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Development and Characterization of Lipid Nanocarriers for Vancomycin HCL Delivery: A Comparative Study of Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC)

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Abstract

Background and Objectives For improved oral drug administration, this work explores the creation and assessment of vancomycin-loaded lipid nanocarriers, such as Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC). In order to evaluate the formulations' potential for drug integration, encapsulation efficiency (EE) and loading capacity (LC) were measured after hot homogenisation and ultrasonication.

Methods To assess the stability of the blank and vancomycin-loaded formulations, measurements were made of their particle size, zeta potential, and polydispersity index (PDI). Vancomycin release patterns were assessed by in vitro release experiments carried out in an enzyme-free artificial intestinal environment. Compared to SLN, NLC showed a more regulated and prolonged release, holding onto a greater release percentage throughout time.

Results The process of lyophilization led to a marginal increase in particle size, but the drug-encapsulating qualities of both nanocarriers were retained. ANOVA statistical analysis verified the significance of variations in the formulations' release behaviours ($P < 0.05$). The results imply that because of its enhanced encapsulation and prolonged release profile, NLC may provide vancomycin administration with higher bioavailability and therapeutic effectiveness.

Conclusions This work demonstrates how lipid-based nanocarriers may be used to create efficient oral delivery systems for medications like vancomycin.

Keywords: Vancomycin, lipid nanocarriers, Solid Lipid Nanoparticles (SLN), Nanostructured Lipid Carriers (NLC), Oral drug delivery.

1. Introduction

In the field of pharmaceutical sciences, nanoparticle drug delivery systems have become a game-changer, offering innovative ways to improve the therapeutic results of a variety of medications, especially those with low bioavailability, poor water solubility, or instability in biological environments. These nanoscale carriers enable the encapsulation of active pharmaceutical ingredients (APIs) within protective matrices, allowing for controlled release, targeted delivery, and reduced systemic toxicity. The unique properties of nanoparticles, such as their small size, high surface area, and ability to cross biological barriers, have made them valuable tools in delivering drugs to specific tissues, overcoming the challenges posed by conventional drug formulations [2,13].

Lipid-based carriers, such as Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC), are among the different kinds of nanoparticles that have attracted a lot of attention because of their biocompatibility, biodegradability, and capacity to shield delicate medication molecules from enzymatic metabolism and chemical degradation. These carriers are particularly advantageous for oral drug delivery, as they can shield drugs from the harsh conditions of the gastrointestinal tract, enhance absorption through the intestinal wall, and achieve sustained or controlled drug release [2].The components of solid lipid nanoparticles (SLN) are solid lipids that solidify at ambient temperature or body temperature. The crystalline structure formed by these lipids serves as a matrix for the encapsulation of lipophilic drugs. This crystalline structure helps to sustain the integrity of the drug contained throughout time and gives stability to the SLN. When the lipid progressively dissolves or degrades, the lipid matrix can both shield the medication from breaking down in acidic environments like the stomach and allow for controlled drug release. These characteristics have prompted extensive research on the delivery of a variety of medicinal substances, including as peptides, antibiotics like vancomycin, and anticancer medications.But SLNs have a major drawback: their high crystallinity might limit the amount of space that can be used to include drugs. Because of this dense lipid molecule packing, the medication's stability may be compromised during storage if the lipid matrix continues to crystallise and causes the encapsulated drug to escape [5,15].

In order to overcome the drawbacks of SLNs, Nanostructured Lipid Carriers (NLC) were created. Compared to SLNs, NLCs have a less crystalline and more flexible lipid matrix because they are made up of a combination of liquid and solid lipids. This mixed lipid composition results in a less ordered structure, providing more room for drug molecules and thus allowing for a higher drug loading capacity. Liquid lipids cause the solid lipid's crystalline structure to break down, which increases the stability of the encapsulated medication and lowers the possibility of drug ejection during storage. Because NLCs are more amorphous, they can hold both lipophilic and hydrophilic medications, which makes them a flexible drug delivery platform [10].Additionally, NLCs provide the ability to modify the ratio of solid to liquid lipids to fine-tune drug release patterns, enabling more sustained and controlled drug release over longer times. This makes NLCs particularly useful for applications where maintaining therapeutic drug levels over time is crucial, such as in chronic conditions requiring long-term medication. NLCs also show enhanced bioavailability compared to SLNs due to their better ability to interact with biological membranes, improving drug absorption and transport across the intestinal barrier[9,10]

The choice between SLNs and NLCs depends on the specific therapeutic needs, the properties of the drug, and the desired release profile. SLNs may be more suitable when stability and protection from degradation are priorities, whereas NLCs are preferred when higher drug loading, reduced expulsion, and prolonged release are needed. For instance, by combining the advantages of improved drug stability and increased bioavailability, NLCs provide a viable option in the delivery of vancomycin, a poorly soluble antibiotic that needs to be protected from degradation in the stomach and released under regulated conditions in the intestines.

The formulations for SLN and NLC can both be developed commercially since they work well with large-scale production techniques including high-pressure homogenisation and ultrasonication. Their use in the oral delivery of drugs addresses key challenges associated with traditional formulations, such as rapid degradation, low absorption, and frequent dosing requirements. By improving the pharmacokinetic profiles of drugs, SLNs and NLCs not only enhance therapeutic efficacy but also contribute to better patient compliance, as they can reduce the frequency of drug administration and minimize side effects. These advantages position lipid-based nanocarriers as a pivotal component in the future of targeted and personalized medicine, offering new possibilities for treating complex diseases and improving the quality of life for patient [13].

