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Archaeosomal nanocarriers improve pharmacokinetics and bioavailability of vancomycin after oral administration

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ABSTRACT

Vancomycin, a glycopeptide antibiotic, is typically administered intravenously (IV) for severe Gram-positive bacterial infections. While the oral route offers higher patient adherence, it is limited by poor mucosal transport, restricting its use to intestinal infections. To address these challenges, this study explores the encapsulation of vancomycin into archaeosomes -phospholipid-based nanocarriers incorporating archaeal lipids, which exhibit exceptional stability in the gastrointestinal environment and interact specifically with enterocytes. Previous work demonstrated that lipid nanocarrier formulations benefit from the incorporation of the archaeal lipid calditol-glycerocaldarchaeol (GCTE), facilitating mucosal barrier penetration and increasing bioavailability *in vivo*. In this study, we investigated the effects of archaeal lipid extract (ALE) and purified caldarchaeol (GDGT) on the pharmacokinetics and oral bioavailability of vancomycin in male Wistar rats. Our findings reveal that archaeosomal formulations significantly increase systemic exposure by prolonging circulation time and enhancing plasma drug concentrations, combined with high biocompatibility. Notably, the oral bioavailability (as measured by the area under the curve, AUC) increased 9-fold with GDGT liposomes and 4-fold with ALE liposomes compared to free vancomycin. These results highlight the potential of archaeal lipid-based drug delivery systems to enable oral administration of therapeutics that are traditionally injection-only.

1. Introduction

Successful drug delivery development is highly dependent on the physicochemical properties of the active pharmaceutical ingredient (API) and its ability to effectively overcome biological barriers (Laffleur and Keckeis, 2020). For oral administration, the harsh environment of the gastrointestinal (GI) tract, along with the presence of degrading enzymes and poor mucosal permeability, often impedes efficient drug absorption. Consequently, oral drug delivery is frequently associated with low bioavailability especially for APIs prone for degradation, such as peptides and proteins (Homayun et al., 2019). This often necessitates subcutaneous (SC) or intravenous (IV) administration of pharmaceuticals, despite their significant drawbacks in terms of patient adherence and high costs (Rangel et al., 2017; Spellberg and Lipsky, 2012). To address these challenges, delivery vehicles are employed to enhance API stability in bodily fluids and facilitate transportation into the bloodstream. Among them, lipid-based nanocarriers, such as liposomes, are particularly promising due to their structural similarity to biological membranes. This resemblance can facilitate interactions with cells and tissues, potentially improving drug delivery and bioavailability (Fricker et al., 2010; He et al., 2019).

Archaeosomes represent a class of liposomes that incorporate either naturally derived isoprenoid-based archaeal lipids, as they are found in the cell membrane of archaea, or synthetic analogs thereof. Due to technical challenges during chemical synthesis, these lipids are however primarily extracted from biomass of extremophilic archaeal organisms, such as *Sulfolobus acidocaldarius* (Rastädter et al., 2020). Archaeal lipids offer unique advantages over conventional lipids: they incorporate ether bonds rather than the ester bond found in fatty acid-based lipids, and exhibit *sn*-2,3 stereochemistry, making them resistant to acid-induced and enzymatic hydrolysis (Kaur et al., 2016). Additionally, they occur as bilayer spanning diether lipids and monolayer spanning tetraether

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lipids (TELs), most prominently the lipids calditolglycerocaldarchaeol (GCTE) and caldarchaeol (GDGT), providing significant rigidity enhancements to the lipid membrane (Romero and Morilla, 2023; Uhl et al., 2017). These features ensure the protection of labile drug molecules against bile salts and low pH in the GI tract (Jensen et al., 2015). This increased structural integrity also contributes to improved colloidal stability during long-term storage (Sedlmayr et al., 2023; Uhl et al., 2017). Furthermore, a high association of archaeal lipid-based vesicles with enterocytes has been reported, promoting oral drug absorption (Morilla et al., 2011; Sedlmayr et al., 2023; Werner et al., 2024b).

Accordingly, archaeal lipids have been studied as excipients for oral drug delivery vehicles, demonstrating increased *in vivo* oral bioavailability of unstable and poorly absorbed substances, such as peptides, proteins, and small molecules (Li et al., 2010; Morilla et al., 2011; Parmentier et al., 2011; Uhl et al., 2016). However, so far, little is known about their effects on the drug pharmacokinetics after oral administration.

Vancomycin, a glycopeptide antibiotic, serves as a last-line treatment for serious infections caused by staphylococci, enterococci and other Gram-positive bacteria (Dinu et al., 2020). Due to its low mucosal penetration as a BCS class III drug, vancomycin is typically administered IV if not used for the local treatment of intestinal infections caused by *Clostridioides difficile* and *Staphyllococcus aureus* (Rubinstein and Keynan, 2014). An oral formulation of vancomycin for systemic therapy could offer benefits in terms of patient convenience and improved compliance in line with antimicrobial stewardship guidelines (Ansari et al., 2024). In addition to its clinical importance, vancomycin represents an excellent model API for studying mucosal transport. It meets key requirements for an effective oral drug delivery system, including high aqueous solubility, resistance to proteolytic degradation, minimal first-pass metabolism, and low conversion by cytochrome P450 (Cao et al., 2018). Besides, it is not a substrate for intestinal influx or efflux transporters, and thus, vancomycin's low oral bioavailability can be attributed primarily to its poor mucosal permeation, making it an ideal candidate for investigating how vesicles and excipients impact on mucosal permeability (Sauter et al., 2020).

