Pyramid-Shaped PEG-PCL-PEG Polymeric-Based Model Systems for Site-Specific Drug Delivery of Vancomycin with Enhance Antibacterial Efficacy

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ABSTRACT: Antibacterial resistance remains a major global problem due to frequent prescripions, leading to significant toxicities. To overcome the limitations of antibiotic therapy, it is highly desirable to provide site-specific delivery of drugs with controlled release. Inspired by the biocompatible, biodegradable, and site-specific mimicking behavior of poly(ethylene glycol) (PEG) and poly(caprolactone) (PCL), we developed vancomycin-PEG-PCL-PEG conjugates to maximize the pharmacological effects and minimize the side effects. Drug-loaded vancomycin-PEG-PCL-PEG conjugates are influenced by size, shape, surface area, encapsulation efficiency, in vitro drug release, hemolysis assay, cytotoxicity, and antibacterial activity against methicillin-resistant Staphylococcus aureus (MRSA) and bacterial kill kinetics. The results demonstrated that vancomycin (VCM) release from PEG-PCL-PEG triblock revealed a biphasic manner. Hemolysis assay showed the nonprescription nature of VCM-PEG-PCL-PEG. Cytotoxicity studies confirmed the biocompatibility of VCM-PEG-PCL-PEG. The in vitro antibacterial results showed enhance activity with minimum inhibitory concentration compared to bare VCM. Molecular dynamics simulation study revealed that binding between VCM and PEG-PCL-PEG by hydrophobic interactions offers molecular encapsulation and steric barrier to drug degradation. This newly developed therapeutic delivery system can offer to enhance activity and delivery VCM against MRSA.

1. INTRODUCTION

There has been a substantial rise in the incidence and occurrence of aggressive methicillin-resistant Staphylococcus aureus (MRSA) and its drug resistance over the past centuries. These MRSA are opportunistic bacterial pathogens, which are widely distributed in the healthcare industry and the community settings as nosocomial pathogens. These opportunistic MRSA can lead to infection from the skin to life-threatening pneumonia as well as bacteraemia, endocarditis, diseases in the skin and smooth tissue, bone, and articulate diseases. The ongoing increasing incidence of antimicrobial resistance has intensified patient situations by extending the hospitalization stay duration, raising the entire overall cost of treatment and rising deaths. The amount of life-threatening bacteria is increasing each year, although Staphylococcus continues to be the world’s leading source of infectious diseases worldwide. According to the United States Center for Disease Control and Prevention, more than 80,000 invasive MRSA diseases and 11,285 associated fatalities happen worldwide in a very short time. Staphylococcus diseases, especially MRSA, have raised concern in the recent years because of their causative role in life-threatening infections.

One of those susceptible pathogens is MRSA, which resists almost all β-lactam antibiotics (penicillin and cephalosporins) and commonly used antibiotics, such as erythromycin, clindamycin, gentamycin, ciprofloxacin, and fusidic acid, and restricts therapy possibilities. In order to meet the challenge of MRSA, an effective therapeutic regimen will likely arise from a drug delivery platform based on nanomedicine and material chemistry, which seeks to contravene the resistance mechanism in target cells and enhance the effectiveness of antibiotics. MRSA’s resistance to low-toxic first-line antibiotics more widely and frequently makes it possible to treat severe infectious disorders with antibiotics such as vancomycin (VCM). The day-by-day increasing incidence of MRSA...
threatened the potential of modern medicine and the efficacy of mainstream antibiotics by antibiotic resistance in bacterial pathogens. The resistance of MRSA to low-toxic first-line antibiotics more commonly and frequently makes it essential to treat severe infectious disorders with the application of antibiotics such as vancomycin. This is a tricyclic glycopeptide antibiotic more commonly used for the treatment of most of the diseases induced by Gram-positive bacteria such as *Clostridium difficile* and *Staphylococcus aureus*. Due to the rigid conformational structure of VCM, there are several factors like pH and temperature that can affect its chemical stability. It is mainly by diminishing the drug in aqueous solutions that ultimately restrict its clinical efficacy by suppressing its anticipated antimicrobial activities.