Strong glycopeptide antibiotic vancomycin is frequently used to treat severe infections brought on by Gram-positive bacteria, particularly those that are resistant to other antibiotics. It is particularly effective against methicillin-resistant *Staphylococcus aureus* (MRSA) and other multi-drug-resistant strains, making it a critical therapeutic option in clinical settings where bacterial resistance is a major concern [7]. Vancomycin works by preventing bacteria from synthesising their cell walls, which results in cell death. Its ability to combat difficult-to-treat infections has made it a preferred choice for treating conditions like endocarditis, osteomyelitis, and severe skin and soft tissue infections. Despite its efficacy, vancomycin's clinical use is challenged by its poor oral bioavailability, which results from its large molecular size and hydrophilic nature, limiting its absorption across the gastrointestinal tract. This necessitates alternative drug delivery strategies to improve its bioavailability and therapeutic effectiveness when administered orally [17]. Vancomycin has been chosen as model drug to produce Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC) due its reduced oral bioavailability and stability. Because vancomycin is not absorbable by the gut, it is generally administered intravenously linking its delivery to symptoms of distress and iatrogenic morbidity (side effects) that are proportional to the oxidized state of the drug in circulation. Vancomycin encapsulation in lipid-based nanocarriers, such as SLN and NLC could attenuate the instability during gastric acidic pHs, the enzymatic degradations by enzymes in the intestine and enhance vancomycin absorption across the intact barrier [9]. Since SLNs is solid lipid matrix, it can be used for vancomycin encapsulation and its slow release to protect drug from degradation in upper GI. Nonetheless, the high crystallinity of SLNs will restrict drug loading. With a mixture of liquid and solid lipids, NLCs presents an adaptable matrix with the advantage of carrying larger drug payload as well as sustained release profile [8,17]. This makes NLCs particularly suitable for achieving prolonged therapeutic levels of vancomycin in the bloodstream. By using SLN and NLC formulations, the goal is to develop an efficient oral delivery system that enhances the bioavailability of vancomycin, allowing for easier administration, reduced dosing frequency, and better patient compliance. This strategy might increase vancomycin's therapeutic uses and increase accessibility for the outpatient treatment of severe infections.

2. Methods

2.1 Materials

Medium-chain triglyceride (MCT) and Imwitor 900 K were obtained as free samples from BASF SE (Ludwigshafen, Germany). Lipoid SPC-3, which is a pure soybean phosphatidylcholine, was sourced from Avanti Polar Lipids, Inc. (Alabaster, Alabama, USA). Tween 80 (Polysorbate 80) and Span 20 were procured from Sigma-Aldrich, India. Vancomycin (>95% purity) was acquired from Sigma-Aldrich, Madrid, Spain. Additionally, pepsin, bile salts, and lipase were also supplied by Himedia, India. All other reagents and chemicals used were of analytical grade.

2.2 Fabrication of lipid nanocarriers loaded with vancomycin

The vancomycin-loaded Solid Lipid Nanoparticles (VSLN) and Nanostructured Lipid Carriers (VNLC) were prepared using a hot homogenization method followed by ultrasonication, tailored to the specific lipid compositions of each formulation [11]. For the VSLN, vancomycin (15 mg) was dissolved in the melted lipid phase after the solid lipid (5.2% w/w) was melted at a temperature that was about 5–10°C above its melting point. The aqueous phase, which included Span 20 (0.6%), lecithin (0.4%), and Tween 80 (4.1%), was heated to the same temperature as the lipid phase concurrently. An initial emulsion was formed by progressively adding the melted lipid mixture to the hot aqueous phase while swirling at 10,000 rpm for five minutes. To guarantee equal dispersion, this mixture was then homogenised at a high speed of 15,000 rpm for ten minutes. To further reduce the particle size and enhance uniformity, the emulsion underwent ultrasonication for 5 minutes. After that, the heated emulsion was cooled to room temperature while being constantly stirred, which helped the lipid to solidify and create nanoparticles. For further characterisation, the suspension that was produced was kept cold, at 4°C.

Similar procedures were used in the manufacture of VNLC;however, the lipid phase was formed by combining liquid and solid lipids (1.2% w/w and 1.1% w/w). This lipid combination was melted and kept at the same high temperature along with 15 mg of vancomycin. Tween 80 (4.0%), lecithin (0.6%), and Span 20 (0.4%) were added to the aqueous phase, which was heated to the same temperature as the lipid phase. The heated aqueous phase was mixed with the melted lipid phase while being stirred at 10,000 rpm to create a pre-emulsion. This was then subjected to a ten-minute high-pressure homogenisation procedure at 15,000 rpm. For five minutes, ultrasonication was used to create smaller, more uniform particles. The lipid mixture solidified and VNLCs were formed when the emulsion was cooled to room temperature while being constantly stirred.For further examination, the final VNLC suspension was kept cold, at 4°C. The same steps were taken to prepare the blank formulations, BLANK-SLN and BLANK-NLC, but vancomycin was not included in either. In addition, the distribution of 46/34/20% of the mono-, di-, and triglycerides in the NLC was calculated using the mixture of solid and liquid lipids. Vancomycin was effectively encapsulated using this preparation technique in both SLN and NLC, resulting in improved stability and a regulated release profile. Vancomycin's therapeutic potential is maximised by these lipid-based carriers because they guard the antibiotic during its passage through the stomach and guarantee focused release in the intestinal [6,11].

