

Article

pH-Dependent Diffusion-Dissolution Transition in Vancomycin-Loaded Calcium Phosphate-Liposome Nanoparticles

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Abstract

Drug delivery systems (DDSs) have attracted significant attention due to their ability to enhance therapeutic efficacy while minimizing side effects. In this study, vancomycin (VCM)-loaded calcium phosphate-liposome (CaPLip) composite nanoparticles were developed as a pH-responsive drug delivery system. The CaPLip nanoparticles were fabricated by in situ calcium phosphate precipitation on preformed liposomal templates in the presence of VCM, allowing the drug to be incorporated within the calcium phosphate matrix and adsorbed onto the CaP-coated surface (VCM-CaPLip). Structural and morphological characterization using FT-IR, XRD, and TEM confirmed the successful formation of calcium phosphate-coated liposomal nanoparticles with particle sizes ranging from 300 to 700 nm and a negative surface charge. The developed system exhibited an overall drug-loading efficiency of 47.28% and effectively reduced the initial burst release under physiological conditions. Equilibrium adsorption studies performed using preformed CaPLip nanoparticles demonstrated that VCM adsorption was well described by the Langmuir isotherm, indicating a high affinity of VCM for the CaP-coated surface under equilibrium conditions. Drug release studies at pH 4.0, 6.5, and 7.4 revealed pronounced pH-dependent behavior, with sustained release at pH 7.4 and accelerated release under acidic conditions. Changes in electrical conductivity provided supporting evidence for calcium phosphate dissolution accompanying drug release under acidic conditions. Kinetic analysis indicated a transition from predominantly diffusion-controlled release at physiological pH to diffusion-dissolution coupled release under acidic conditions. These findings demonstrate that CaPLip nanoparticles provide an effective pH-responsive antibiotic delivery platform and show potential for controlled drug release in infection-associated mildly acidic microenvironments.

Keywords: controlled drug release; liposome; calcium phosphate; vancomycin



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1. Introduction

Over the past decades, there has been a growing demand for controlled drug delivery systems (DDSs) capable of improving therapeutic efficacy while minimizing side effects. DDSs have been extensively developed to overcome the limitations of conventional treatments by enabling controlled and sustained drug release, enhanced bioavailability, and targeted delivery to diseased or infected tissues [1–6]. Significant progress has been made in applications such as cancer therapy and bone-related inflammatory diseases. Among the various DDS platforms, polymer-based carriers, nanoparticles, hydrogels, and

liposomal systems have been widely explored [7–12]. Liposomes, in particular, have attracted considerable attention due to their excellent biocompatibility, structural similarity to biological membranes, and ability to encapsulate a wide range of therapeutic agents with different physicochemical properties, including hydrophilic and amphiphilic drugs such as vancomycin (VCM) and doxorubicin (DOX) [12–15]. However, despite these advantages, conventional liposomal delivery systems still suffer from several limitations, including premature drug leakage, limited stability under physiological conditions, and insufficient responsiveness to pathological microenvironments. In addition, many current antibiotic delivery systems rely on passive release mechanisms and fail to exploit physiological triggers such as pH variations commonly found at infection or inflammation sites. These challenges have driven the development of stimuli-responsive DDSs capable of releasing therapeutic agents in response to specific environmental cues. In this context, calcium phosphate (CaP)-based systems have emerged as promising candidates due to their excellent biocompatibility, biodegradability, and structural similarity to the mineral phase of bone [13,14]. Importantly, CaP exhibits pH-dependent solubility, remaining stable under physiological conditions (pH ~7.4) while undergoing accelerated dissolution in mildly acidic environments typically associated with bacterial infection and inflammatory responses [2–6]. Under acidic conditions, protonation of phosphate ions shifts the equilibrium from PO_4^{3-} to HPO_4^{2-} and H_2PO_4^- , reducing the activity of phosphate species available to maintain calcium phosphate equilibrium [16]. The integration of liposomes with CaP matrices offers a synergistic approach to drug delivery by combining the high drug-loading capacity of liposomes with the structural stability and pH-responsive behavior of CaP. In such hybrid systems, the CaP layer acts as a protective shell that suppresses premature drug release and enhances carrier stability, while enabling controlled release under acidic conditions through dissolution of the inorganic matrix. Vancomycin (VCM) has been widely investigated and clinically used as a local antibiotic in antibiotic-loaded bone cement formulations for the prevention and treatment of orthopedic infections [17–20], represents an ideal model drug for evaluating such systems. The development of VCM-loaded CaP-liposome composites is therefore of significant interest for localized antibacterial therapy, particularly in the treatment of bone infections, implant-associated infections, and chronic inflammatory diseases.

In this study, calcium phosphate-liposome (CaPLip) nanoparticles were investigated as a pH-responsive hybrid platform for controlled vancomycin (VCM) release. The CaPLip system was prepared by direct precipitation of calcium phosphate (CaP) in the presence of preformed liposomes and VCM, resulting in a CaP-liposome composite. The objectives of this work were to: (i) synthesize the CaPLip composite via a controlled precipitation method; (ii) investigate VCM loading into the CaPLip system; (iii) characterize the physicochemical properties, morphology, and structural features of the resulting composite; and (iv) investigate pH-dependent VCM release and conductivity behavior under the experimentally investigated pH conditions. The results demonstrate that the developed VCM-CaPLip system provides enhanced structural stability under physiological conditions and exhibits pH-triggered drug release in acidic environments, highlighting its potential as a promising platform for controlled and targeted antibiotic delivery. This work provides insights into the design of inorganic-organic hybrid DDSs with tunable release behavior for infection-targeted therapy.

2. Materials and Methods

2.1. Chemicals and Materials

Egg phosphatidylcholine (Egg-PC) and cholesterol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Calcium chloride (CaCl_2) was obtained from Fluka (Buchs,

Switzerland), and dipotassium hydrogen phosphate (K_2HPO_4) was obtained from Fisher Scientific (Loughborough, Leicestershire, UK), and were used as calcium and phosphate sources, respectively. Chloroform and methanol (Fisher Scientific (Waltham, MA, USA)) were used as organic solvents for liposome preparation. Phosphate-buffer solution (PBS, pH 7.4, 0.1 M) and acetate buffer solution (pH 4, 0.1 M). All chemicals were of analytical grade and used without further purification. Deionized water was used throughout the experiments. Vancomycin (VCM) (Fluka Biochemika (Buchs, Switzerland)) was used as the model antibiotic for drug loading and release studies.

2.2. Preparation of Liposomes (Lip)

Liposomes were prepared using the reverse evaporation method [21,22]. Briefly, Egg-PC and cholesterol (molar ratio 2:1) were dissolved in a chloroform/methanol mixture (1:2, v/v) in a round-bottom flask. The organic solvent was removed using a rotary evaporator to form a thin lipid film on the inner wall of the flask. Residual solvent was further removed by purging with nitrogen gas for several minutes. The lipid film was then rehydrated with 10 mL of deionized water, resulting in the formation of liposomal vesicles suspended in aqueous solution.