VCM has been revealed to have lost its clinical effectiveness due to the development of resistance by MRSA, reduced susceptibility to VCM, and the side effects caused by the choice of drug. VCM administration has been revealed to be cytotoxic to disease sites due to dose frequency and inadequate concentration of drugs. The toxicity risk or side effects associated with high serum are well known. There is an urgent need to develop better therapies to overcome the limitation of drug choice. We have two choices: One is to discover new antibacterial drugs that are better than the existing. Alternately, well-established and approved last-resort antibiotics could be delivered selectively to the affected location to boost local drug effectiveness and decrease other side effects. To address the challenges and limitations of the existing therapy, the alternate is the best way to go. An efficient therapy regimen can be established to tackle the challenge of MRSA therapy and the drug delivery platform. Demands for the development of efficient drug delivery systems will continue to grow significantly in the foreseeable future due to the growing number of patients and treatments. A controlled or site-specific drug delivery system is a new way of offering a predetermined level of concentration of the drug in the body for a specific time within the desired therapeutic window.

The growth of bacterial species with multidrug resistance has benefited the local availability of antibiotics with the Food and Drug Administration (FDA)-approved biodegradable polymers due to enhanced biocompatibility, biodegradability, bioactivity, and reabsorption. Therefore, small amphiphilic polymeric nanoparticles (NPs) containing both hydrophobic and hydrophilic groups with controlled hemolytic behavior is extremely imperative for targeted VCM drug delivery. Polymeric NPs from amphiphilic block copolymers have developed significant value in drug delivery over the past centuries. These polymeric NPs usually have a core–shell architecture characterized by an inner hydrophobic core surrounded by a hydrophilic corona that is strongly water-bound. The most significant advantages are outstanding self-assembly, effective hydrophobic agent solutions, enhanced pH, and passive accumulation of tissue by enhanced permeability and retention (EPR) impact. The polymer NPs are frequently researched and are made from PEG-R-amphiphilic. R reflects the biodegradable hydrophobic section, such as poly(lactic-co-glycolic acid) (PLGE), poly(lactide (PLA), poly(caprolactone) (PCL), and their copolymers.

To accomplish this, we developed hydrophobic and hydrophilic clusters of VCM-PEG-PCL-PEG conjugates small amphipathic molecules. Small molecules with structural features are thus viewed as a prominent approach for discovering small molecular antibacterial drug delivery of VCM-PEG-PCL-PEG.

![Figure 1. Schematic illustration of the VCM-PEG-PCL-PEG conjugated nanoparticles synthesis.](image-url)
1.1. Choice of Materials: Formulation Fundamentals.

At present, triblock copolymer PEG-PCL-PEG polymers were synthesized and are typically used as drug delivery vectors for delivery of most reliable and effective antibiotics of the past decades, that is, VCM. To carry out this study, two polymers (PEG and PCL) are selected for the synthesis of copolymer PEG-PCL-PEG. Both the selected polymers have been approved by the Food and Drug Administration (FDA).

PEG is a water-soluble polyether with a broad spectrum of molecular weights that have been discovered to be biocompatible and used as an adhesive molecule for surface modification of other polymers to acquire co-polymers. PEG is exceptionally physicochemical and toxicity free, has excellent biocompatibility, and decreases nonspecific protein adsorption and cell adhesion. These properties make PEG as a unique scaffold, and it can be considered a very suitable material for bioactive fabrication and has been selected as a hydrophilic macrodiol.\(^ {11}\)

However, PCL is a semicrystalline, hydrophobic material that is compatible with a wide range of other materials polymers. Due to its polyvalent nature, fabrication ease, good biocompatibility, it is established as a polymer of choice with a wide range of applications in targeted novel drug delivery and tissue engineering.

When we consider the unmodified form of both the polymers, however, there are significant limitations to the use. Therefore, approaches have been rendered to solve these unnecessary features by various kinds of alterations mentioned for promising implementation in pharmaceutical drug delivery formulations. The polymeric transition behavior is mainly governed or controlled by the ratio of different polymers used.\(^ {12}\)

In this research, we advocated a PEG-PCL-PEG amphiphilic triblock copolymer system composed of a covalently connected main hydrophobic PCL block section to two terminating hydrophilic PEG segments as shown in Figure 1. This newly synthesized amphiphilic triblock copolymer system can be used as a single blend and produce products with few novel applications in novel drug delivery by facilitating more effective drug encapsulation or loading and higher biocompatibility.

This work aimed to evaluate the polymeric blend of PCL and PEG with an incorporated VCM as a model drug. Results of the in vitro study showed that amphiphilic block copolymers are capable of forming various nanostructures useful for drug delivery.