Table 1 Make-up of lipid nanocarriers loaded with vancomycin and blank

Formulation	Solid Lipid (%)	Liquid Lipid (%)	Tween 80 (%)	Lecithin (%)	Span 20 (%)	Water (%)	Mono/Di/Tri Glycerides (%)*	Vancomycin (mg)
Vancomycin-Free								
BLANK-SLN	4.8	0	3.8	0.6	0.4	90.4	56/44	-
BLANK-NLC	1.2	4.1	3.9	0.6	0.6	90.6	45/35/19	-
Vancomycin Loaded								
VSLN	5.2	0	4.1	0.4	0.6	89.7	54/46	15
VNLC	1.1	4.2	4.0	0.6	0.4	90.3	46/34/20	15

***Note:** The proportion of various lipids utilised in the production process was utilised to determine the mono-, di-, and triglyceride content in NLC. Blank solid lipid nanoparticles, or BLANK-SLN. BLANK-NLC: Lipid carriers with a blank nanostructure. Solid lipid nanoparticles loaded with vancomycin, or VSLNs. Vancomycin-loaded nanostructured lipid carriers are known as VNLCs.

2.3 Particle size and zeta potential

Measurement of zeta potential and particle size: Using dynamic light scattering (DLS) and a zeta potential analyser, the particle size and zeta potential of the vancomycin-loaded Solid Lipid Nanoparticles (VSLN) and Nanostructured Lipid Carriers (VNLC) were determined. To counteract the effects of repeated scattering, the formulations were diluted with distilled water before being examined in a cuvette. Particle size was measured using the DLS approach, which yielded average nanoparticle diameters and utilised the polydispersity index (PDI) to quantify the homogeneity of the size distribution.A monodispersed suspension is said to be represented by a PDI value less than 0.3. We assessed the colloidal suspension's stability by measuring the zeta potential, or the NPs' surface charge. To guarantee accuracy, the samples were performed in triplicate and data were taken at 25 °C. As previously reported, these studies were also employed to assess the dispersibility and stability of lipid nanocarriers in different formulations [16].

2.4 Evaluation of Encapsulation efficiency (EE) and loading capacity (LC)

An indirect technique was used to measure the loading capacity (LC) and encapsulation efficiency (EE) of vancomycin within the Nanostructured Lipid Carriers (VNLC) and Solid Lipid Nanoparticles (VSLN). Following the production of the nanoparticles, the dispersion was centrifuged for 30 minutes at 4°C at 15,000 rpm in order to extract the free vancomycin from the medication that was encapsulated. At the proper wavelength of 279 nm, UV-Visible spectrophotometry was used to determine the concentration of the supernatant, which included the unencapsulated vancomycin. The formula was used to determine the EE: EE (%) = [(Initial quantity of vancomycin - Quantity of free vancomycin in the supernatant) / Initial quantity of vancomycin] × 100. This calculation provided the percentage of vancomycin successfully encapsulated within the lipid matrix.

By measuring the quantity of vancomycin encapsulated within the nanoparticles in relation to their overall weight, the loading capacity (LC) was ascertained. LC (%) was calculated using the formula: LC (%) = [Quantity of encapsulated vancomycin / Total weight of nanoparticles] × 100. To assure consistency and precision, these measurements were made three times. The outcomes were utilised to assess the vancomycin incorporation efficiency of the lipid

nanocarriers as well as their potential for therapeutic dosage delivery. Elevated EE signifies the successful assimilation of vancomycin, whilst maximum LC signifies the formulation's capacity to provide the medication effectively within the intended dose form [14].

2.5 Lipid digestion in vitro under circumstances modelled by the stomach and intestines

Studying the behaviour and release properties of lipid-based nanocarriers, including Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC), in vitro lipid digestion under artificial stomach and intestinal conditions is crucial. These experiments assess how well the lipid carriers degrade and release their encapsulated medication by simulating the physiological conditions of the gastrointestinal system [1].

2.5.1 Simulated stomach condition

The simulated stomach condition is designed to replicate the acidic environment of the stomach, typically characterized by a low pH and the presence of digestive enzymes such as pepsin. The study begins by preparing a simulated gastric fluid (SGF) with a pH of around 1.2–2.0, using hydrochloric acid (HCl) and sodium chloride (NaCl) to adjust the pH. Pepsin, at a concentration of around 0.32% w/v, is added to mimic the proteolytic activity in the stomach. A measured amount of the lipid nanocarrier dispersion is then added to the SGF and incubated at 37°C with gentle shaking to simulate stomach churning. The digestion process is typically monitored over 1–2 hours to assess how the lipid matrix behaves in the acidic environment. Samples are taken at regular intervals, and the lipid digestion is evaluated by measuring changes in particle size, zeta potential, or by quantifying the release of the encapsulated drug using spectrophotometric or chromatographic methods. This phase simulates the initial breakdown of the lipid matrix as the formulation passes through the stomach [1].