2.3. Preparation of VCM-Loaded Calcium Phosphate-Liposome (CaPLip) (VCM-CaPLip) Composite Nanoparticles

VCM-loaded calcium phosphate-liposome (CaPLip) nanoparticles were synthesized via direct precipitation of calcium phosphate in the presence of VCM onto liposome surfaces (Figure 1). Briefly, 600 μ L of liposome stock solution was added to 15 mL of $CaCl_2$ solution (2.5×10^{-2} M) containing VCM at final concentrations of 0.1, 0.2, 0.3, 0.5, 1.0, and 1.5 mg/mL under magnetic stirring. Subsequently, 15 mL of K_2HPO_4 solution (1.5×10^{-2} M) was added dropwise to initiate calcium phosphate formation on the liposome surface (the final pH of the reaction mixture after mixing the calcium chloride and potassium hydrogen phosphate solutions was measured to be 7.8). The reaction mixture was stirred at 200 rpm at room temperature. The resulting suspension was collected by centrifugation, and the supernatant was retained for VCM concentration analysis. The collected precipitate was freeze-dried to obtain VCM-CaPLip composite microspheres.

2.4. Characterization of CaPLip Nanoparticles

The crystalline structure of the samples was analyzed using X-ray diffraction (XRD) (Bruker D8 Advance, Billerica, MA, USA) with Cu $K\alpha$ radiation operated at 40 kV and 40 mA. Diffraction patterns were recorded over a 2θ range of 20 – 60° with a step size of 0.037° . Fourier transform infrared (FT-IR) spectra were obtained using a Spectrum GX spectrometer (PerkinElmer, Waltham, MA, USA). Samples were mixed with KBr and pressed into pellets under 8 tons of pressure for 1 min. Spectra were collected over the range of 400 – 4000 cm^{-1} with 16 scans. Morphological and structural analysis was performed using transmission electron microscopy (TEM) (JEOL JEM-2010, Tokyo, Japan), and electron diffraction patterns were also recorded. Particle size distribution and zeta potential of liposomes and CaPLip nanoparticles were measured using a Zetasizer Nano ZS (Malvern, UK).

2.5. In Vitro VCM Drug Adsorption

Adsorption studies were conducted by varying the initial VCM concentration from 0 to 1.5 mg/mL at pH 7.0. Briefly, a fixed amount of CaPLip particles was incubated with 5 mL of VCM solution at the desired initial concentration under controlled conditions until equilibrium was reached. The particles were then separated by centrifugation, and the supernatant was collected for determination of the residual VCM concentration. The

separated particles were washed with phosphate-buffered saline (PBS) to remove loosely bound VCM. The equilibrium VCM concentration remaining in the solution was quantified, and the VCM adsorption capacity was calculated from the mass balance before and after adsorption according to Equation (1):

$$q_e = \frac{(C_0 - C_e)V}{W} \quad (1)$$

where q_e is the adsorption capacity of VCM per unit mass of particles (mg/g), C_0 is the initial VCM concentration (mg/mL), C_e is the equilibrium VCM concentration (mg/mL), V is the volume of the VCM solution (mL) (5 mL), and W is the mass of the particles added to the solution (mg).

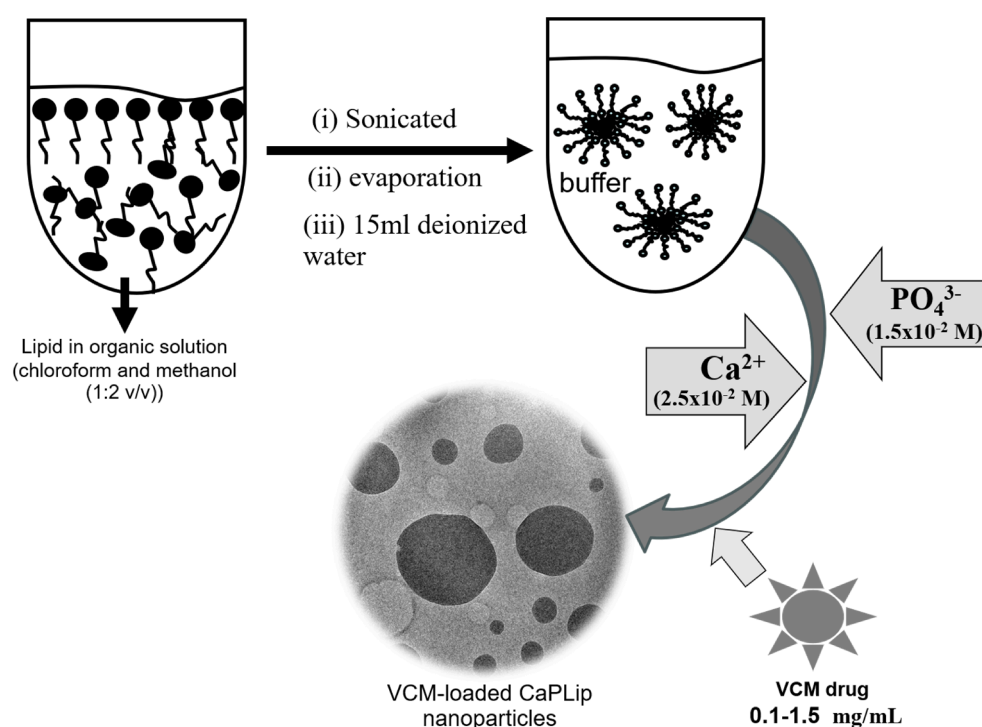


Figure 1. Conceptual schematic illustration of the synthesis of VCM-loaded calcium phosphate-liposome (CaPLip) (VCM—CaPLip) nanoparticles via the direct precipitation of calcium phosphate in the presence of VCM and liposomes, resulting in the formation of a calcium phosphate-liposome composite structure.

2.6. In Vitro Drug Release Study

In vitro release of VCM from CaPLip nanoparticles were conducted using 0.1 M acetate buffer at pH 4.0 and 0.1 M PBS at pH 7.4. For the mildly acidic condition, PBS was adjusted from pH 7.4 to pH 6.5 using HCl at 37 °C. Briefly, 50 mg of VCM-loaded CaPLip particles were dispersed in 15 mL of buffer solution and incubated in a shaking bath (SK-300 shaker). At predetermined time intervals (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, and 25 h), 3 mL of supernatant was withdrawn for analysis. The VCM concentration was determined using a UV-Vis spectrophotometer (Jenway 6405, London, UK) at a wavelength of 280 nm. After each measurement, the sampled solution was returned to the system, assuming negligible disturbance due to the short measurement time relative to the release duration. The VCM concentration (C_i) was calculated from a calibration curve using the equation: $C_i = 0.1042 A_i + 0.00018$; $R^2 = 0.9907$, where A_i is the absorbance at 280 nm.