2. RESULTS AND DISCUSSION

2.1. Characterization of (PEG-PCL-PEG) Triblock Copolymer. Different techniques were used for the characterization of PEG-PCL-PEG copolymer, as shown in Figure 2. Figure 2A shows the Fourier transform infrared (FTIR) spectrum of the newly synthesized PEG-PCL-PEG copolymer triblock. A C=O stretching vibration in the ester carbonyl group is attached to the absorption band at 1722.85 cm\(^{-1}\). The 1109 and 1238 cm\(^{-1}\) absorption bands are attributed to the C−O−C stretching vibrations of the repeated −OCH\(_2\)CH\(_2\)−O− units of PEG and the C−O stretching vibrations, respectively. The 2883 cm\(^{-1}\) absorption band is attributed to the copolymer terminal hydroxyl clusters (−OH).

The \(^{1}\)H NMR spectrum of synthesized triblock copolymer PEG-PCL-PEG is shown in Figure 2B. Two separate triplet signals at approximately 3.80 and 2.14 ppm (\(J = 6.4\) and 7.3 Hz) for CH\(_2\) (OH−CH\(_2\)−CH\(_2\)) and (−CH\(_2\)−CH\(_2\)−COO−) were noted for the \(^{1}\)H NMR spectrum of (400 MHz, CDCl\(_3\)).
of the initial compounds. Furthermore, for two \( \text{CH}_2 \) protons (OH\( ^{-} \text{CH}_2\text{−CH}_2\text{−CH}_2\text{−CH}_2\text{−COO}^{-} \)), the most informative wide singlet signal, resonated around 1.58 ppm.

Figure 2C indicates the PEG-PCL-PEG triblock copolymer differential scanning calorimetry (DSC) thermograms. The PEG-PCL-PEG copolymer thermograms showed an endothermic peak at 59.70 °C, which shows the melting of the crystalline copolymer section.

Figure 2D shows scanning electron microscopy (SEM) pictures of typical agglomerates generated from the PEG-PCL-PEG triblock copolymer synthesized by the ring activation polymerization (ROS) method. In the size range of approximately 2 μm, agglomerates were achieved.

Brunauer−Emmet−Teller (BET) surface area and porosity estimates of Barrett−Joyner−Halenda (BJH) were determined at 77 K by nitrogen adsorption−desorption isotherms and are shown in Figure 2E,F, respectively. The PEG-PCL-PEG triblock copolymer surface area was found to be 0.8227 m\(^2\)/g, and its pore volume was found to be 0.03866 cm\(^3\)/g.

Different characterization methods verified the development of the conjugates. The presence of hydrophilic blocks and hydrophobic blocks confirmed the existence of amphiphilic polymers with unique molecular constructions. Outcomes of the findings (FTIR, NMR, SEM, BET, and BJH) are in line with one another.

2.2. Characterization of Triblock PEG-PCL-PEG Copolymer Conjugated Vancomycin Nanoparticles. 2.2.1. Morphology, Particle Size, Surface Charge, and Surface Area Investigations. In this experimental research work, we have developed VCM-PEG-PCL-PEG conjugated polymeric nanoparticles, and they were characterized by different techniques as shown in Figure 3. SEM was used to characterize the morphology of the VCM-PEG-PCL-PEG conjugate. Image of the SEM revealed that particle was of pyramid shape with smooth surface and edge lengths 200 nm in size as shown in Figure 3A. Xie et al. revealed in their study that nanotriangles exhibited the greatest cellular uptake as compared to other nanoparticles.13

The VCM-PEG-PCL-PEG conjugate’s polydispersity index (PDI) is displayed in Figure 3B. The average particle size of the VCM-PEG-PCL-PEG conjugate came out to be 78.59 nm with the PDI within an acceptable range of 0.279 ± 0.015. Further, it was also observed that the nanoparticles were randomly distributed throughout the surface and the distance between the particles was not uniform.

The zeta potential of the VCM-PEG-PCL-PEG conjugate, which is indicative of the surface charge of the particles, was −19 ± 5, as shown in Figure 3C.

A nitrogen adsorption−desorption isotherm was used for the measurements of the surface area at 77 K and is presented in Figure 3D. The BET surface area of synthesized and lyophilized nanoparticles was found to be 1.1818 m\(^2\)/g. We observed a major change in the surface area of the synthesized polymer (Figure 2E) and VCM-PEG-PCL-PEG conjugate (Figure 3D), which can be hypothesized because of sonication and homogenization techniques used in the preparation of formulation. The larger surface area gives rise to a more considerable drug flux per unit volume.

