenoid absorption is limited by two interconnected processes: (i) solubilization into bile salt-phospholipid mixed

micelles and (ii) translocation across the unstirred water layer to the enterocyte membrane, where uptake into chylomicron assembly pathways occurs. Formulation choices that improve the abundance, stability, and diffusivity of the mixed-micellar pool are therefore expected to enhance early-phase exposure [5,8,11].

At the oil-water interface, PC provides better organization of the interfacial film and stabilizes emulsions, helping micelle formation compared to neutral triglycerides alone. PS, on the other hand, adds a negatively charged headgroup, increasing the interfacial zeta potential during digestion. Elevated negative charge is linked to smaller, more numerous colloidal particles and increased electrostatic repulsion between droplets, which reduces coalescence and maintains a higher surface area for bile salt extraction [10, 11]. Practically, this can speed up lutein's partitioning into the aqueous micellar phase, leading to earlier and higher plasma levels. Our exploratory, non- inferential analysis (DLS/zeta, Section 2) supports this view and aligns with reports from lipid digestion models where charge modulation shifts micelle size distributions and uptake kinetics [10-12,16].

The liposome approach offers a useful comparison. While vesicular encapsulation can protect sensitive actives before dosing, reconstituted liposomes often face bile salt-induced destabilization in the intestinal environment. Their components, phospholipids and actives, then follow the same mixed-micelle pathway that governs oil absorption. Improvements in exposure depend on whether the liposome's specific composition and reconstitution conditions maintain surface benefits during digestion. In our study, the liposomal powder showed a modest improvement over MCT. However, it did not surpass PC and clearly lagged behind PS, consistent with rapid vesicle breakdown and the dominance of mixed-micellar transport.

Comparison with prior literature

Although direct head-to-head comparisons of PS, PC, and liposome for lutein/zeaxanthin are limited, the current ranking aligns with broader principles of lipid-based delivery. Zwitterionic phospholipids (e.g., PC) and short-to medium-chain triglycerides often enhance dispersion and facilitate the formation of mixed micelles, which improve carotenoid bioavailability compared to long-chain neutral oils used alone [3,7-9]. The role of interfacial charge has been emphasized in digestion models for other lipophiles, where anionic interfaces shift the colloidal environment toward smaller, more diffusive particles [10,11]. The benefits of liposomes, when observed, tend to be specific to the product and relate to vesicle stability during the gastric-intestinal transition [13-15]. Our findings extend these concepts to an *in vivo* setting for lutein under controlled, dose-matched conditions, highlighting matrix chemistry as the main factor.

Translational and formulation implications

The key message from these data is simple: if the goal is to increase and accelerate lutein exposure without using complex structures, adding an anionic phospholipid like PS to the oil phase is a sensible default. In formulation terms, PS functions Less as a "special ingredient" and more as an interfacial environment, helping keep digestive colloids small, numerous, and mobile exactly when the intestine is ready to absorb them. This interfacial setting can be adjusted with modest PS levels (around five to fifteen percent of the oil phase) without affecting manufacturability. In pilot tests, formulators can fine-tune within that range to balance viscosity, pumpability, and capsule fill behavior, then fix the level once *in-vitro* dispersion and early pAUC benefits are consistently reproduced [8,11].

Translating this into label and dose decisions, the PS advantage promotes a dose-saving approach rather than an arms race of milligrams. A 20% increase in AUC at a fixed dose indicates that a PS product may achieve the desired exposure with a significantly smaller lutein dose. A concise crossover study can be designed (fasted and fed) comparing bioavailability from a legacy MCT soft gel to a PS-enriched successor, supporting claims based on absorption metrics, "faster uptake," and "greater bioavailability," while avoiding clinical promises that the data do not yet justify [17,18]. The earlier Tmax observed here emphasizes convenience and speed: consumers taking the product with breakfast or a small fat bolus can expect to see systemic lutein availability sooner after dosing.

The choice of dosage form follows the same logic. PS-in-MCT fills can be used for soft gel production and storage if handled under low light, with nitrogen headspace, and regular peroxide/anisidine testing. Self-emulsifying concentrates can also produce the PS effect if the surfactant system keeps an anionic interfacial character upon dilution; here, screening with dynamic light scattering and zeta potential in simulated intestinal fluid provides quick feedback before moving to animal or human PK [11,16].