2.5.2 Simulated intestinal condition

The simulated intestinal condition aims to replicate the neutral pH and enzymatic environment of the small intestine, which is critical for the further digestion of lipid-based formulations. The study uses simulated intestinal fluid (SIF) with a pH of around 6.8–7.5, typically prepared using phosphate buffer and bile salts. Pancreatin, an enzyme mixture that mimics the activity of pancreatic lipase and other digestive enzymes, is added at a concentration of approximately 0.1–0.4% w/v. The lipid nanocarrier dispersion, pre-treated under simulated stomach conditions, is transferred to the SIF and incubated at 37°C with continuous gentle stirring or shaking to mimic peristaltic movements. The digestion process in the simulated intestinal phase is monitored over 2–4 hours, depending on the study design. Samples are taken at regular intervals to evaluate lipid digestion by measuring the release of fatty acids, changes in particle size, and zeta potential, as well as the release profile of the encapsulated drug. Analytical methods such as titration with sodium hydroxide (NaOH) can be used to quantify the extent of lipid digestion through the release of free fatty acids. Together, these in vitro digestion studies provide valuable insights into how lipid nanocarriers like SLN and NLC behave as they transit through the gastrointestinal tract. The results suggest the formulation of lipid-based delivery systems for enhanced drug release and absorption, ultimately contributing to improved therapeutic outcomes [1].

2.6 In vitro bioaccessibility

In vitro bioaccessibility studies are conducted to evaluate how effectively a compound, such as a drug or nutrient, becomes available for absorption after undergoing digestion. These studies simulate the conditions of the human gastrointestinal tract to predict the release of the compound from its formulation and its subsequent readiness for intestinal uptake [3]. For lipid-based nanocarriers like Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC), understanding in vitro bioaccessibility is crucial, as it can indicate the potential of these carriers to enhance the delivery and absorption of encapsulated drugs, such as vancomycin in the present study [3].The bioaccessibility assessment typically involves a two-step digestion

process, starting with simulated gastric digestion. In this step, the nanocarrier formulation is incubated in simulated gastric fluid (SGF), which mimics the acidic environment of the stomach with a pH of 1.2–2.0, created using hydrochloric acid (HCl) and sodium chloride (NaCl). Pepsin, a digestive enzyme, is also added to simulate proteolytic activity at a concentration of approximately 0.32% w/v. The formulation is mixed with the SGF and maintained at 37°C with gentle agitation for 1–2 hours, representing the time the formulation would spend in the stomach. During this phase, the stability of the nanocarrier and the initial release of vancomycin are monitored [12].

Following gastric digestion, the formulation is exposed to simulated intestinal fluid (SIF) to replicate the conditions of the small intestine, which has a pH of 6.8–7.5. The SIF is prepared using a phosphate buffer and bile salts, and pancreatin is added to simulate the action of digestive enzymes. The digestion mixture is incubated at 37°C with continuous stirring for 2–4 hours, mimicking the intestinal environment where lipids are further broken down, and the drug is released from the nanocarriers. Samples are collected at specific time intervals throughout this phase to determine the amount of vancomycin released into the aqueous phase, representing the bioaccessible fraction.The percentage of bioaccessibility is calculated by comparing the amount of vancomycin released into the aqueous phase to the total amount initially encapsulated in the formulation, using the formula: Bioaccessibility (%) = [(Amount of drug in the aqueous phase after digestion) / (Total amount of drug in the formulation)] × 100. The concentration of vancomycin in the aqueous phase ismeasured using UV-Visible spectrophotometry at 279 nm [12].In vitro bioaccessibility studies are valuable as they provide a predictive model for understanding the release behaviour of the drug during digestion and its availability for absorption. For SLN and NLC, this studyindicates whether the lipid matrix effectively facilitates the release of vancomycin in the presence of digestive enzymes and bile salts. A higher bioaccessibility percentage suggests that the formulation more effectively releases vancomycin in a form ready for absorption, potentially leading to improved bioavailability when administered orally. These insights can guide the optimization of nanocarrier formulations, making them more effective for therapeutic applications and ensuring better delivery of vancomycin to the target site.

2.7 Drug release under simulated intestinal conditions

The study of drug release under simulated intestinal conditions is crucial for understanding how effectively a drug is released from its delivery system in the intestinal environment, which is often the primary site of absorption for orally administered drugs. For lipid-based nanocarriers like Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC), this process helps evaluate their potential to enhance the bioavailability of encapsulated drugs, vancomycin[4].The simulated intestinal conditions are typically modelled using a simulated intestinal fluid (SIF) with a pH of approximately 6.8–7.5, prepared using a phosphate buffer that mimics the neutral to slightly alkaline environment of the small intestine. To replicate the enzymatic activity encountered in the intestine, pancreatin is added to the SIF. Pancreatin is a mixture of digestive enzymes that includes lipase, amylase, and proteases, which play a vital role in breaking down the lipid matrix of the nanocarriers and facilitating drug release. Bile salts are also included to simulate the emulsifying action of bile, which aids in the solubilization of lipids and enhances the digestion of lipid-based formulations.During the release study, the nanocarrier formulation containing vancomycin is mixed with the SIF and incubated at 37°C, mimicking body temperature, with continuous gentle stirring to replicate intestinal motility. This process is typically monitored over a period of 2–6 hours to simulate the time the formulation would spend in the small intestine. At predetermined intervals, samples are withdrawn from the mixture and replaced with fresh SIF to maintain sink conditions, which ensures that the concentration gradient remains favourable for continued drug release.The amount of vancomycin released into the SIF at each time point is quantified using analytical

technique, UV-Visible spectrophotometry. The cumulative percentage of drug release is calculated over time to generate a release profile. The release behaviour of vancomycin from SLN and NLC under simulated intestinal conditions provides insight into the effectiveness of the lipid matrix in controlling drug release. SLN, with its more crystalline lipid structure, may exhibit a slower and more sustained release compared to NLC, which has a more amorphous structure due to the inclusion of liquid lipids, allowing for a faster initial release. This differential release behaviour can be advantageous depending on the therapeutic requirements, such as immediate release or extended-release formulations.Studying drug release under these conditions helps predict how the formulation will perform in vivo, providing a basis for optimizing the design of nanocarriers to achieve the desired release rate and bioavailability of vancomycin. Such in vitro release studies are essential for guiding the development of effective oral drug delivery systems that can deliver therapeutic concentrations of the drug directly to the absorption site in the intestines.