2.2.2. Thermal Properties and FTIR Spectroscopy Analysis. DSC is an appropriate technique for analysis of purity and
The determination of polymorphic forms and melting point of a sample. An overview of DSC thermograms of PEG, PCL, vancomycin, and lyophilized formulation is shown in Figure 4A. The characteristic peak of PEG and PCL contained characteristic peaks at 56.28 and 68.95 °C. The peak of vancomycin was found at 106.61 °C. However, in the case of final formulation, we observed characteristic peaks at 54.30 and 60.92 °C. This observation supported the assumption that the polymer formed a matrix-type semisolid system. The comparatively high melting point of the final formulation showed their consistency to remain semisolid at ordinary temperatures for the storage and use of pharmaceutical formulations.

The presence of vancomycin in the VCM-PEG-PCL-PEG conjugate and its potential interactions with excipients with loaded drugs have been investigated with FTIR spectroscopy. FTIR analysis was carried out to verify the sample’s functional groups, as shown in Figure 4B–E. Phenolic OH at 3200 cm⁻¹, aromatic C=C stretching at 1650 cm⁻¹, and C=O stretching at 1400 cm⁻¹ were reported in the FTIR spectra of vancomycin. The peaks at 2869 and 2950 cm⁻¹ are due to the C−H stretching. C=O stretching corresponded to absorption at 933 and 1247 cm⁻¹. Poly(ethylene glycol) is allocated between 1750 and 1765 cm⁻¹ to C=O, 1090−1300 cm⁻¹ to C−C and C−O, and 1085−1150 cm⁻¹. The distinctive peaks for VCM have shown greater intensity after the conjugate formation between vancomycin-PEG-PCL-PEG. The peaks ranging from 2800 to 3100 cm⁻¹ belong to the C−H stretching bands. The peak at the 1650−1800 cm⁻¹ range is due to the C=O stretching vibration group. The peak between 1350 and 900 cm⁻¹ is the C−O stretching vibration mode. The peaks within 2950−3000 cm⁻¹ are allocated to the CH₂ and CH₃ stretching vibrations. The peak at 1763 cm⁻¹ corresponds to the ester vibration of the C=O. The peaks observed with excellent intensity showed similar special peaks for the nanoparticles’ spectrum. The alterations and changes in IR peaks give confirmation of conjugate formation between PEG-PCL-PEG and vancomycin and further vancomycin encapsulation within it.

2.2.3. Determination of Encapsulation Efficiency. UV-visible spectroscopy analyzed the encapsulation of the drug molecules, and the outcomes were indicated as encapsulation effectiveness (in %). The maximum encapsulation effectiveness of VCM in VCM-PCL-PEG-PCL conjugates was found to be high, that is, 77.58% at 6.25 mg/mL. The higher percentage encapsulation effectiveness of VCM shows a covalent association between VCM and PEG-PCL-PEG and ionic loading of VCM. The excellent encapsulation shows a reliable sustainable directed vancomycin drug delivery with minimal chances of drug leakage and low toxicities.

2.2.4. In Vitro Drug Release. To investigate the influence of chemical and biochemical factors on the release of VCM from the VCM-PCL-PEG-PCL conjugate, the release study was conducted at pH 7.4 on drug-loaded conjugates, as shown in Figure 5. The release patterns were accomplished by the VCM release proportion with respect to the total amount of vancomycin encapsulated. The release of free VCM was analyzed in order to verify that the diffusion of drug molecules through the membrane of dialysis during the release step was not a rate limitation phase. Free vancomycin was observed to be released rapidly and reached a peak of more than 90% of the total in the first 5 h. In the case of VCM-PEG-PCL-PEG conjugates, the pattern of the drug release of VCM had to be followed by initial burst release and sustained release after 10 h. The total VCM released over 10 h was 41%, and it may be mainly due to drug dissolution and distribution on or near the surface of nanoparticles. The complete vancomycin release after 48 h was 81%. This was aligned with the release pattern.
observed with the release pattern of 5-fluorouracil\textsuperscript{,14} The sustained release of vancomycin may be linked to the trapping of vancomycin in the core of the newly modified hybrid polymer. In the release of VCM from the core of the modified polymer, there are several procedures including polymer matrix allocation, release by polymer degradation, solubilization, and diffusion via microchannels that are in or created by erosion in the polymer matrix. It was evident from VCM’s drug release pattern from VCM-PEG-PCL-PEG conjugates that modification found its implication in modulating the encapsulated drug release profile.