Powder systems liposomes or beadlets should be designed with humility, recognizing that vesicles rarely remain intact through the upper intestine; success comes from ensuring that, upon reconstitution, the interface still appears anionic, even if the vesicle itself has broken down into mixed micelles. Food-effect studies should be approached cautiously. Endogenous phospholipids increase with meals and may partially reduce the difference between matrices. Instead of claiming superiority under all meal conditions, it is more reasonable to position PS formulations as reliable "with or without food," then gather data to confirm that the early-phase benefit persists or at least does not reverse, under a standardized fed challenge. When rapid uptake is needed (for stacking with other fat-soluble actives), recommending co-administration with a small amount of fat (five to ten grams) is a sensible, consumer- friendly guideline [3,7].

Quality control connects the narrative. Since the benefit begins at the interface, lot release should consider more than just assays. PS content in the fill, oxidative markers, and simple in vitro dispersion metrics (droplet size and zeta potential after standardized dilution) are practical critical quality attributes that ensure the product performs like the batch that passed the PK study [8,11,18]. Photo stability and low-oxygen packaging are essential; they help preserve the label claim long enough for the interfacial advantage to be meaningful.

Finally, a pragmatic path to market emerges to screen PS levels on the bench; run a small, well-powered crossover PK to confirm the exposure lift and earlier tmax; scale with guardrails that protect the interface; and speak in the language the data support [16-18]. In doing so, developers can convert a mechanistic insight into a reliable, manufacturable product that delivers earlier and higher lutein exposure in real-world use.

Practical formulation guidance

The present data support several practical recommendations for xanthophyll formulations. Favour anionic interfacial character (e.g., PS at ~5%-15% of the oil phase) when the goal is to maximize early-phase delivery and overall exposure under fasted or light-fed conditions. Combine PS with an appropriate oil phase (e.g., MCT) to balance viscosity, capsule fill behaviour, and oxidative stability; include antioxidants (e.g., mixed tocopherols) to protect carotenoids without compromising micellar transfer. Control reconstitution for powder systems: if liposomes are used, align vesicle size and membrane composition with anticipated GI conditions or accept that benefits may be modest unless vesicle stability is engineered to persist through early intestinal digestion. Mitigate light and oxygen throughout manufacturing and packaging to preserve potency, as absorption gains are only meaningful if labelled content is maintained.

Strengths of the study

Several design features enhance interpretability: Dose-matched arms and a common lutein source eliminate content variability between groups. Uniform bio-analytical methods (calibration, QC, ISR) minimize measurement error and enable precise between-group comparisons. Randomization with analytical blinding reduces allocation and detection biases. Low CV%across matrices increases confidence that observed differences are mechanistically fundamental rather than stochastic.

Limitations

This fasted, single-dose rat study cannot fully generalize to human fed-state digestion. Only one reconstituted liposomal product was tested; alternative vesicle chemistries might differ. The 24-hour window limits the precision of terminal parameters. Mechanistic conclusions are inferential (no direct micellar fraction/transport measurements). These constraints do not affect the primary finding that PS improves absorption compared to PC, liposome, and MCT.

Future work

Key next steps are: (i) INFOGEST-aligned digestion comparing PS *vs* PC to measure bio accessibility and zeta potential; (ii) a small human crossover PK (fed/fasted) study to confirm effect size and variability; and (iii) optimizing PS level (5%-15%) within stability/QC guidelines, using selective transporter probes as needed. These experiments should compare against the PS-in-MCT reference to establish translational relevance.

Clinical and regulatory considerations

In nutraceutical settings, formulation choices are often guided by label constraints, stability, and cost rather than mechanistic optimization. The present data argue that modest inclusion of PS-widely available as a dietary ingredient-can deliver meaningful exposure gains at practical inclusion levels, potentially justifying its use as a differentiating excipient. From a regulatory standpoint, claims should be carefully framed around bioavailability or pharmacokinetic parameters rather than therapeutic outcomes unless supported by clinical endpoints. Batch-to-batch controls (PS content, peroxide values, lutein assay) will be essential for consistent performance.