Previously, we have demonstrated the beneficial effects of archaeal lipids on the oral delivery of vancomycin. The incorporation of 5 mol% GCTE to lecithin-cholesterol-based liposomes nearly doubled the vancomycin plasma concentration in rodents one hour after oral administration compared to liposomes without GCTE. Moreover, blood levels were tripled compared to the unencapsulated substance (Uhl et al., 2017). The oral efficacy of the formulation can be further enhanced by using 1 mol% of a phospholipid conjugated with a cyclic and enzymatically stable cell penetrating peptide (Uhl et al., 2021).

Based on these promising preliminary results, in the current study, we investigated the performance of archaeal lipid-based formulations on the pharmacokinetics and oral bioavailability of vancomycin. Therefore, we examined the effect of an archaeal lipid extract (ALE) of *S. acidocaldarius*, a mixture of five archaeal lipids (Quehenberger et al., 2020), and an additional formulation containing purified GDGT on the oral delivery of vancomycin in male Wistar rats. Relevant pharmacokinetics of the liposomal formulations were determined and compared to the free drug. Our results demonstrate the substantial impact of archaeal lipids on the pharmacokinetics of vancomycin, such as prolonged circulation time and increased oral bioavailability, and indicate promising use with other difficult to administer therapeutics.

2. Materials and methods

2.1. Materials

Lecithin (EPC) was obtained from AppliChem GmbH (Germany) and NAP™−5 columns were obtained from GE Healthcare (United Kingdom), while cholesterol, Triton™ X-100, acetonitrile, chloroform, methanol and all other solvents were obtained from Sigma Aldrich (Germany). Archaeal lipid extract (ALE) and caldarchaeol (GDGT) were provided by NovoArc GmbH (Austria). The composition of ALE was as described previously (Quehenberger et al., 2020). Vancomycin hydrochloride was obtained from DEMO Pharmaceutical Gmbh (Germany). 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N- [amino(polyethylene-glycol)-2000] (DSPE-PEG(2000)) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) (18:1 Liss Rhod PE) were purchased from Avanti Research (USA). Dulbecco's phosphate buffered saline (DPBS), Dulbeccso's modified Eagle medium (high glucose) (DMEM) and Hank's buffered salt solution (HBSS) were obtained from Thermo Fisher Scientific (USA). For permeability experiments, Transwell™ inserts consisting of polyethylene-terephthalate membrane filters (0.4 μm pore size, 1.12 cm^2 growth area) inside translucent 12-well inserts (cellQART®) were used and purchased from Sabeu (Germany). ZO-1 Monoclonal Antibody (ZO1–1A12), Alexa Fluor™ 488 and NucBlue™ Live ReadyProbes™ Reagent (Hoechst 33,342) were obtained from Invitron (USA).

2.2. Preparation of archaeosomes via dual asymmetric centrifugation (DAC)

Archaeosomes were prepared either using a SpeedMixer™ (DAC150FVZ Hausschild Engineering GmbH & Co. KG, Hamm, Germany) or a ZentriMix 380 R (Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). First, the lipids were dissolved in chloroform/methanol 9:1 to obtain 100 mM stock solutions of the individual components. Subsequently, the stock solutions were mixed in microtubes (2 mL, polypropylene) to obtain lipid mixtures with the desired molar ratios (85 mol- % EPC, 10 mol- % cholesterol, 5 mol- % ALE/ GDGT). The organic solvent of the lipid mixtures was evaporated by a nitrogen stream. Afterwards, the resulting lipid film was dried overnight in a vacuum chamber. Vancomycin was dissolved in DPBS and added in the first speed mixing step. Before starting the speedmixing process, 94 mg of 1.00–1.20 mm glass beads were added. Archaeosomes were prepared by speed mixing in 3 runs. The first run (15 min; 1467 x g; 20 °C) was performed using 30 μL of a 50 g/L vancomycin solution in DPBS. For the subsequent runs (5 min; 1467 x g; 20 °C), 100 μL and 120 μL DPBS were added to the sample. Samples used in the permeability assay additionally included 0.2 mol- % of 18:1 Liss Rhod PE). After formulation, samples were purified using Sephadex G-25 gel filtration chromatography (NAP™−5 columns) (Cytiva, Sweden).