2.2.5. Molecular Dynamics Simulations. To understand the binding between VCM and the PEG-PCL-PEG polymer, we have calculated the time evolution of center of mass (COM) distance and interaction energies and its components between these molecules. The COM distance (Figure 6A) between these molecules revealed that these molecules interacted three times during the 100 ns simulation time (Figure 7). The first time these molecules interacted was at \( \sim 28 \) ns, and they remained bound at \( \sim 12 \) ns and separated at \( \sim 40 \) ns. The second time these molecules interacted was at \( \sim 62 \) ns, and they remained bound at \( \sim 15 \) ns before getting separated at around \( \sim 77 \) ns. The third time these molecules interacted was at \( \sim 91 \) ns, and they remained bound until the end of the simulation time. The time evolution of interaction energy (\( \Delta E_{\text{total}} \)) and its components (\( \Delta E_{\text{VdW}} \) and \( \Delta E_{\text{Elec}} \)) between these molecules revealed that \( \Delta E_{\text{VdW}} \) energy (Figure 6B, green line) played a major role in the interaction between these molecules followed by \( \Delta E_{\text{Elec}} \) (Figure 6B, red line) in all the three binding events during the simulations. Overall, this data suggested that these molecules can bind spontaneously, and van der Waals (VdW) interaction played a crucial role in binding between these molecules.

2.2.6. In Vitro Hemolysis. The hemolysis proportion of VCM-PEG-PCL-PEG conjugates was evaluated using fresh sheep blood at different concentrations, as shown in Figure 8. As for VCM-PEG-PCL-PEG conjugate solutions up to 250 mg/mL, the hemolysis rates were very less (25%), indicating their excellent blood compatibility and potential for intravenous injection. Hemolysis is very low due to treatment and indicates that drug delivery is hemocompatible.

2.2.7. In Vitro Antibacterial Activity. The minimum inhibitory concentration (MIC) of VCM-PEG-PCL-PEG conjugates was determined based on the result of in vitro antibacterial activity of comparative results of bare VCM, VCM-PEG-PCL-PEG conjugates, and PEG-PCL-PEG. The 96-well broth dilution technology was utilized. It confirms and compares the potency and improvement of VCM when it makes conjugates with VCM-PEG-PCL-PEG conjugates against MRSA as given in Table 1. Even at the highest concentration, PEG-PCL-PEG alone did not indicate any
Table 1. In Vitro Antibacterial Activity of the VCM-PEG-PCL-PEG Conjugates

| Compound                | MIC (μg/mL) | 24 h | 48 h | 72 h |
|-------------------------|-------------|------|------|------|
| PEG-PCL-PEG             | NA          | NA   | NA   | NA   |
| bare VCM                | 8.2         | 8.2  | 8.2  | 8.2  |
| VCM-PEG-PCL-PEG         | 1.13        | 1.13 | 1.13 | 1.13 |

“NA, not applicable.

activity. After completion of 24 h of study, MIC values were calculated to be 8.2 and 1.13 μg/mL for bare VCM and VCM-PEG-PCL-PEG conjugates against MRSA at physiological pH (7.4), respectively. The results of MIC values give an indication that newly modified polymers with VCM showed best activity as compared to bare VCM. However, we could not clarify this outcome completely, and this may be due to the very slow release of the drug, which limits the quantity of drugs accessible to kill the bacteria owing to a retarded release. Thus, the PEG-PCL-PEG complexation of VCM did not adversely influence VCM’s antimicrobial behavior. Overall, results from our antimicrobial activity study suggest that VCM-PEG-PCL-PEG conjugates can open a new door to research, therapy, and management of MRSA.

2.2.8. In Vitro Cytotoxicity. The cytotoxicity of the prepared formulation was evaluated to establish and confirm the biocompatibility of drug and excipients by MTT assay, as shown in Figure 9. In vitro cytotoxicity experiments on chosen cell lines for VCM-loaded nanoparticles were performed to check if the released VCM is still pharmacologically effective or not. The biosafety of the VCM-PEG-PCL-PEG conjugates in an in vitro cell culture system was assessed using three cell line models: A549, HEK293, and HEP G2. The findings of the study indicated that cell viability ranged from 78.02 to 80% across all the selected cell lines. The outcomes indicated that cells could develop well and not be influenced by the newly synthesized VCM-PEG-PCL-PEG conjugates. The findings showed that the VCM-PEG-PCL-PEG conjugates had low toxicity and had excellent biocompatibility. The low toxicity and good biocompatibility of VCM-PEG-PCL-PEG conjugates show high potential application for in vivo antibacterial therapy.