2.8 Statistical analysis

The differences in the release patterns and other measurable properties of Nanostructured Lipid Carriers (VNLC) and vancomycin-loaded Solid Lipid Nanoparticles (VSLN) were assessed statistically. The data were presented as mean ± standard deviation (SD) and all experiments were run in triplicate (n = 3).One-way analysis of variance (ANOVA) was used to compare the statistical values between various formulations and time points. Tukey's post hoc test was then used for multiple comparisons to discover significant differences. A statistically significant difference was defined as a p-value of less than 0.05 ($P < 0.05$), signifying a substantial difference between the groups. GraphPad Prism Version 8 statistical software was used for the analysis, verifying that the changes were real variations in the formulations' properties and performance rather than the result of chance.

3. Results and Discussion

Physicochemical characterization of SLN and NLC

Particle size, zeta potential, and polydispersity index (PDI) analyses of the lyophilised, vancomycin-loaded, and blank lipid nanocarriers offer important information on their encapsulation properties and stability. For the blank formulations, the particle size of the blank solid lipid nanoparticles (BLANK-SLN) was 105 ± 7.34 nm, while the blank nanostructured lipid carriers (BLANK-NLC) were smaller, measuring 49 ± 3.55 nm. The smaller size of BLANK-NLC suggests a more flexible matrix due to the inclusion of liquid lipids, in contrast to the more rigid structure of BLANK-SLN. Upon loading with vancomycin, both formulations experienced changes in particle size. The size of vancomycin-loaded SLN (VSLN) increased to 130 ± 8.50 nm, indicating a slight expansion of the solid lipid matrix as it accommodated the drug. Interestingly, the vancomycin-loaded NLC (VNLC) showed a reduction in size to 37 ± 5.67 nm, possibly due to tighter packing of the lipid molecules around the vancomycin or a structural reorganization within the lipid matrix. This difference suggests varying capacities between the SLN and NLC for drug encapsulation and stabilization.

Lyophilization had a notable impact on particle size, with the lyophilized vancomycin-loaded SLN (LV-SLN) increasing to 238 ± 14.35 nm and the lyophilized NLC (LV-NLC) increasing to 182 ± 20.58 nm. The increase in size is typical of the lyophilization process, which can cause aggregation or partial restructuring of nanoparticles upon rehydration. The larger size of LV-SLN compared to LV-NLC indicates that the solid lipid matrix is more susceptible to such changes during lyophilization and subsequent reconstitution. In terms of particle uniformity, the PDI values of the blank formulations were 0.42 for BLANK-SLN and 0.31 for BLANK-NLC, indicating a relatively homogeneous particle size distribution. The PDI values slightly decreased for vancomycin-loaded formulations, with V-SLN at 0.39 and V-NLC at 0.32, suggesting improved uniformity upon drug loading. The lyophilized formulations showed even

lower PDI values (0.21 for LV-SLN and 0.29 for LV-NLC), reflecting a more uniform particle size distribution after the lyophilization process.

Zeta potential measurements revealed moderate positive surface charges, with BLANK-SLN and BLANK-NLC having values of 18 mV and 21 mV, respectively. This indicates a degree of electrostatic stability that helps prevent particle aggregation. Upon vancomycin loading, the zeta potential of VSLN increased to 22 mV, which could suggest enhanced stability due to the slight increase in surface charge. Conversely, VNLC exhibited a slight reduction in zeta potential to 19 mV, possibly indicating changes in surface properties upon drug incorporation. The zeta potential values of the lyophilized formulations were not determined, as reconstitution after lyophilization can alter surface charges, requiring separate analysis. Nevertheless, the observed trends in particle size and PDI indicate that the lyophilized formulations maintain an overall stable and uniform structure.

Overall, the incorporation of vancomycin into both SLN and NLC affects their structural properties, with NLC showing a more compact size and improved uniformity even after lyophilization. These characteristics make NLC a potentially more stable and efficient system for delivering vancomycin. The observed stability in terms of particle size and charge suggests that both nanocarrier types can maintain their integrity during formulation, making them suitable for further development as oral drug delivery systems.

Table 2. Vancomycin-loaded and lyophilised lipid nanocarriers' particle size, polydispersity index, surface charge, encapsulation efficiency, and loading capacity.

Formulation	Size (nm)	PDI	Zeta Potential (mV)	EE (%)	LC (%)
Vancomycin-Free					
BLANK-SLN	105 ± 7.34	0.42	18	-	-
BLANK-NLC	49 ± 3.55	0.31	21	-	-
Vancomycin Loaded					
VSLN	130 ± 8.50	0.39	22	90 ± 2.66	0.7 ± 0.10
VNLC	37 ± 5.67	0.32	19	89 ± 3.45	0.8 ± 0.11
Lyophilized					
LV-SLN	238 ± 14.35	0.21	n.d.[a]	87 ± 2.90	0.7 ± 0.11
LV-NLC	182 ± 20.58	0.29	n.d.	90 ± 1.88	0.8 ± 0.10

Note: *Statistically significant, $P < 0.05$. a not determined (n.d.); EE stands for encapsulation efficiency; LC for loading capacity; PDI for polydispersity index. Blank solid lipid nanoparticles, or BLANK-SLN. BLANK-NLC: Lipid carriers with a blank nanostructure. Solid lipid nanoparticles loaded with vancomycin, or VSLNs. Vancomycin-loaded nanostructured lipid carriers are known as VNLCs. LV-SLN: Solid lipid nanoparticles loaded with vancomycin and lyophilised. Lyophilised vancomycin-loaded nanostructured lipid carriers are known as LV-NLCs.