2.3. Liposome characterization

Particle size and polydispersity (PDI) were determined by dynamic light scattering with a ZetaSizer Nano ZS (Malvern Panalytical, United Kingdom) (cell type: DTS0012; $T = 25$ °C; number of measurements; 3; number of runs: 15; run duration: 10 s; measurement angle: 173° backscatter). Zeta potential measurements were conducted on the same device (cell type: DTS1070; 25 °C, number of measurements: 3; number. of runs: 30). Size and PDI were measured after the dilution to a concentration of 0.076 mg mL−1 with PBS while the zeta potential was determined after the dilution to a concentration of 0.95 mg mL−1 by a 50 mmol phosphate buffer as described previously (Uhl et al., 2021). The measurements were conducted by using the automatic mode and the average of three measurements.

2.4. Encapsulation efficiency

Encapsulation efficiency was determined by analyzing the vancomycin concentration before the purification step and after the purification step. The concentration of vancomycin was determined via reversed phase HPLC analysis on a Varian Pro Star HPLC system (Varian, USA). Separation of analytes was carried out on a Chromolith Performance RP-18e (100 × 3 mm) column (Supelco, USA). The mobile phase was composed of (A) water + 0.1 % (v/v) trifluoroacetic acid and (B)

acetonitrile + 0.1 % (v/v) trifluoroacetic acid. After 0.5 min of 100 % (A), a 5.5 min gradient to 0 % (A) was performed. After a 1.5 min cleaning step at 0 % (A), a gradient to 100 % (A) was performed, which was followed by a re-equilibration step 2.75 min. Retention time was 3.24 min.

2.5. Cryo-Transmission electron microscopy (TEM)

Cryo-TEM of both liposomal formulations was performed as reported previously (Werner et al., 2024a). In brief, the archaeosomes at a concentration of 10 mg/mL were applied onto holey carbon-coated grids (Lacey, USA), blotted and vitrified vogueish liquid ethane. Subsequently, electron micrographs were recorded with a Talos 200 Electron microscope (FEI, USA) using a 4 $K \times 4$ K Ceta CMOS camera.

2.6. Preparation of Caco-2 monolayer for permeability assay

The human colon adenocarcinoma cell line Caco-2 was used at passage 17–20. Cells were cultured in DMEM supplemented with 10 % fetal bovine serum (FBS) and 1 % antibiotic solution (1×10^4 UI/mL penicillin, 10 mg/mL streptomycin) (Pen-Strep) in a humidified incubator 5 % CO_2/95 % air atmosphere at 37 °C. Cells were plated on T75 flasks, trypsinized using trypsin-EDTA at a confluency of around 75 % and seeded on 0.33 cm^2 surface area Transwell™ inserts with a pore size of 0.4 μm at a concentration of 1×10^5 cells/insert. The culture medium was replaced every two days for 2 weeks and every day thereafter (0.5 mL per insert, 1.5 mL per well). Prior to the experiments, cells were washed twice with HBSS for 15 min at 37 °C. The transepithelial resistance (TER) was measured with a STX-100 M probe connected to a Millicell-ERS voltohmmeter (Merck Millipore, USA). Background resistance was corrected by subtracting the resistance obtained with cell-free inserts from the TER of filters with Caco-2 monolayers. Only cell monolayer with TER higher than 250 Ω cm^2 were used.

2.7. In vitro permeability assay

The transport of the two archaeosomal formulations and free vancomycin were studied in the apical to basolateral direction on Caco-2 cells. Samples were diluted in pre-warmed HBSS to a vancomycin concentration of 150 μM. 0.5 mL of the vancomycin containing samples were added to the apical side, while 1.4 mL of HBSS was added to the apical side to start the experiment. After 2 h of incubation at 37 °C, 250 μL sample was taken from the basolateral side and stored at −80 °C until analysis via LC-MS/MS.

2.8. Fluorescence microscopy

At the end of the experiment, cells were washed twice with HBSS and fixed using 4 % paraformaldehyde. After washing, cells were permeabilized with a 0.1 % Triton X-100 solution.

After one further washing step, cells were incubated in a blocking solution (4 % FBS in HBSS) overnight at 4 °C. The monolayer was washed with HBSS and labeled with ZO-1 Monoclonal Antibody (ZO1–1A12), Alexa Fluor™ 488 (1:500 dilution). Nuclei stain was conducted using NucBlue™ Live ReadyProbes™ Reagent (Hoechst 33,342) after another washing step. Rhodamine labeled nanocarriers, ZO-1 and nuclei were visualized using a Keyence BZ8100 Fluorescence microscope (Keyence, Japan) equipped with a Nikon SPLan Fluor 20 × /0.45 objective (Nikon, Japan).

2.9. Cytotoxicity assay

Cytotoxicity was examined as described previously (Uhl et al., 2021). In short, Caco-2 cells were cultivated in 96 well plates on DMEM supplemented with 10 % FBS and 1 % Pen-Strep and grown for 14 days after the formation of a monolayer. The medium was replaced every two days. On the day of viability measurement, the liposomal suspensions were added at appropriate concentrations and incubated for 3 h. Subsequently, the medium was replaced by a growth medium supplemented with 10 % PrestoBlue HS Cell Viability Reagent (Invitrogen, USA) and cells were incubated for 3 h. Fluorescence measurement was performed on an Infinite F200 Pro (Tecan, Switzerland). Normalization of the cell viability was done with respect to the signal of wells containing untreated cells.