2.7. Electrical Conductivity Analysis of Ion Release from CaPLip Nanoparticles

The release of ions from CaPLip nanoparticles was investigated in phosphate buffer solutions. Typically, 0.15 g of as-prepared CaPLip nanoparticles was dispersed in 15 mL of buffer solutions at pH 4.0, 6.5, and 7.4 at room temperature. Measurements were conducted at predetermined time intervals of 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, and 25 h. At the end of each incubation period, the change in conductivity (Δ conductivity), which reflects the concentration of released ionic species, was measured using a digital conductivity meter (HI9813-5 portable pH/EC/TDS meter, HANNA Instruments, Woonsocket, RI, USA). Prior to each measurement, the conductivity meter was calibrated using certified standard conductivity solutions following the manufacturer's instructions. Blank conductivity values obtained from buffer solutions without samples were measured under identical conditions and subtracted from the sample measurements before data analysis. The ion release profiles were evaluated by plotting Δ conductivity as a function of time. All measurements were performed in triplicate, and the data are presented as mean $\pm$ standard deviation.

3. Results and Discussion

3.1. Physico-Chemical Properties of CaPLip and VCM-CaPLip Nanoparticles

The zeta potential of liposomes (Lip) (Figure 2a) was measured to be -26.75 ± 3.32 mV. This highly negative surface charge, originating from the phosphate groups in Egg-PC, promotes the formation of calcium phosphate (CaP) [23] by facilitating the localization of Ca^{2+} ions around the liposome surface and enhancing local supersaturation. After CaP coating (Figure 2b), the surface charge of CaPLip decreased to -15.52 ± 2.28 mV, indicating successful deposition of the CaP layer. The increase in zeta potential from -26.75 to -15.52 mV, together with the structural characterization by TEM, FTIR, and XRD, supports the successful deposition of a calcium phosphate layer on the liposomal surface.

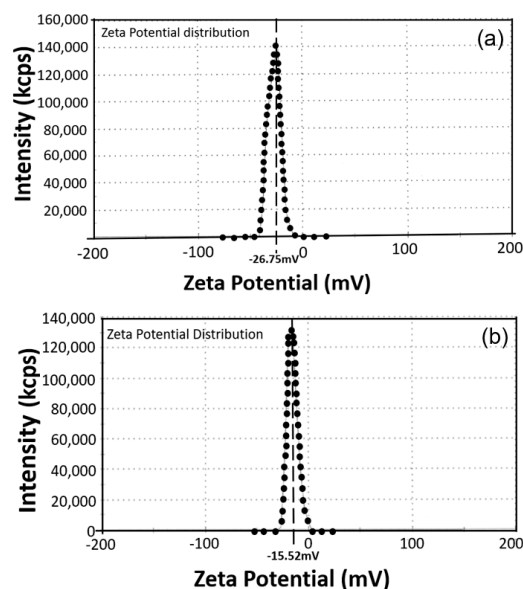


Figure 2. Zeta-potential of (a) liposome (Lip) and (b) calcium phosphate-liposome (CaPLip) nanoparticles.

TEM images and the corresponding electron diffraction pattern of CaPLip nanoparticles are shown in Figure 3a and Figure 3b, respectively. The TEM images reveal that most particles appear opaque, while some exhibit a core-shell structure. This opacity is likely due to the relatively thick CaP layer formed on the liposome surface, which limits electron transmission. Meanwhile, core-shell morphology is advantageous for drug delivery, as the CaP shell can act as a protective barrier while enabling pH-responsive dissolution. The

electron diffraction pattern does not exhibit well-defined diffraction rings, indicating that the CaP phase has a poorly resolved or low degree of crystallinity. The absence of distinct diffraction rings also suggests that the crystalline domains are either very small and/or insufficiently ordered to produce clearly resolved diffraction features. This observation is consistent with the broad diffraction peaks observed in the XRD pattern, further supporting the presence of a poorly crystalline or nanocrystalline CaP phase. The particle size distribution measured by dynamic light scattering (DLS) using a zeta potential analyzer (Figure 3c) indicates that the CaPLip nanoparticles have hydrodynamic diameters ranging from 300 to 700 nm, with an average hydrodynamic diameter of approximately 475 nm.

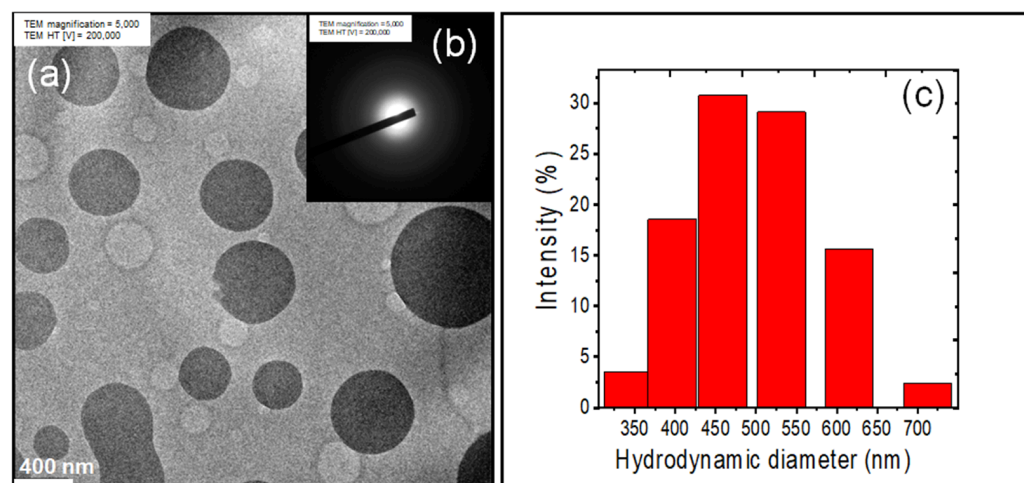


Figure 3. Microstructure of CaPLip nanoparticles. (a) TEM images, (b) electron diffraction patterns and (c) size distribution.

The chemical composition of the mineral phase deposited on the liposomes was analyzed by FT-IR spectroscopy (Figure 4). The characteristic bands at 1088, 1035, and 961 cm^{-1} correspond to PO_4^{3-} stretching vibrations, while the doublet peaks at 602 and 562 cm^{-1} are attributed to PO_4^{3-} bending modes. The bands around 2900 cm^{-1} are assigned to $-\text{CH}_2$ and $-\text{CH}_3$ groups. After VCM loading, additional bands at 1449, 1470, 1494, and 1639 cm^{-1} corresponding to C–C stretching vibrations of VCM were observed [24]. Based on the FTIR spectra, no significant changes in the characteristic absorption bands were observed, indicating that no major chemical bond formation or structural alteration was detected under the present experimental conditions. The phase composition of CaPLip nanoparticles was further investigated using XRD (Figure 5), which reveals a poorly crystalline apatite structure. This is evidenced by the broad diffraction peaks, indicating low crystallinity of the CaP layer formed on the liposome surface. The low crystallinity of the CaP phase is beneficial for biomedical applications due to its enhanced biodegradability and dissolution under acidic conditions. The average crystallite size along the (002) plane ($2\theta \sim 26^\circ$) was estimated using the Scherrer equation, $d = k\lambda/(\beta\cos\theta)$, where d is the crystallite size, λ is the X-ray wavelength (0.154 nm), k is the shape factor (~ 0.89), β is the full width at half maximum (FWHM, in radians), and θ is the Bragg angle. The calculated crystallite size was approximately 36 nm. Overall, the combined XRD and FT-IR results confirm the formation of poorly crystalline, nanostructured apatite on the liposome surface. The successful formation of the CaP coating is further supported by the observed change in zeta potential during the synthesis process (Figure 2).