3.1 Encapsulation efficiency and loading capacity

The encapsulation efficiency (EE) and loading capacity (LC) of vancomycin-loaded and lyophilized lipid nanocarriers provide insight into the ability of these formulations to incorporate and deliver vancomycin effectively. For the vancomycin-loaded formulations, the encapsulation efficiency of the vancomycin-loaded solid lipid nanoparticles (VSLN) was 90 ± 2.66%, while the vancomycin-loaded nanostructured lipid carriers (VNLC) showed a slightly lower EE of 89 ± 3.45%. These high EE values indicate that both types of lipids nanocarriers are highly effective in encapsulating vancomycin within their lipid matrices, ensuring that a

significant proportion of the drug is retained during the formulation process.

The loading capacity (LC), which represents the amount of vancomycin incorporated relative to the total weight of the nanoparticles, was 0.7 ± 0.10% for VSLN and slightly higher at 0.8 ± 0.11% for VNLC. This slight difference in LC suggests that the NLC formulation may have a marginally greater capacity to carry vancomycin compared to the SLN, possibly due to its mixed lipid matrix providing more flexibility and space for drug incorporation. Upon lyophilization, the encapsulation efficiency of the lyophilized vancomycin-loaded solid lipid nanoparticles (LV-SLN) decreased slightly to 87 ± 2.90%, while the lyophilized vancomycin-loaded nanostructured lipid carriers (LV-NLC) maintained an EE of 90 ± 1.88%. The minimal change in EE for LV-NLC suggests that the lyophilization process has less impact on the integrity of NLC, allowing it to retain its encapsulated vancomycin more effectively than LV-SLN. This stability is crucial for ensuring that the drug remains protected within the carrier during storage and reconstitution.

The loading capacity of the lyophilized formulations remained consistent with their pre-lyophilization values, with LV-SLN at 0.7 ± 0.11% and LV-NLC at 0.8 ± 0.10%. This consistency indicates that the lyophilization process did not significantly alter the amount of vancomycin relative to the weight of the nanoparticles, preserving the formulation's efficiency in delivering the drug. Overall, the data highlights the efficiency of both SLN and NLC in encapsulating and delivering vancomycin, with NLC showing a slight advantage in maintaining encapsulation efficiency and loading capacity through the lyophilization process. This makes NLC potentially more suitable for formulations that require stability during storage and efficient drug release upon administration.

3.2 Stability of lipid nanocarriers in simulated gastrointestinal fluid

For Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC) to be successful in drug administration, they must be stable in simulated environments like Simulated Gastric Fluid (SGF, pH 2.0) and Simulated Intestinal Fluid (SIF, pH 7.0). The initial sizes of SLN and NLC after lyophilization were 256 ± 2.24 nm and 298 ± 2.35 nm, respectively. Lyophilization slightly increased the particle sizes, which is common as the drying process can induce minor structural changes or aggregation in nanoparticles. NLC exhibited a slightly larger size compared to SLN, likely due to its combination of solid and liquid lipids, which creates a more flexible and less compact structure.

In SGF, both SLN and NLC showed a decrease in particle size, with SLN reducing to 197 ± 2.11 nm and NLC to 162 ± 2.09 nm. This reduction suggests that both formulations remain stable in the acidic environment, possibly due to structural compaction or a decrease in hydrodynamic diameter. The size decrease indicates that SLN and NLC can maintain their integrity in acidic conditions, making them suitable for oral drug delivery where protection during stomach passage is essential.However, in SIF, a significant increase in particle size was observed, with SLN reaching 1189 ± 12.25 nm and NLC increasing to 1084 ± 11.45 nm. This enlargement, marked by statistical significance, suggests that both formulations swell or aggregate in the neutral pH of the intestinal environment. The neutral pH and ionic strength of SIF likely contribute to this swelling, as the nanoparticles interact more with the fluid, leading to changes in surface charge and possible aggregation. This behaviour could enhance the release of the encapsulated drug in the intestines, indicating a potential advantage for formulations targeting intestinal drug release.Comparatively, SLN demonstrated a slightly larger increase in size in SIF than NLC, which could be due to its more rigid lipid structure. In contrast, the mixed lipid composition of NLC results in a more flexible structure, potentially making it more suitable for controlled release applications. The observed size reduction in SGF and subsequent size increase in SIF highlight the potential of both SLN and NLC for targeted drug delivery, where the drug remains protected in the acidic environment of the stomach and is then released upon reaching the more neutral conditions of the intestines. Overall, the choice

between SLN and NLC depends on the desired release profile, with NLC offering a more adaptable matrix for controlled release and SLN providing a stable structure suitable for sustained release formulations.