2.10. Animal experiments

The animal study was conducted in accordance with local authority guidelines (approved by the Regierungspräsidium Karlsruhe: 35.9185.81/G-223/21) using male Wistar rats, each weighing between 200 and 250 g Vancomycin was administered orally via gavage, with each rat receiving 1.5 mL of solution containing 9 mg of vancomycin. To explore the pharmacokinetics of vancomycin, blood samples were taken over the course of 8 h. Plasma was separated in heparinized tubes via centrifugation in (5 min, 3000 rpm) and stored at −80 °C until further analysis.

2.11. Determination of vancomycin concentrations

Vancomycin was extracted from 35 μL of rat plasma samples. Proteins were precipitated with 70 μL of ice-cold acetonitrile containing 2 μM of internal standard [$^2H_{12}$]-vancomycin (Alsachim, Illkirch-Graffenstaden, France). The samples were then vortexed for 30 s and centrifuged for 5 min, 15 000 x g at 4 °C. Afterwards, the supernatants were filtered using Supelco 96-Well Protein Precipitation Filter Plate, 0.2 μm pore size (Merck KGaA, Darmstadt, Germany). Then, 60 μL of the filtrates were mixed by vortexing with 30 μL of H2O, and the samples were pipetted to LC-MS vials. The calibration curve was prepared using 30 μL of clean rat plasma and spiking the plasma with 5 μL of vancomycin standard solutions at eight different concentrations. For analysis of vancomycin concentration from the permeability assay, HBSS containing samples were diluted 1:5 in a mixture of acetonitrile and water (1:1).

For the LC-MS/MS analysis of vancomycin an LC system consisting of a Waters 2695 pump, a Waters autosampler, and a column oven was employed (Waters, USA). The liquid chromatography was performed by a C18 reversed phase PerfectSil Target ODS-3HD (100 mm x 2.1 mm, 3 μm) column (MZ-Analysentechnik GmbH, Germany). The MS analysis was performed with a Waters Micromass Quattro Ultima Pt mass spectrometer equipped with an electrospray ionization source (Waters, Milford, MA, USA). MassLynx V4.1 software was used for data acquisition and processing. Analyte detection was performed using multiple reaction monitoring, the transitions being 725 → 144.1 and 730 → 144.1, for vancomycin and [$^2H_{12}$]-vancomycin, respectively. The used injection volume was 10 μL for plasma samples and 30 μL for samples derived from the *in vitro* permeability assay. The chromatographic separation was done using a mobile phase consisting of LC-MS grade acetonitrile with 0.1 % (v/v) formic acid (Eluent A) and LC-MS grade water with 0.1 % (v/v) formic acid (Eluent B). A gradient elution 5–90 % A was applied over 0–3 min followed by 2 min isocratic elution, and finally, the column was equilibrated for 8 min before the next injection. The flow rate was 0.2 mL/min. The lower limit of quantification for the used method was 50 nM.

2.12. Determination of pharmacokinetic parameters

The elimination rate k was determined via an exponential fit of the vancomycin plasma level decay according to the Eq. (1), where c_t is the plasma concentration at timepoint t and c_0 the initial plasma level concentration. For oral administration, the physiological parameters were determined using sampling time points where elimination of vancomycin exceeded the absorption, and a decay of vancomycin could

be recognized.

$$c_t = c_0 * \exp(-k * t) \quad (1)$$

The half-life $t_{1/2}$ was determined via Eq. (2).

$$t_{1/2} = \log(2) * k \quad (2)$$

3. Results

3.1. Formulation of archaeosomes

Table 1 shows the physicochemical properties of the archaeosomes investigated in this study incorporating 5 mol- % ALE (ALE liposomes) and 5 mol- % GDGT (GDGT liposomes). Both formulations resulted in particles with a comparable size of 108 nm, exhibited monodisperse distributions and a slightly negative Zeta potential. Also, the encapsulation efficiencies of the two formulations were similar at approximately 40 %.

Fig. 1 shows Cryo-TEM images of ALE liposomes and GDGT liposomes. A mixture of primarily unilamellar and to a lower extent multilamellar vesicles were obtained for archaeosomes incorporating ALE and GDGT. The overall lamellarity is low.