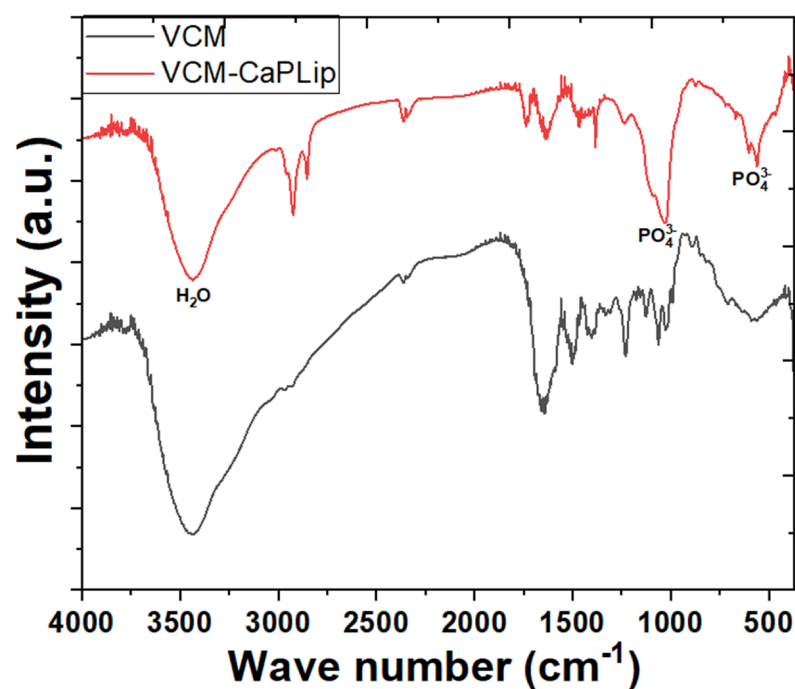


Figure 4. FT-IR spectra for pure vancomycin (VCM) and VCM-loaded calcium phosphate-liposome (CaPLip) (VCM-CaPLip) nanoparticles.

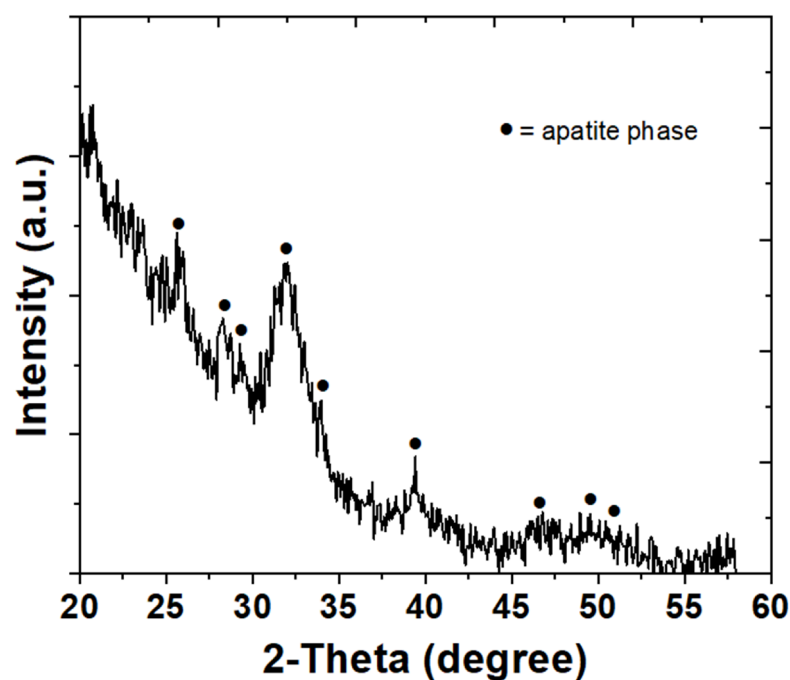


Figure 5. X-ray diffraction (XRD) pattern of calcium phosphate-liposome (CaPLip) nanoparticles. • indicates the apatite phase.

3.2. *In Vitro* VCM Drug Loading and Release from CaPLip Nanoparticles

The results shown in Figure 6 indicate that the amount of VCM adsorbed onto the CaPLip nanoparticles increases rapidly when the VCM concentration is in the range of 0.1–0.5 mg/mL and increases more slowly at concentrations above 1.0 mg/mL.

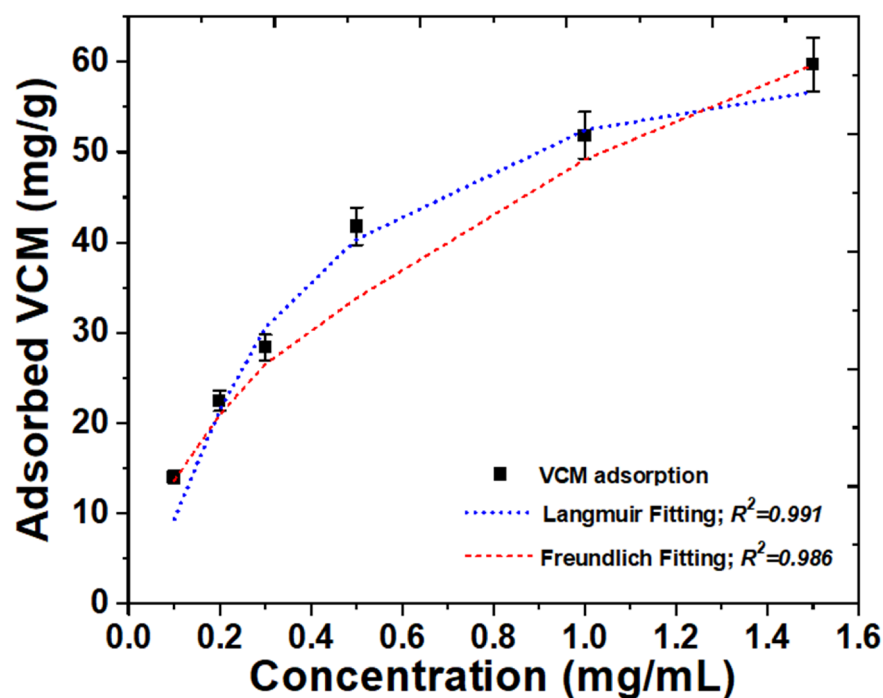


Figure 6. The amount of VCM adsorbed onto the calcium phosphate-liposome (CaPLip) nanoparticles and the adsorption data were fitted using both Langmuir and Freundlich isotherm models within the range of 0–1.5 mg/mL. The data points are expressed as mean $\pm$ SD; $n = 3$.