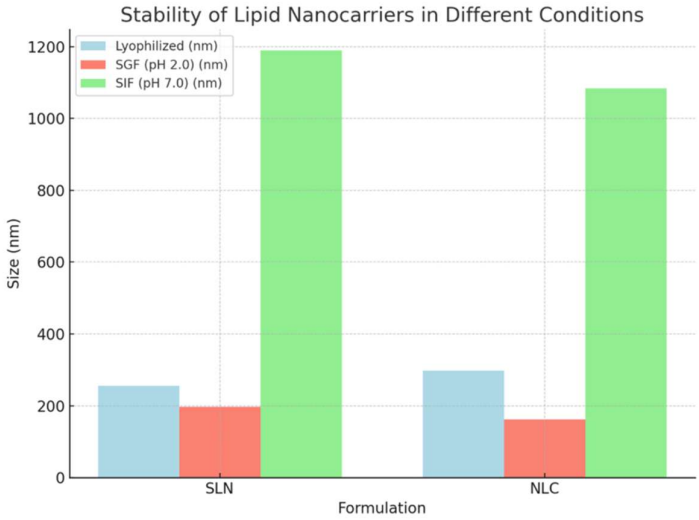

Fig.1. Nanocarriers' stability in SGF and SIF. Solid Lipid Nanoparticles, or SLNs. Nanostructured Lipid Carriers, or NLCs. Simulated Gastric Fluid (SGF) has a pH of 2.0. Simulated Intestinal Fluid (SIF) has a pH of 7.0. * denotes changes that are statistically significant ($P < 0.05$).

3.3 Bioaccessibility

The dispersion of vancomycin in solution (VS), solid lipid nanoparticles (SLN), and nanostructured lipid carriers (NLC) exhibit notably varied time-dependent bioaccessibilities in simulated intestinal fluid (SIF). Over a period of 120 minutes, the bioaccessibility of vancomycin is notably higher when encapsulated in SLN or NLC compared to when it is dispersed in solution. At 30 minutes, the bioaccessibility of vancomycin is relatively low for the solution form (4.2 ± 0.10%), while SLN and NLC show significantly higher bioaccessibility values of 30.8 ± 1.10% and 36.4 ± 1.21%, respectively. These differences suggest that both SLN and NLC enhance the initial release and availability of vancomycin compared to its unencapsulated form, with NLC showing the highest initial release.

As time progresses to 60 minutes, the trend continues, with the solution form achieving a marginal increase in bioaccessibility to 5.6 ± 0.21%, while SLN and NLC reach 32.6 ± 1.15% and 46.5 ± 1.15%, respectively. This indicates that encapsulation within lipid-based carriers, particularly NLC, significantly improves vancomycin's bioavailability in the intestinal environment. At 120 minutes, the bioaccessibility of vancomycin in solution remains low (5.7 ± 0.22%), whereas SLN and NLC further increase to 38.8 ± 1.16% and 47.7 ± 1.09%, respectively. The consistent superiority of NLC over SLN across all time points suggests that the more flexible structure of NLC may facilitate better drug release and absorption in the simulated intestinal environment. The statistically significant differences ($P < 0.05$) among these formulations indicate that the improved bioaccessibility observed with SLN and NLC is not random but is likely due to the enhanced protective and release properties of the lipid-based carriers. The ability of these carriers to maintain stability in SIF and control the release of vancomycin contributes to the increased bioaccessibility, making SLN and NLC more effective formulations for delivering vancomycin to the intestinal tract. Among these, NLC appears to offer the best enhancement of vancomycin bioaccessibility, making it a promising candidate for improving the therapeutic efficacy of vancomycin through oral delivery

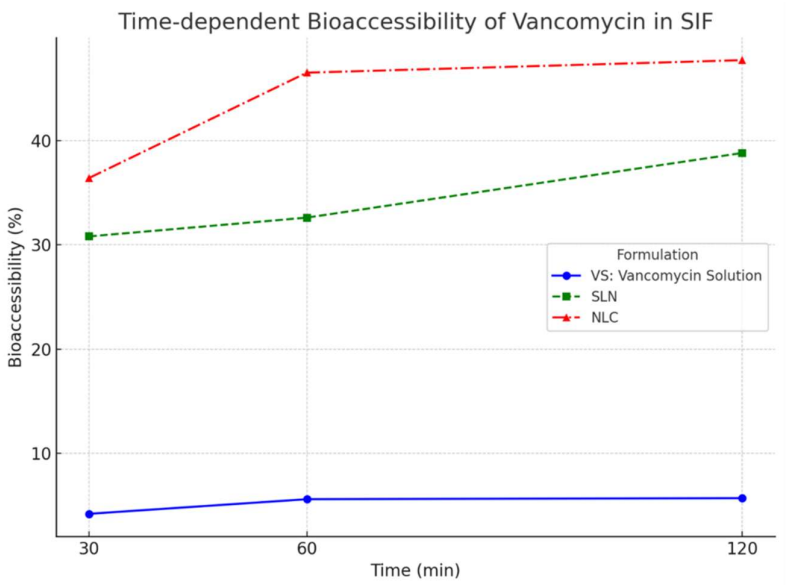

Fig.2. Time dependent bioaccessibility of vancomycin in SIF. Vancomycin dissolved in a solution is VS. Solid Lipid Nanoparticles, or SLNs. Nanostructured Lipid Carriers, or NLCs. The differences are statistically significant ($P < 0.05$).