3.2. In vitro testing of archaeosomal vancomycin

Fig. 2A depicts the results of the viability assay for the liposomal formulations investigated in this study. Viability of Caco-2 cells remained unaffected for nanocarriers at concentrations of up to 500 mg/L archaeal lipids. As shown in Fig. 2A, no adverse effect on cell viability was observed. Fig. 2B depicts a comparison of the apparent permeability coefficient (P_{app}) of free vancomycin and the two liposomal formulations. P_{app} for free vancomycin was $(0.45 \pm 0.13) \times 10^{-6}$ cm/s, indicating low permeability. No significant difference could be determined for ALE liposomes ($(1.44 \pm 2.78) \times 10^{-6}$ cm/s) and GDGT liposomes ($(1.11 \pm 0.18) \times 10^{-6}$ cm/s). Fig. 2C presents the individual channels analyzed using fluorescent microscopy, along with a merged image. For GDGT liposomes, the signal for rhodamine labelled particles align closely with the signal for the tight junction protein ZO-1, suggesting predominantly paracellular transport. Conversely, ALE liposomes exhibit a diffuse signal for rhodamine, indicating a pronounced combination of paracellular and transcellular transport mechanisms.

3.3. Oral delivery of vancomycin

Fig. 3A-C shows the vancomycin plasma concentration in male Wistar rats over time after oral administration for ALE liposomes, GDGT liposomes and free vancomycin. In all groups, vancomycin plasma levels peaked 1-hour post-administration. Following c_{max}, elimination of vancomycin exceeded absorption, resulting in a decline in plasma levels. The highest vancomycin level was observed for the formulation containing GDGT, reaching 0.83 μM, compared to 0.28 μM with ALE liposomes and 0.083 μM with free vancomycin. After this peak, vancomycin plasma levels declined rapidly for GDGT liposomes. After 6 h, no vancomycin was detected in the free drug group, while the detection limit was reached for both liposomal formulations at 8 h.

Table 2 summarizes the pharmacokinetic parameters of the orally administered samples. Bioavailability increased by a factor of 4.1 with ALE liposomes and 8.9 with GDGT liposomes, compared to free vancomycin. The elimination rate was reduced substantially for the liposomal formulations of vancomycin, while the half-life increased 5-fold with ALE liposomes and more than doubled with GDGT liposomes.

Table 1
Sizes, polydispersity index (PDI), and Zeta potential of archaeosomes incorporating 5 mol% ALE (ALE liposomes) and 5 mol% GDGT (GDGT liposomes). ($n = 3$).

Sample	Size [nm]	PDI[a]	Zeta-potential [mV]	Encapsulation efficiency [%]
ALE liposomes	107.5 ± 2.2	0.122 ± 0.028	−3.24 ± 0.21	41.7 ± 1.6
GDGT liposomes	107.6 ± 2.3	0.159 ± 0.013	−4.04 ± 0.74	37.6 ± 3.0

[a] A polydispersity index of < 0.2 indicates a monodisperse distribution (Khadke et al., 2020).

4. Discussion

Archaeal lipids have been extensively explored as excipients in liposomal formulations to enhance the therapeutic efficacy of various APIs after oral administration. These lipids offer notable advantages, including significantly improved bioavailability of peptides and enhanced immunological responses in vaccine formulations (Romero and Morilla, 2023). Our previous studies revealed, that the oral delivery of vancomycin benefits significantly from the incorporation of GCTE, with plasma concentrations tripling one hour after administration compared to administration of the free drug (Uhl et al., 2017). Moreover, the advantages of archaeosomes over conventional ester lipid-based liposomes in oral drug delivery have been demonstrated for multiple APIs (Parmentier et al., 2011; Uhl et al., 2017, 2016). Despite these findings, limited data exist on the long-term pharmacokinetics of vancomycin when formulated with archaeal lipids compared to free drug administration. This gap in knowledge is critical for the preclinical development of lipid-based formulations. Additionally, the *in vivo* performance of ALE and GDGT has not yet been assessed. Unlike other archaeal lipids, GDGT lacks headgroup modifications and has recently been investigated for its potential fusogenic properties (Bhattacharya et al., 2024; Sedlmayr et al., 2024). A comparison with ALE could provide valuable insights into how headgroup modifications affect *in vivo* transfection.

Dual asymmetric centrifugation (DAC) enables the formulation of lipid nanocarriers with high encapsulation efficiency (EE %) (Pantze et al., 2014; Tremmel et al., 2016). In this study, these findings were transferred to dual centrifugation (DC), indicating that both methods result in promising particle characteristics and encapsulation efficiencies of peptide therapeutics. The obtained particles, incorporating either 5 mol- % ALE or GDGT, exhibited a mean particle diameter that was reduced by approximately 40 nm compared to previous formulations incorporating 5 mol- % GCTE (Uhl et al., 2017). Cryo-TEM analysis revealed the formation of predominately unilamellar vesicles with a low content of multilamellar vesicles, a feature advantageous for encapsulating hydrophilic therapeutics such as vancomycin. Importantly, the lamellarity of the two formulations was comparable.

Transwell™ experiments using Caco-2 cell monolayers is a commonly employed method to determine the permeability and to predict the absorption of drug molecules, showing good correlation with *in vivo* applicability in humans (Hubatsch et al., 2007). Despite the high potential of archaeal lipid-based liposomes in oral drug administration, this method has not yet been applied extensively to study archaeosomes. In our experiments, we could confirm a low permeability of vancomycin through the monolayer, while it significantly increased utilizing archaeosomal formulations reaching a value of beyond 1×10^{-6} cm/s, indicating moderate permeability (Sevin et al., 2013). No significant difference between the two archaeosomal formulations was found in the permeability experiments.