2.2.9. Bactericidal Time-Kill Kinetics. The rate of microbial killing by bare VCM and VCM-PEG-PCL-PEG conjugates is represented in Figure 10. We selected five times dose of MIC of each treatment over a 24 h incubation period at 37 °C. The VCM-PEG-PCL-PEG conjugates showed a fast bactericidal impact with a 3-log decrease (99.9% clearance) within 10 h as compared to the bare VCM resulting in a bactericidal impact after 24 h. The killing kinetics of vancomycin is comparable to those reported in the literature. This could result in the rapid elimination of bacteria in the blood, thus decreasing the length of treatment and the amounts needed to accomplish effective therapy.

3. CONCLUSIONS

We developed a polymeric modification-based triblock polymer (PEG-PCL-PEG) by using two FDA-approved polymers PEG and PCL. We alter the polymeric behavior by modification in the route of synthesis and addition of reagents. Incorporation of VCM in PEG-PCL-PEG and conjugate formation between VCM-PEG-PCL-PEG showed a good encapsulation efficiency and sustained drug release. Hemolysis study and MTT cytotoxicity cell line study results conformed the biosafety and biocompatibility of the formulation. Results of in vitro antibacterial and bacterial kill kinetics revealed that prepared formulation was more potent against MRSA bacterial strains. Molecular dynamics (MD) simulations suggested that these molecules can bind spontaneously and VdW interaction played a crucial role in binding between VCM and PEG-PCL-PEG molecules. MD simulation results showed that the interaction is hydrophobic, which promotes a biphasic pattern of drug release and prevent drug leakage. Based on the results, we can conclude that this system (i) can offer a solution to the problem of antimicrobial resistance, (ii) is helpful in preventing rapid absorption into the bloodstream and its undesirable toxicities, and (iii) can provide specificity and selectivity toward MRSA. This proposed approach can offer the advantage of minimizing the limitations of the existing therapy and might open up a new avenue to develop effective therapy for treatment of MRSA.

4. EXPERIMENTAL SECTION

4.1. Chemicals, Reagents, and Instrumentation. VCM were purchased from Sinobright Import and Export Co. Ltd. (China). Poly(ethylene glycol) (MW = 2,000), poly(caprolactone) (MW = 14,000), and sodium dihydrogen orthophosphate dehydrate were purchased from Merck, South
Africa. Sodium phosphate dibasic dehydrate (Sigma-Aldrich, Germany), a dialysis tubing of MWCO 14,000 Da (Sigma-Aldrich, USA), and 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl-tetrazolium bromide (MTT) used in the cytotoxicity study were obtained from Merck Chemicals (Germany). Mueller-Hinton agar (MHA), Mueller-Hinton broth (MHB), and nutrient broth used for antibacterial testing were Biobal (South Africa) items. The bacterial culture used was MRSA (Rose-nbach ATCC BAA 1683). Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich, South Africa. Double distilled (DD) water was used throughout the experiment. All other chemicals and solvents were of analytical quality and were used without further purification.

The instruments used are as follows: Fourier transform infrared spectrometer (Bruker Alpha-P ATR FT-IR, Germany), ¹H NMR spectrometer (measured at 400 MHz on a Bruker-400 spectrometer using TMS as an internal standard and CDCl₃ as a solvent), differential scanning calorimeter (Shimadzu DSC-60, Japan), field emission scanning electron microscope (ZEISS Ultra Plus, Germany), BET analyzer (Micromeritics Tristar II 3020 2.00, USA), and UV spectrophotometer (UV-1800, Shimadzu, South Africa). Proton and carbon nuclear magnetic resonance (¹H NMR and ¹³C NMR) measurements were performed on a Bruker 400 and 600 UltraShield (United Kingdom) NMR spectrometer.