The absorption reaches a plateau at approximately 1.5 mg/mL. To analyze the adsorption behavior of VCM onto CaPLip nanoparticles, two commonly used isotherm models were applied to describe the relationship between adsorption capacity and VCM concentration. One is the Langmuir adsorption isotherm [25]:

$$\frac{1}{q_e} = \frac{1}{q_{\max}} + \frac{1}{q_{\max} b C_e} \quad (2)$$

where $q_{\max}$ is the maximum monolayer adsorption capacity (mg/g) and b is the Langmuir equilibrium constant (mL/mg), which reflects the affinity between adsorbate and adsorbent. The other is the Freundlich adsorption isotherm [25]:

$$\ln q_e = \frac{1}{n} \ln C_e + \ln K_F \quad (3)$$

where K_F and $1/n$ are Freundlich constants related to adsorption capacity and surface heterogeneity, respectively. A value of $1/n$ close to zero indicates highly heterogeneous surface adsorption. The Freundlich model is an empirical equation describing adsorption on heterogeneous surfaces. The adsorption data were fitted using both the Langmuir and the Freundlich isotherms. As shown in Figure 6, the adsorption equilibrium of VCM onto pre-formed CaPLip nanoparticles at pH 7.4 and 37 °C is well described by both models. The calculated isotherm parameters are summarized in Table 1. The correlation coefficients (R^2) are 0.991 for the Langmuir and 0.986 for the Freundlich model. The Langmuir model provides a slightly better fit, suggesting that VCM adsorption occurs predominantly as a saturated monolayer on the pre-formed CaPLip surface, with a maximum adsorption capacity of 65.096 mg/g. These results also indicate that VCM adsorption is strongly dependent on the drug concentration in solution within the range of 0–1.5 mg/mL. However, the adsorption experiment was conducted using pre-formed CaPLip nanoparticles, whereas the actual VCM-CaPLip nanoparticles were prepared through co-precipitation in

the presence of VCM. Therefore, the Langmuir analysis provides evidence for the surface adsorption behavior and monolayer adsorption assumption, but does not by itself establish the complete mechanism of VCM incorporation during CaP co-precipitation.

Table 1. Isotherm constants for VCM adsorption onto calcium phosphate-liposome (CaPLip) nanoparticles.

Sample	Langmuir Isotherm Parameter			Freundlich Isotherm Parameter		
	b	q_{max}	R^2	K_F	n	R^2
pH 7.4	5.606	65.096	0.991	55.450	2.5	0.986

To further explain the controlled release behavior of VCM from CaPLip nanoparticles, it is noted that apatite (as confirmed by XRD) is stable under physiological pH conditions (~7.4) but becomes unstable in acidic environments. Therefore, drug loading is effectively achieved under near physiological conditions (pH ~7.0). An overall drug-loading efficiency into the CaPLip nanoparticles was determined using UV-Vis spectroscopy, and the loading efficiency was calculated as follows:

$$\text{Loading efficiency}(\%) = \frac{W_i - W_s}{W_i} \times 100\% \quad (4)$$

The amount of free vancomycin (VCM) (W_s) in the supernatant after centrifugation was determined using Beer-Lambert's law [26], $A = \epsilon cl$, where A is the absorbance, ϵ is the molar absorptivity, c is the VCM concentration, and l is the path length of the quartz cuvette (1 cm). The VCM loading efficiency calculated using Equation (4) was 47.28% ($W_i = 10$ mg).

The concentration of released drug was determined by immersing VCM-loaded CaPLip nanoparticles in phosphate buffer solutions with different pH values (4.0, 6.5 and 7.4), followed by measuring the UV-Vis absorbance of the samples. Figure 7 presents the cumulative release profile of VCM as function of time at the three pH conditions. At physiological pH 7.4, VCM release from CaPLip exhibits a sustained release behavior, with only 39.51% of the drug released after 25 h. At pH 6.5, a similar sustained release profile is observed, with 67.08% of VCM released over the same period. A comparison between these two conditions indicates that the amount of drug released increases as the pH decreases. In contrast, at pH 4.0, a markedly different release behavior is observed. A rapid burst occurs, reaching approximately 82.17% within the first 5 h. These results clearly demonstrate the pH-dependent release behavior of VCM from CaPLip nanoparticles. Two distinct release mechanisms can be identified: (i) a slow and sustained release at physiological and mildly acidic conditions (pH 7.4 and 6.5), and (ii) a burst release under strongly acidic conditions (pH 4.0). This behavior is primarily attributed to the stability of the calcium phosphate (CaP) layer. At higher pH values, the CaP layer remains structurally intact, thereby restricting drug diffusion. However, under acidic conditions, the increased concentration of H^+ ions shift the dissolution equilibrium of CaP, leading to structural degradation. This phenomenon has been supported by the increased Ca^{2+} concentration observed in solution, as reported by Matsumoto et al. [27]. At pH 4.0, the crystallinity and structural integrity of the CaP layer rapidly deteriorate, resulting in a more porous structure that facilitates drug release. In contrast, when the CaP layer remains intact (pH 7.4 and 6.5), the encapsulated drug is retained within the CaPLip nanoparticles, leading to controlled release behavior. However, repeated sampling and replenishment of the release medium may introduce minor cumulative dilution errors during extended-release experiments. Future studies will employ dynamic dialysis or continuous-flow release systems to minimize sampling-related artifacts and improve the accuracy of release kinetics measurements.

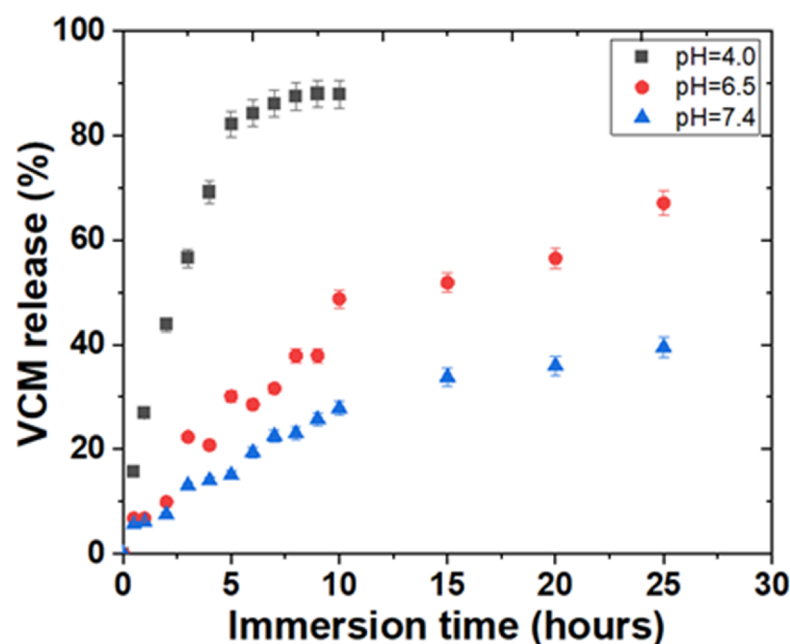


Figure 7. Release profiles of vancomycin (VCM) from calcium phosphate-liposome (CaPLip) nanoparticles in phosphate buffer solutions at different pH (4.0, 6.5, and 7.4). The data points are expressed as mean $\pm$ SD; $n = 3$.