Broader implications for lipid-based delivery

While lutein/zeaxanthin served as the test case, the principles here extend to other amphipathic, poorly water-soluble actives that rely on mixed-micelle formation and lymphatic transport for absorption (e.g., certain tocotrienols, ubiquinone, and phytosterols). The interfacial charge and phospholipid head group composition are often underutilized variables in many commercial formulations; strategically adjusting these factors can enhance exposure to levels comparable to, or greater than, more complex architectures.

In summary, an anionic PS-enriched matrix provides a consistent absorption-phase advantage for lutein compared to PC, liposome, and neutral MCT carriers, as indicated by earlier Tmax and higher Cmax and AUC0–t without affecting the elimination phase. The findings are mechanistically consistent, statistically solid, and readily applicable for formulation development. Follow-up human studies and detailed mechanistic analysis under physiologically relevant digestion conditions are the next logical steps to translate these results into approved bioavailability benefits and, ultimately, into measurable improvements in retinal nutritional health.

CONCLUSION

This controlled rat study demonstrates that the interfacial chemistry of the lipid carrier is a key factor in determining lutein exposure under fasted conditions. At a fixed dose, enriching MCT oil with Phosphatidylserine (PS) resulted in the highest Cmax and AUC0–t, as well as an earlier tmax, compared to Phosphatidylcholine (PC), a reconstituted liposomal powder, or neutral MCT. The elimination half-life was similar across groups, indicating that the advantage lies in absorption rather than clearance.

Practically, modest PS inclusion (5-15%) enhances bioavailability and offers a simple, scalable way to improve bioavailability without complex structures. Potential benefits include dose reduction, faster onset, and possibly lower variability pending confirmation in humans. For powder or liposomal formats, performance depends on whether reconstitution maintains an anionic interface during early intestinal digestion; without this, PS-in-oil provides a reliable benchmark. In summary, PS-enriched matrices outperform PC, liposome, and MCT in delivering lutein in vivo and serve as a logical default for xanthophyll formulations aiming for earlier and higher systemic

and higher systemic exposure. Follow-up studies, including INFOGEST experiments and small crossover human PK trials, should now verify the magnitude, food effect, and durability of these benefits, helping to establish credible claims and quality standards.

AUTHOR CONTRIBUTIONS

Conceptualisation: S. Mehkri, K. Bopanna. Methodology: S. Mehkri, K.G. Dinesh, G. Ashok. Investigation: K.G. Dinesh, G. Ashok; Formal Analysis: S. Mehkri, K. Bopanna. Data Curation: K.G. Dinesh, G. Ashok; Writing-Original Draft: K. Bopanna, S. Mehkri. Writing-Review & Editing: All authors; Visualisation: S. Mehkri; Supervision: K. Bopanna; Project Administration: S. Mehkri; Funding Acquisition: This work was supported by internal R&D resources of Bio-gen Extracts Pvt. Ltd. No external funding was received.

FUNDING STATEMENT

This work was supported by internal R&D resources of Bio-gen Extracts Pvt. Ltd..

COPE-compliant

S. Mehkri is affiliated with Bio-gen Extracts Pvt. Ltd., which provided lutein/zeaxanthin (LZ) active used in the study; this may be perceived as a competing interest relevant to formulation outcomes. K.G. Dinesh and G. Ashok are affiliated with Radiant Research, which conducted preclinical and bioanalytical work under contract research agreements. K. Bopanna is affiliated with Tejhana Consulting LLP, which received consulting fees related to study design and data analysis. The authors declare no additional financial or personal relationships that could inappropriately influence this work. Disclosures are provided in accordance with journal ethics and COPE-aligned policies.

ETHICS STATEMENT (ANIMALS)

All animal procedures were reviewed and approved by the Institutional Animal Ethics Committee (Radiant Research Services, Bangalore; protocol ID: RR250056-65/PC/PK/01-2025) and were compliant with CCSEA guidelines for care, anaesthesia, humane endpoints, and euthanasia. Animals were housed in SPF conditions with environmental enrichment; fasting and dosing procedures, anaesthesia, and blood sampling are detailed in Methods. This section adheres to ethical expectations for animal research manuscripts at journals with COPE-aligned policies and should meet JBHS requirements.