3.4 Drug release under simulated intestinal conditions

Under enzyme-free simulated intestinal circumstances, the in vitro release profile of vancomycin from Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC) demonstrates considerable variations in the release rates between the two formulations. The study, conducted over a period of 360 minutes, demonstrates that NLCs have a consistently higher release percentage of vancomycin compared to SLNs.At the initial time point (0 minutes), both formulations show no release of vancomycin, as expected. After 60 minutes, the release of vancomycin from SLN reaches 12.76 ± 1.09%, whereas NLC shows a higher release of 17.56 ± 1.02%. This suggests that the NLC formulation enables a more rapid release of vancomycin in the early phase, which could be due to the more flexible and less crystalline structure of NLC, allowing the drug to diffuse more readily.As time progresses to 120 minutes, the release from SLN increases to 27.86 ± 1.02%, while NLC releases 38.92 ± 1.03%. The difference in release continues to be notable, with NLC providing a faster and more substantial release profile. At 180 minutes, the release from SLN reaches 43.55 ± 1.10%, whereas NLC achieves 60.55 ± 1.13%. This trend highlights the greater efficiency of NLCs in facilitating the release of encapsulated vancomycin.By the 240-minute mark, the release percentages further increase to 56.75 ± 1.21% for SLN and 73.45 ± 1.12% for NLC. The enhanced release from NLC suggests that the presence of liquid lipids in its matrix may contribute to a more favourable environment for vancomycin diffusion. At 300 minutes, the release from SLN is 66.90 ± 1.23%, compared to 82.50 ± 1.31% from NLC. By the end of the study (360 minutes), the SLN achieves a release of 72.31 ± 1.22%, while NLC nearly completes the release of vancomycin with 89.70 ± 1.28%.

Thereby, both formulations were able to sustain the release of vancomycin for a long period of time based on the results obtained and the NLCs provided a more efficient and greater extent regarding the total kinetic profile than SLN. These may be beneficial for applications where a quicker release of vancomycin is needed, such as in severe infections that need fast therapeutic

levels. Another possible reason for the better release of vancomycin from NLC is the amorphous structure, which have fewer obstacles for drug release than the more crystalline SLN. Therefore, NLC could be a suitable nanosystem for the formulations having dual behaviour to release the antibiotic in its ephemeral form with rapid uptake in plasma along with sustained release at intestinal locales and thus higher bioavailability.

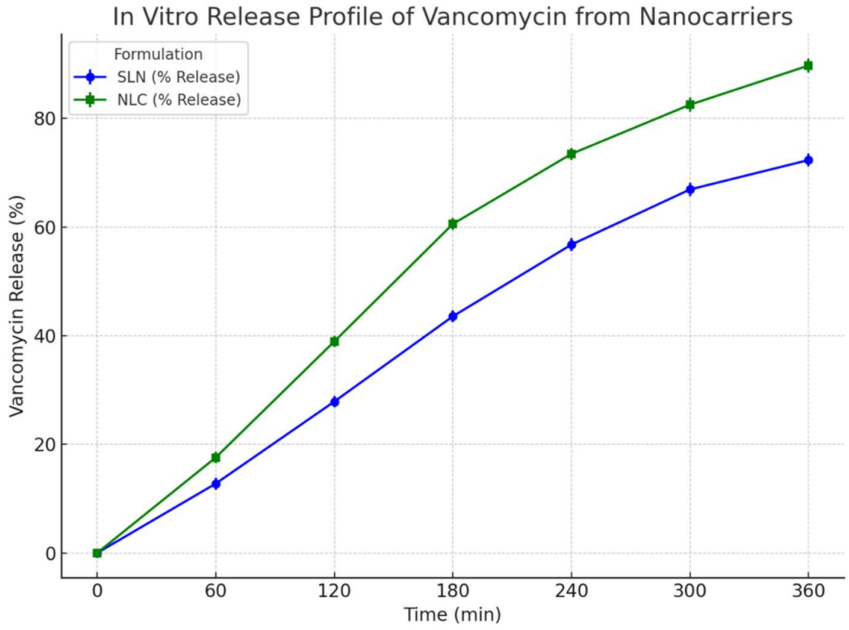

Fig. 3. Vancomycin release profile in vitro from nanocarriers in an intestinal environment mimicked without enzymes (mean ± SD; n = 3). NLC: nanostructured lipid carriers; SLN: solid lipid nanoparticles.

4. Conclusion

The study has proven that SLN and NLC lipid nanocarriers can be promising carriers for oral vancomycin delivery. NLC formulations were slightly superior to SLN impact on the preservation of encapsulation efficiency after cryoprotector removal and lyophilization, however both reached quite high scores for encapsulations. Particle size analysis data indicated the increase in SLN size with vancomycin loading whereas NLC maintained a more compact structure, resulting in higher stability. The in vitro release experiments confirmed the sustained release behaviours of the NLC given a much slower cumulative release regarding to SLN, with a slightly increased percent released on intestinal stage. The statistical analysis of release profile differences confirmed the significance ($P < 0.05$) between formulations, what is also an indication in favour of a long-term controlled delivery from NLC for vancomycin. Lyophilized formulations-maintained stability and encapsulation ability, qualifying them for long-term storage. In summary, NLC formulations may be a feasible alternative for increasing the oral bioavailability of vancomycin and can be used as potential base in the development of further advanced drug delivery systems of poorly absorbable antibiotics.

Declarations

Author contributions All the authors participated in designing and performing this study. VS, RG and AK wrote the first version of this paper, and RR reviewed it. AD was the leading researcher to finalize this study.

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Conflict of interests Authors declare that they have no competing interest.

Availability of code, data and material The source data for all figures are available upon request from the corresponding author.

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