The immunocytochemistry assay, visualizing ZO-1, a human tight junction protein, allows for the estimation of the degree of trans- and paracellular transportation through the monolayer (Roger et al., 2009). Significant differences between ALE- and GDGT-containing formulations were observed, suggesting that paracellular transport was predominant for GDGT liposomes, whereas ALE promoted a high degree of both para- and transcellular transport. This observation might also explain previous results, indicating higher particle internalization of

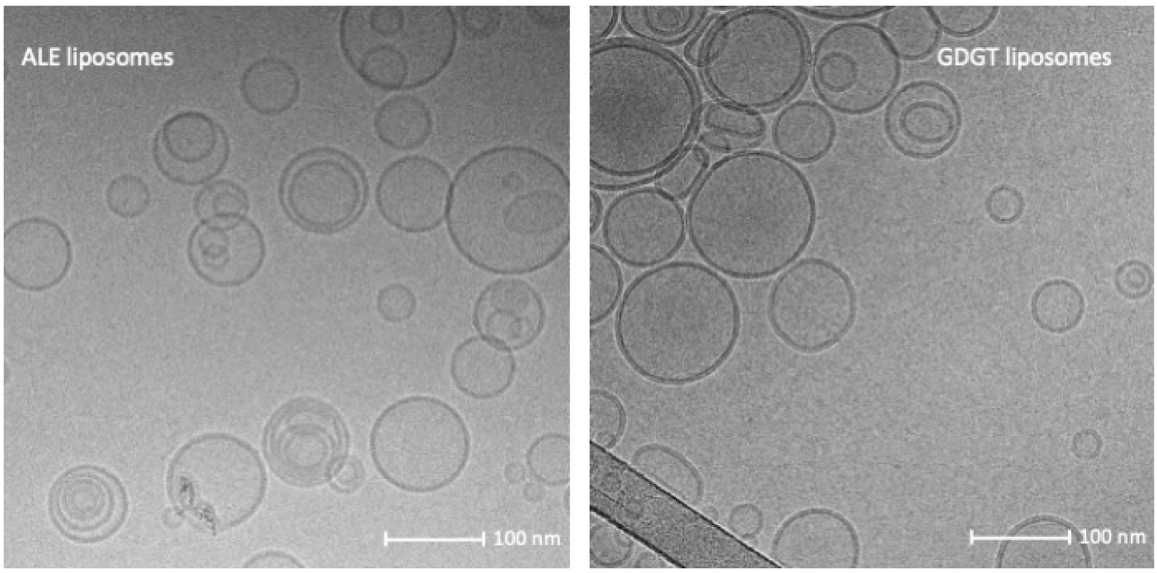

Fig. 1. Cryo-TEM images in 64,0000 x magnification of ALE liposomes (left) and GDGT liposomes (right). The scale bar corresponds to 100 nm. For both formulations, a mixture of unilamellar and multilamellar liposomes could be found, while the overall lamellarity is low.