4.2. Preparation and Characterization of PEG-PCL-PEG. The PEG-PCL-PEG polymer was synthesized by the earlier reported method with slide variation in the procedure and addition of the catalyst.¹⁵ The ROS technique was used to prepare the PEG-PCL-PEG triblock copolymer. A solution of 0.01 mol of ε-caprolactone and 0.01 mol of poly(ethylene glycol) 2000 was first melted. After proper melting of both polymers, 1 mL of 0.05% (w/w) copper acetate under a dry nitrogen atmosphere was added to a reaction vessel, and the reaction temperature was raised to 130 °C and kept for 12 h. After 12 h, the reaction mixture was completed with the addition of 0.01 mol of hexamethylene disiocyanate (HMDI). The mixture solution was stirred for 6 h at 80 °C after a few minutes. The resulting copolymer was then cooked to room temperature after 1 h of degassing under vacuum. The recently obtained triblock copolymers were first dissolved in dichloromethane and reprecipitated from the filtrate using excess cold petroleum ether. Then, the mixture was filtered and vacuum-dried to a constant weight at room temperature. Before further purification, the purified copolymers were kept in airtight containers. FTIR analyses were conducted in ATR mode using the Perkin-Elmer Spectrum 1000 with an accuracy of ±0.0088 and 0.9992, respectively. UV spectrophotometry (Shimadzu UV 1601, Japan) at 280 nm was used to determine the encapsulation effectiveness using a pre-established calibration curve. Lyophilized nanoparticles were dissolved in phosphate-buffered solution (PBS; 0.01 M, pH 7.4). It was sonicated for 20 min, and after that, it was centrifuged at 1000 rpm for 10 min. Two hundred microliters of filtrate was taken off and diluted to 10 mL with the phosphate buffer solution, and the amount of encapsulated drug was estimated using UV spectrophotometry.¹⁷ The equation of correlation and linearity (r²) were y = 0.0318 × −0.0088 and 0.9992, respectively. The encapsulation efficiency percentage (% EE) was calculated based on eq 1.

4.3. Conjugation and Characterization of Vancomycin to the PEG-PCL-PEG Copolymer. Nanoparticles loaded with VCM were developed using the technique of nanoprecipitation. Ten milligrams of VCM and 66 mg of triblock copolymer PEG-PCL-PEG were dissolved in 4 mL of tetrahydrofuran (THF).¹⁶ This solution was rapidly introduced to 15 mL of deionized water. Then, it was sonicated for 10 min. After 10 min, it was homogenized to create an adequate VCM ligand, a copolymer conjugate, by an Ultra-Turrax homogenizer at 1000 rpm. Due to the formation of nanoparticles, the solution instantly became opalescent. The resulting conjugates were performed with two-step purification: In the first step, the solution was centrifuged at 10,000 rpm for 1 h. The resulting solution was filtered through a 5 μm pore size microfilter to remove non-incorporated drugs and copolymer aggregates. Subsequently, for further use, the dispersed solution was lyophilized. Similarly, nondrug-containing nanoparticles were prepared to omit the drug.

4.4. Material Characterization. 4.4.1. Morphology, Surface Area, Particle Size, and Surface Charge Investigations. The nanoparticles’ shape and morphology were examined using a scanning electron microscope equipped with the detector of EDS (Oxford X-max). BET was used in measuring the lyophilized powder’s specific surface area. The Malvern Zetasizer Nano ZS (Malvern Instruments GmbH) was used to determine the mean diameter, polydispersity index (PDI), and zeta potential (ZP) for dispersions of VCM-loaded PEG-PCL-PEG nanoparticles. All DLS measurements were done with a laser wavelength of 633.0 nm at 25 °C with a detection angle of 90 °C. ZP with a Zetasizer (Nano-ZS 90, Malvern Instruments Corp, UK) was used at 25 °C after diluting the dispersion to an appropriate volume with deionized water. All analyses were tripled, and the outcomes are three cycles on average.

4.4.2. Thermal Properties and FTIR Spectroscopy Analysis. A differential scanning calorimeter (DSC Q100 TA Instrument, Germany) was used to measure the thermotropic characteristics of polymers and the PEG-PCL-PEG nanoparticles with VCM loading. Approximately 3–5 mg of each sample was weighed into an aluminum pan and sealed hermetically, and the thermal properties were investigated at a heating rate of 5 °C/min in the range of 20–220 °C. The baselines were determined using an empty pan, and all the thermograms were baseline-corrected. The infrared (IR) spectra were acquired from a Perkin Elmer 100 FTIR spectrometer with a universal ATR sampling accessory.