DATA AVAILABILITY STATEMENT

De-identified raw data (plasma concentration–time values), processed PK outputs (Cmax, AUC_0–t, t½), and analysis code (if any in R/WinNonlin exports) are available from the corresponding author upon reasonable request, subject to institutional and contractual policies. Key tabulated results are reported and included.

ACKNOWLEDGEMENTS

The authors thank the analytical and vivarium teams at Radiant Research Services, Bangalore, for executing sample handling, LC–MS/MS validation, and PK sampling, and the formulation support team at Bio-gen Extracts Pvt. Ltd. for matrix preparation. We appreciate critical reading by colleagues at Tejhana Consulting LLP for statistical diagnostics and figure QA.

REFERENCES

1. Bernstein PS, Li B, Vachali PP, Gorusupudi A, Shyam R, Henriksen BS, et al. Lutein, zeaxanthin, and meso-zeaxanthin: The basic and clinical science underlying carotenoid-based nutritional interventions for age-related macular degeneration. Prog Retin Eye Res. 2016;50:34-66.
2. Ma L, Lin XM. Effects of lutein and zeaxanthin on aspects of eye health. J Sci Food Agric. 2010;90(1):2-12.
3. Borel P, Desmarchelier C. Bioavailability of fat-soluble vitamins and related compounds: Absorption and metabolism. Nutrients. 2017;9(1):E43.
4. Johnson EJ. Role of lutein and zeaxanthin in visual and cognitive function throughout the lifespan. Nutr Rev. 2014;72(9):605-612.
5. Reboul E. Mechanisms of carotenoid intestinal absorption: Where do we stand? Nutrients. 2019;11(4):838.
6. During A, Dawson HD, Harrison EH. Carotenoid transport is regulated by the Scavenger Receptor class B, type I (SR-BI) in Caco-2 cells. J Nutr. 2005;135(5):1083-1089.
7. van het Hof KH, West CE, Weststrate JA, Hautvast JG. Dietary factors that affect the bioavailability of carotenoids. J Nutr. 2000;130(3):503-506.
8. Porter CJH, Trevaskis NL, Charman WN. Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. Nat Rev Drug Discov. 2007;6(3):231-248.
9. McClements DJ. Food Emulsions: Principles, practices and techniques. 3rd ed. Boca Raton: CRC Press; 2015.
10. Sarkar A, Murray B, Holmes M, Ettelaie R, Abdalla A, Yang X. Colloidal aspects of digestion of Pickering emulsions: Role of particle type and interfacial charge. Soft Matter. 2016;12(15): 3558-3569.
11. Salvia-Trujillo L, Qian C, Martín-Belloso O, McClements DJ. Modulating lipid digestion by structural design of edible emulsion droplets. Annu Rev Food Sci Technol. 2017;8:439-466.
12. Armand M. Lipases and lipolysis in the human digestive tract: where do we stand? Curr Opin Clin Nutr Metab Care. 2007;10(2): 156-164.
13. Mozafari MR, Johnson C, Hatziantoniou S, Demetzos C. Nano-liposome technologies for the solubilization and delivery of poorly soluble drugs. J Nanosci Nanotechnol. 2008;8(7): 3247-3269.
14. Nunes R, Silva C, Choudhury H, et al. Lipid-based nanocarriers for the oral delivery of poorly water-soluble drugs. Nanomaterials. 2021;11(4):1084.
15. Tan CP, Nakajima M. Influence of surfactant and oil composition on nanoemulsions formation and stability. J Food Eng. 2005;80(2):471-480.

16. Minekus M, Alminger M, Alvito P, Ballance S, Bohn TO, Bourlieu C, et al. A standardised static *in vitro* digestion method suitable for food-an international consensus. Food Funct. 2014;5(6):1113-1124.
17. Gabrielsson J, Weiner D. Non-compartmental analysis. In: Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications. 5th ed. Stockholm: Swedish Pharmaceutical Press; 2016.
18. European Medicines Agency. Guideline on bio-analytical method validation. 2011.