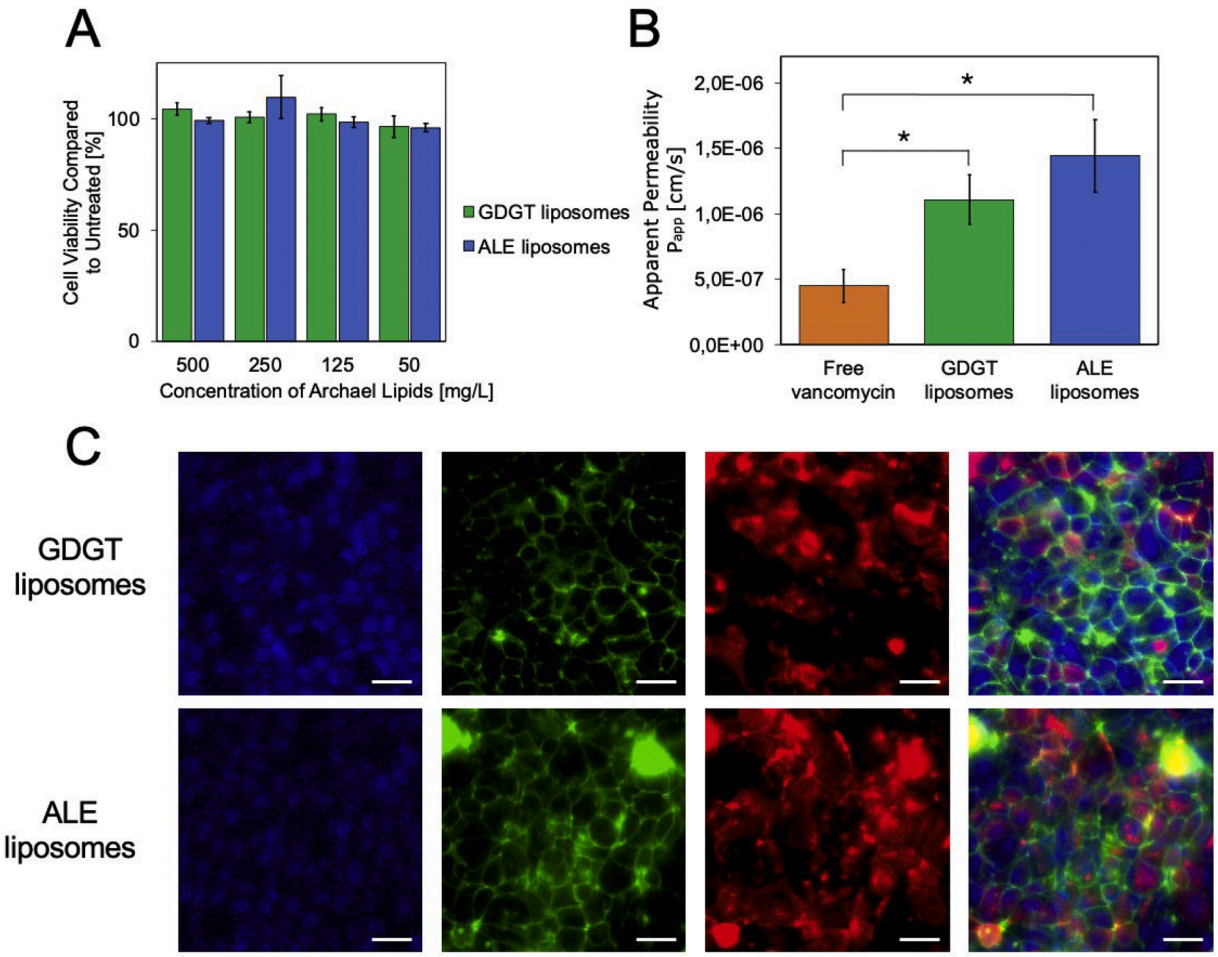

Fig. 2. (A) Results of Presto blue assay using Caco-2 cells for the two liposomal formulations investigated in this study. Archaeosomes were composed of 85 mol- % egg phosphatidylcholine and 10 mol- % cholesterol, incorporating 5 mol- % archaeal lipid extract (ALE liposomes) or 5 mol- % caldarchaeol (GDGT liposomes), respectively. ($n = 3$). (B) Apparent permeability coefficients (P_{app}) of free vancomycin, ALE liposomes and GDGT liposomes. ($n = 3$). Statistical analysis was done using one-way variance ANOVA followed by post hoc Tukey test. A single asterisk (*) indicates $p < 0.005$. (C) Fluorescent microscopy images showing cell nuclei (blue), ZO-1 (green) and rhodamine labeled particles (red).

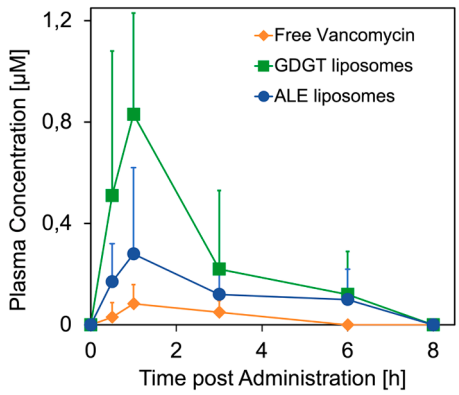

Fig. 3. Plasma concentration of vancomycin in male Wistar rats after oral administration of vancomycin encapsulated in archaeal lipid extract (ALE) liposomes (A) and caldarchaeol (GDGT) liposomes (B) compared to free drug (C). Data is represented as mean ± standard deviation ($n = 3$ for free vancomycin and ALE liposomes; $n = 2$ for GDGT liposomes).

ALE-containing nanocarriers by Caco-2 cells for mRNA delivery (Sedlmayr et al., 2024). The internalization process of archaeosomes remains largely unexplored; although not within the scope of this study, our findings strongly support future research efforts aimed at elucidating this mechanism. No negative influence on cell viability could be found for the two formulations investigated in this study in the applied concentrations. These results are consistent with earlier reports demonstrating good biocompatibility of archaeal lipids (Kaur et al., 2016). The natural exposure of humans to archaeal organisms in both the environment and microbiome, along with the absence of known pathogenic species, supports their biocompatibility and lack of toxicity (Lurie-Weinberger and Gophna, 2015).