To place the present findings in the context of previously reported VCM delivery systems, the release behavior of VCM-CaPLip nanoparticles was compared with representative calcium phosphate- and liposome-based carriers. Dion et al. reported VCM release from amorphous calcium polyphosphate matrices designed for osteomyelitis treatment, demonstrating that the gelation and drying conditions of the calcium polyphosphate matrix could substantially reduce the initial release and extend the effective release period [18]. In another approach, Lukina et al. developed brushite-monetite-newberyite cements as VCM carriers, in which the release rate was strongly influenced by cement composition, porosity, and dissolution behavior; approximately 85–97% of VCM was released within 24 h, followed by a plateau at approximately 72 h [19]. These studies demonstrate the important role of calcium phosphate matrix structure, porosity, and dissolution in controlling VCM release.

Liposome-based VCM delivery systems provide a different strategy for prolonging drug retention. Gao et al. developed VCM-loaded multivesicular liposomes and subsequently incorporated them into a thermoresponsive PLGA-PEG-PLGA hydrogel. The VCM-loaded multivesicular liposomes exhibited an encapsulation efficiency of approximately 92%, sustained release for more than 12 days, and the liposome-hydrogel system extended drug release and antibacterial activity to approximately 20 and 21 days, respectively [20]. In contrast to these systems, the present VCM-CaPLip platform integrates a liposomal component with a calcium phosphate phase within a composite architecture. Rather than relying solely on prolonged diffusion through a liposomal or hydrogel barrier, the present results suggest that the CaP component contributes to pH-dependent modulation of VCM release. Importantly, the present system exhibited a gradual change in release behavior with environmental pH. At pH 7.4, VCM release was predominantly diffusion-dominated, whereas decreasing the pH to 6.5 was associated with an increasing contribution from CaP dissolution. Under more acidic conditions (pH 4.0), enhanced CaP dissolution was accompanied by more rapid VCM release. Therefore, the principal distinction of the present CaPLip system from previously reported VCM-loaded CaP matrices and liposomal carriers is not simply prolonged release, but the integration of diffusion and

pH-dependent CaP dissolution within a single hybrid platform. This provides a mechanistic basis for developing VCM delivery systems capable of adapting drug release to changes in the surrounding pH.

To further analyze the release kinetics, the Peppas model was applied to describe the initial drug release behavior [5,28]:

$$\frac{M_t}{M_0} = kt^n \quad (5)$$

where M_t and M_0 represent the amount of drug released at time t and the initial drug content, respectively. The fractional release (M_t/M_0) is expressed as kt^n , where k is the release rate constant (h^{-1}) and n is the release exponent. This model is typically used for systems in which drug dissolution is faster than diffusion.

Fitting the experimental data (Figure 8) shows good agreement with the Peppas model for immersion times less than 5 h across all pH conditions. However, deviations from the model become more pronounced at longer times, particularly at pH 4.0, due to the progressive dissolution of the CaP layer. The release exponent (n) values obtained from the Korsmeyer-Peppas model were 0.688, 0.683, and 0.515 at pH 4.0, 6.5, and 7.4, respectively (Table 2). The values at pH 4.0 and 6.5 lie within the range of $0.5 < n < 1.0$, indicating anomalous (non-Fickian) transport, in which drug release is governed by a combination of diffusion and matrix relaxation or dissolution processes [5,28]. In contrast, the n value at pH 7.4 (0.515) is very close to the theoretical Fickian diffusion boundary ($n = 0.5$), suggesting that drug release under physiological conditions is predominantly diffusion-controlled, with only a limited contribution from structural relaxation or calcium phosphate dissolution. This observation is consistent with the relatively high stability of the calcium phosphate coating at neutral pH, where dissolution is minimal and diffusion remains the dominant release mechanism. Furthermore, the release constant k decreases with increasing pH, confirming the pH-sensitive nature of the system.

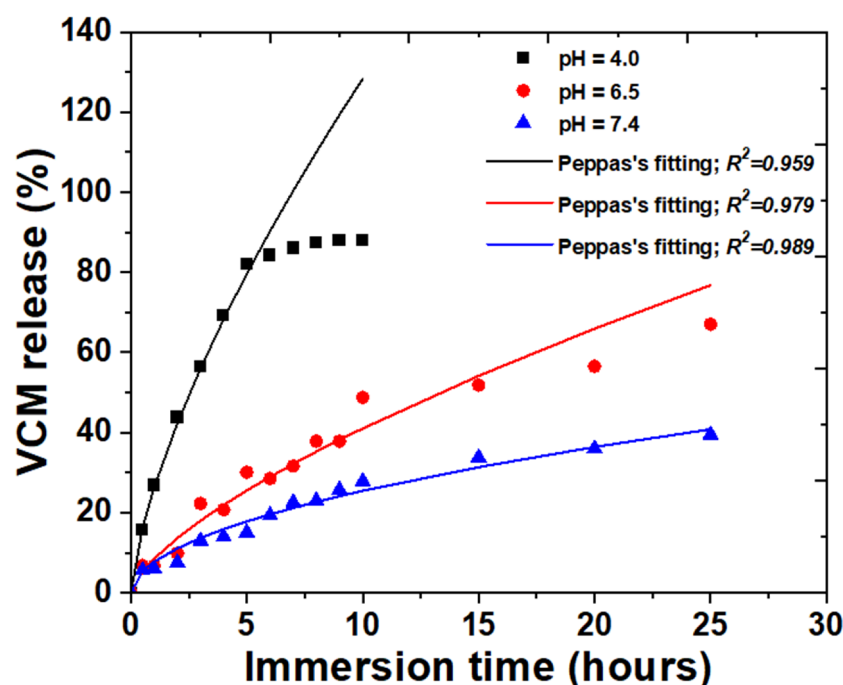


Figure 8. Fitting of experimental VCM release data using Peppas model ($M_t/M_0 = kt^n$). Solid symbols represent experimental data, while solid lines correspond to model fitting. Good agreement is observed at early release times ($t < 5$ h), suggesting anomalous transport behavior governed by a combination of diffusion and matrix relaxation mechanisms.

Table 2. Release characteristic parameters of vancomycin (VCM)-loaded calcium phosphate-liposome (CaPLip) (VCM-CaPLip) composite microspheres.

pH	$M_t/M_0 = kt^n$			$M_t/M_0 = \varphi_0(1 - \exp(-k_b t))$		
	k (h^{-1})	n	R^2	φ_0	k_b (h^{-1})	R^2
4.0	26.314	0.688	0.959	93.806	0.3405	0.996
6.5	8.5035	0.683	0.979	96.818	0.0699	0.987
7.4	7.7701	0.515	0.989	55.793	0.0645	0.990

φ_0 is the fraction of drug released through initial burst.

To better describe the overall release profiles, a first-order kinetic model proposed by Batycky et al. [29] was also applied:

$$\ln\left(1 - \frac{M_t}{M_0}\right) = -k_b t \quad (6)$$

where M_t/M_0 represents the fractional drug release and k_b (h^{-1}) is the burst release constant. The fitting results (Figure 9) show improved agreement with the experimental data compared to the Peppas model.

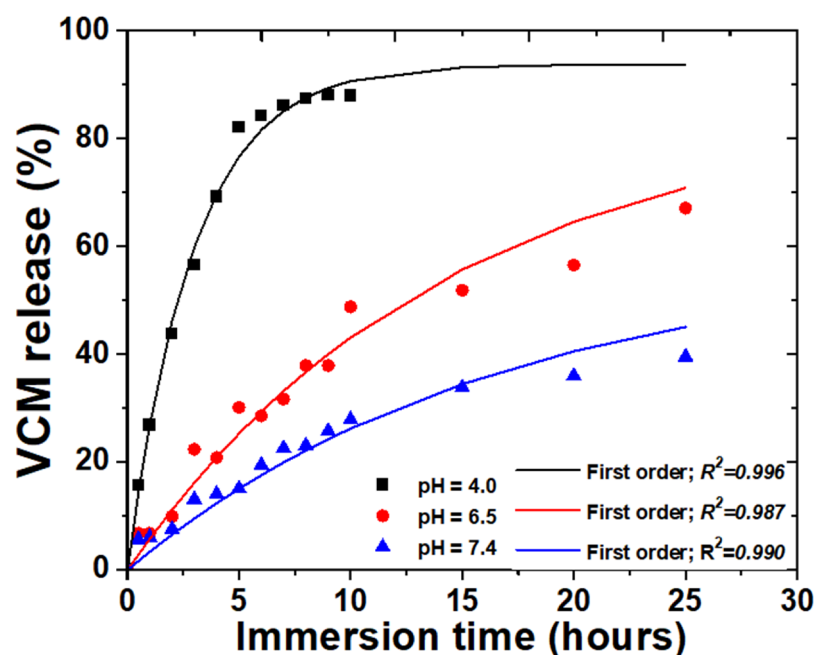


Figure 9. Fitting of experimental VCM release data using first-order kinetic model ($\varphi_0(1 - \exp(-k_b t))$). The model demonstrates improved agreement with experimental data across the entire time range compared to the Peppas model, particularly under acidic conditions, indicating that dissolution-driven processes significantly influence drug release kinetics.

The corresponding fitting parameters (φ_0 , k_b , and R^2) are summarized in Table 2. Overall, the first-order model provided the best fit to the experimental release profiles (the values of the φ_0 and k_b , which lead to the best fits are ($93.806, 0.3405 \text{ h}^{-1}$), ($96.818, 0.0699 \text{ h}^{-1}$) and ($55.793, 0.0645 \text{ h}^{-1}$) for the pH 4.0, 6.5, and 7.4), respectively), as evidenced by the higher correlation coefficients compared with the other kinetic models, indicating that the release rate is dependent on the amount of drug remaining within the CaPLip matrix. The fitted parameter φ_0 represents the theoretical equilibrium release predicted by the model and therefore may exceed the cumulative drug release measured within the finite experimental period, which explains why the fitted values are slightly higher than the experimental

observations. The release profiles, characterized by an initial burst followed by a gradual exponential release, suggest that drug diffusion predominates under physiological conditions, whereas matrix dissolution increasingly contributes under acidic conditions. Although the fitted first-order rate constants at pH 6.5 and pH 7.4 are comparable, the proposed release mechanisms were not assigned solely based on the k_b values. Instead, the mechanistic interpretation was derived from the combined evidence of the Korsmeyer-Peppas release exponent, conductivity measurements, and the well-established pH-dependent dissolution behavior of calcium phosphate. At pH 7.4, the release exponent ($n = 0.515$) is close to the Fickian diffusion boundary, indicating that diffusion is the predominant release mechanism because calcium phosphate remains relatively stable under physiological conditions. In contrast, the higher n value at pH 6.5 ($n = 0.683$), together with the increase in conductivity, suggests the onset of calcium phosphate dissolution, indicating that matrix dissolution contributes to drug release in addition to diffusion. Therefore, the release behavior at pH 6.5 is more appropriately described as an intermediate diffusion-dissolution regime rather than purely diffusion-controlled. This behavior is consistent with the spherical morphology of the CaPLip nanoparticles observed by TEM (Figure 3a) and supports the proposed pH-dependent transition from diffusion-dominated to coupled diffusion-dissolution-controlled release.

To elucidate the underlying release mechanism, the change in conductivity (Δ conductivity) of the release medium was monitored as an indirect indicator of CaP dissolution. As shown in Figure 10, Δ conductivity increased significantly with decreasing pH, indicating enhanced release of ionic species, particularly Ca^{2+} , from the calcium phosphate phase, as reported in similar biomaterial systems [30–32]. Notably, at pH 4.0, a rapid increase in Δ conductivity was observed during the initial stage, corresponding closely to the burst release of VCM. Throughout the incubation period, the Δ conductivity at pH 4.0 ranged from 0.39 to 1.22 mS/cm. At mildly acidic pH (6.5), the Δ conductivity profile exhibited a gradual increase (0.08–0.76 mS/cm), reflecting sustained ion release governed by a coupled diffusion-dissolution mechanism, which is consistent with the absence of burst release behavior observed at lower pH (4.0). In contrast, minimal changes in Δ conductivity were detected at pH 7.4 (0.01–0.08 mS/cm), consistent with sustained, diffusion-controlled release behavior. These results provide compelling evidence that the CaPLip system exhibits a pH-dependent transition from diffusion-controlled to dissolution-driven release, governed by coupled transport mechanisms. The dissolution of the CaP coating not only increases ionic conductivity but also dynamically alters the structural barrier, thereby accelerating drug release under acidic conditions.