In accordance with previous studies, we demonstrated an increased oral bioavailability of peptides when using lipid nanocarriers incorporating archaeal lipids (Li et al., 2010; Parmentier et al., 2014, 2011; Uhl et al., 2016). Similar to the findings by Parmentier et al. for human growth hormone, plasma concentration upon oral administration peaked after 1 h (Parmentier et al., 2014). Both archaeosomal formulations significantly enhanced the pharmacokinetics of vancomycin, leading to increased systemic plasma concentrations and reduced clearance. ALE liposomes exhibited prolonged drug half-life and slower elimination, whereas GDGT liposomes resulted in higher overall blood levels of vancomycin, suggesting a more efficient absorption mechanism via the intestine. Considering the results from the permeability experiments, this supports the relevance of transcellular particle absorption via the tight junctions.

While several case studies confirm the efficacy of oral archaeosomal drug formulations, the underlying mechanisms remain insufficiently understood. It is known, that in contrast to conventional phospholipids, which upon ingestion are rapidly hydrolyzed by pancreatic phospholipase A 2 and thus lose their colloidal stability, archaeosomes maintain their integrity due to the resistance of archaeal lipids to bile salts, phospholipases, and the acidic environment of the gut (Mumtaz Virk and Reimhult, 2018; Romero and Morilla, 2023). Accordingly, their interaction with enterocytes differs from commonly proposed mechanisms for phospholipid-based liposomal formulations. Parmentier et al. hypothesized an adsorption-release mechanism based on observations of the low amounts of radiolabeled archaeal lipids detected in inner organs and the bloodstream following oral administration of archaeosomes (Parmentier et al., 2011). This hypothesis is further supported by a recent article by Vidakovic et al., showing strong adhesion of archaeosomes to cell membranes (Vidakovic et al., 2024). Conversely, a study conducted by Morilla et al. claimed drug leakage during transcytosis, based on their findings following the delivery of radiolabeled DTPA (Morilla et al., 2011).

Despite the enhanced oral bioavailability, the plasma concentrations achieved with archaeosomal vancomycin remain below the recommended minimum inhibitory concentration (MIC) (Martin et al., 2010). Nevertheless, the increased bioavailability observed in this study may enable clinically relevant absorption of poorly permeable but highly active APIs, such as hormones or GLP-1 analogues. Previous studies have demonstrated that combinations of phospholipids and archaeal lipids offer a versatile platform for the delivery of various pharmaceuticals (Herbster et al., 2025; Jacobsen et al., 2017; Parmentier et al., 2011; Sedlmayr et al., 2024) As discussed earlier, vancomycin exhibits key characteristics of an ideal model peptide drug for studying mucosal transport and the impact of formulation components (Sauter et al., 2020). To enhance vancomycin delivery, further optimization of the formulation, particularly targeting improved mucosal penetration, may be advantageous. In this context, the incorporation of bioenhancers, such as cetylpyridinium chloride or cell-penetrating peptides, could facilitate the achievement of therapeutic plasma concentrations (Parmentier et al., 2014; Uhl et al., 2021; Werner et al., 2024b).

Our findings highlight the distinct advantages of archaeal lipid-based formulations in enhancing oral drug delivery of APIs. The observed improvements in pharmacokinetics, including increased systemic bioavailability and prolonged drug retention, emphasize the potential of ALE and GDGT as highly beneficial drug delivery systems.

5. Conclusion

This study demonstrates the potential of ALE and GDGT as versatile excipients in liposomal formulations, improving the pharmacokinetics and bioavailability of vancomycin. The incorporation of 5 mol- % GDGT had the most profound effect, enhancing the relative bioavailability 9-fold compared to free vancomycin administration. Pharmacokinetics were influenced substantially by enhancing c_{max} and half-life, while drug elimination of vancomycin was reduced. The enhanced oral delivery of vancomycin using ALE and GDGT containing liposomes underscores their potential as effective drug carriers. Further studies are needed to elucidate the precise mechanisms governing archaeosomal absorption and internalization, paving the way for their broader application in pharmaceutical formulations.

CRediT authorship contribution statement

Viktor Sedlmayr: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Julian Quehenberger:** Writing – review & editing, Resources, Project administration, Methodology, Formal analysis, Conceptualization. **David Wurm:** Writing – review & editing, Project administration, Methodology, Formal analysis, Conceptualization. **Mikko Gynther:** Writing – review & editing, Project

Table 2
Area under the curve (AUC), elimination rate, and half-life following oral administration of free vancomycin *versus* vancomycin encapsulated in archaeal lipid extract (ALE) liposomes and caldarchaeol (GDGT) liposomes. Values are represented as means ($n = 3$).

Sample	AUC [µM*h]	Improvement of bioavailability vs. free drug	Elimination rate [h^{-1}]	Half-life [h]
Free Vancomycin	0.24	–	1.00	0.70
ALE liposomes	0.99	4.1	0.19	3.56
GDGT liposomes	2.14	8.9	0.40	1.73

administration, Methodology, Investigation, Formal analysis, Data curation. **Rebecca Vieth:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Valerie Dürr:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Pascal Gesse:** Writing – review & editing, Formal analysis, Data curation. **Oliver Spadiut:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization. **Gert Fricker:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Formal analysis, Conceptualization. **Philipp Uhl:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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Data availability

Data will be made available on request.

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