Based on the VCM release kinetics and changes in conductivity (Δ conductivity), Figure 11 presents a proposed pH-responsive release mechanism for the VCM-CaPLip nanoparticles. Under physiological conditions (pH 7.4), the relatively limited dissolution of the CaP phase favors predominantly diffusion-controlled VCM release. As the pH decreases to 6.5, the increase in conductivity together with the release kinetics suggests an increasing contribution of CaP dissolution to drug release, resulting in an intermediate coupled diffusion-dissolution regime. Under more acidic conditions (pH 4.0), enhanced dissolution of the CaP phase is expected to facilitate more rapid VCM release, indicating a greater contribution of matrix dissolution. This interpretation is consistent with the broader concept of phosphate-based inorganic nanomaterials as pH-responsive platforms, in which dissolution of the phosphate-containing phase under acidic conditions can contribute to the release of therapeutic components [33]. Although the material composition and therapeutic payload differ from those of the present CaPLip system, recent work on a core-shell manganese phosphate/siPD-L1 modulator similarly demonstrated pH-responsive behavior associated with the dissolution of a phosphate-based inorganic phase

under acidic conditions [33]. Thus, the present results support a gradual pH-dependent transition from predominantly diffusion-controlled release at physiological pH toward coupled diffusion–dissolution-controlled release under acidic conditions. Importantly, Figure 11 is intended as a conceptual representation of the proposed release mechanism rather than a direct depiction of VCM localization within the CaPLip structure. The present study does not directly establish whether VCM is located within the aqueous liposomal core, associated with the CaP phase, or distributed at the CaP-liposome interface. Therefore, the proposed mechanism primarily describes the contribution of CaP dissolution and diffusion to VCM release, while the precise spatial distribution of VCM within the CaP-liposome composite remains to be determined by direct localization studies.

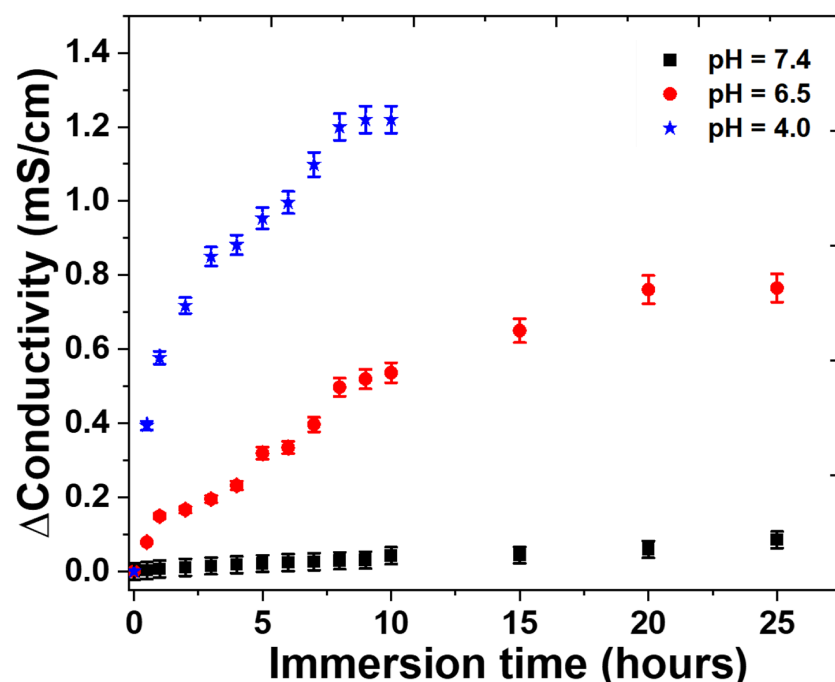


Figure 10. Time-dependent the change in conductivity (Δ conductivity) of the release medium at different pH conditions (4.0, 6.5, and 7.4). The increase in conductivity at lower pH reflects enhanced dissolution of the CaP and release of ionic species. The data points are expressed as mean $\pm$ SD; $n = 3$.

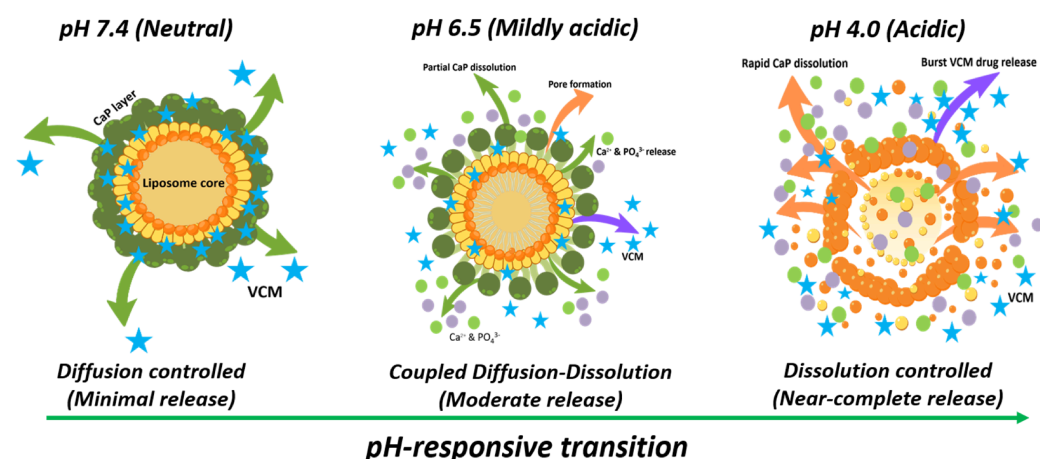


Figure 11. Conceptual schematic of the proposed pH-responsive VCM release mechanism from CaP-liposome (CaPLip) nanoparticles under physiological (pH 7.4), mildly acidic (pH 6.5), and acidic (pH 4.0) conditions. The schematic illustrates the proposed transition from predominantly diffusion-controlled release to increasing CaP dissolution-assisted release as the environmental pH decreases.

4. Conclusions

VCM-loaded CaPLip nanoparticles were successfully prepared through the direct precipitation of calcium phosphate in the presence of vancomycin onto liposome surfaces, yielding composite particles with diameters ranging from 300 to 700 nm. Adsorption studies demonstrated that the interaction of VCM with preformed CaPLip nanoparticles was better described by the Langmuir isotherm than by the Freundlich model, suggesting that surface adsorption predominantly occurs as monolayer adsorption. Drug release experiments performed under physiological (pH 7.4), mildly acidic (pH 6.5), and acidic (pH 4.0) conditions demonstrated clear pH-responsive release behavior, with sustained release at physiological pH and accelerated release under acidic conditions. The conductivity measurements, together with the kinetic analyses, further support the contribution of calcium phosphate dissolution to drug release under acidic environments. Overall, the results indicate a gradual transition from diffusion-dominated release at physiological pH to diffusion–dissolution coupled release as the medium becomes increasingly acidic. These findings demonstrate that the CaPLip system is a promising pH-responsive drug delivery platform for localized antibiotic delivery in infection-associated acidic microenvironments. Nevertheless, further biological validation and release studies under additional physiologically relevant conditions are required to fully establish its therapeutic potential.

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Abbreviations

The following abbreviations are used in this manuscript:

DDSs	Drug delivery systems
VCM	Vancomycin
CaPLip	Calcium phosphate-liposome
DOX	Doxorubicin
CaP	Calcium phosphate
Lip	Liposomes

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