4.5. Encapsulation Efficiency (% EE). UV spectrophotometry (Shimadzu UV 1601, Japan) at 280 nm was used to determine the encapsulation effectiveness using a pre-established calibration curve. Lyophilized nanoparticles were dissolved in phosphate-buffered solution (PBS; 0.01 M, pH 7.4). It was sonicated for 20 min, and after that, it was centrifuged at 1000 rpm for 10 min. Two hundred microliters of filtrate was taken off and diluted to 10 mL with the phosphate buffer solution, and the amount of encapsulated drug was estimated using UV spectrophotometry.¹⁷ The equation of correlation and linearity (r²) were y = 0.0318 × −0.0088 and 0.9992, respectively. The encapsulation efficiency percentage (% EE) was calculated based on eq 1.
\[(%\text{EE}) = \frac{\text{total amount of VCM} - \text{amount of VCM in supernatant}}{\text{total amount of VCM}} \times 100 \quad (1)\]

4.6. In Vitro Drug Release. In vitro vancomycin release from vancomycin-PEG-PCL-PEG conjugates was achieved using the PBS method of dialysis bags (pH 7.4) at 37 °C. Both free VCM and VCM-PEG-PCL-PEG conjugates (1 mL each) were packed individually into dialysis containers (MWCO 14,000 Da). The loaded tubings were tightly sealed and dialyzed against 40 mL of PBS at 37 ± 0.5 °C in a shaking incubator at 100 rpm. Three milliliters of both samples was taken from the dissolution media at a specified interval of time and replaced with an equal amount of fresh PBS in order to preserve a standardized quantity of volume and sink condition. The quantity of VCM present in the samples was evaluated at a wavelength of 280 nm by UV–vis spectrophotometry. The study was performed in triplicate.

4.7. Binding of VCM and PEG-PCL-PEG Polymer: Insight from Molecular Dynamics (MD) Simulations. To understand the binding between VCM and the PEG-PCL-PEG polymer, MD simulation was performed. The structures of VCM were taken from PDB ID 1QD8, and the PEG-PCL-PEG structure was constructed using ChemDraw.18 Charmm general force field parameters were used for both the molecules.19 In the present simulation, one molecule of VCM and one molecule of PEG-PCL-PEG polymer were used. The simulation system was solvated using the TIP3P water model and contained 6861 water molecules.20 The steepest descent algorithm was employed for energy minimization,21 and two short sequential equilibrations (10 ps each) were performed using canonical ensemble (NVT) and isobaric–isothermal ensemble (NPT), respectively. The production simulation was performed using the NPT ensemble for 100 ns at 310.15 K and 1 atm pressure. The velocity rescale coupling. The Parrinello–Rahman method23 was used for the pressure coupling. The particle mesh Ewald method was used for long-range electrostatic interactions, and the Verlet cutoff scheme was used for neighbor searching.22 A time step of 2 fs was used to integrate Newton’s equation of motion using the leap-frog integrator.25 The simulation was performed using the GROMACS simulation package.26 The center of mass (COM) distance between VCM and the polymer was calculated using the in-house Tcl script, and interaction energy was calculated using the g_mmpbsa tool,27 which has been previously used to calculate interaction binding energies.28,29

4.8. In Vitro Hemolysis Activity of Triblock PEG-PCL-PEG Copolymer Conjugated Vancomycin Nanoparticles. The biocompatibility of the nanoparticles was conducted through blood hemolysis testing, which was conducted with slide alteration in the method from the previous protocol indicated.30 We are thankful to Dr. Thirumula Govender’s lab at the University of KwaZulu-Natal, Westville, South Africa, for the sheep blood serum sample, which was used to carry out further studies. A sample of sheep serum (5 mL) was drawn from a covered bottle of ethylenediaminetetraacetic acid (EDTA) glass tube and used within an hour. At 1800 rpm for 10 min, centrifugation was performed to distinguish red blood cells (RBCs) from blood samples. Separate RBCs were washed three times with a PBS solution diluted with 0.9% saline solution to act as a negative control. In a round-bottom 96-well plate, 100 μL of samples containing the RBC suspension was treated with different concentrations of nanocarriers in the range of 0.0, 50, 100, 150, 200, and 250 μg/mL. Eventually, the plate was stirred gently and further incubated for 3 h at 37 °C. The obtained supernatant was determined by UV–vis spectrophotometry via a plate reader at 541 nm. The percentage of hemolysis was calculated by eq 2.

\[\text{hemolysis(%) = (At - Ac)/(Ax - Ac)} \quad (2)\]

where At is the absorbance of the treated supernatant, Ac is the absorbance of the negative control, and Ax is the absorbance of the positive control.

4.9. In Vitro Cytotoxicity. Using adenocarcinoma human alveolar basal epithelial cells (A549), embryonic kidney cells (HEK-293), and liver hepatocellular carcinoma (Hep G2) cell lines, the MTT assay was used to determine the cytotoxicity of the VCM-PEG-PCL-PEG conjugated nanoparticles. The cells were cultured and seeded in 96-well cell culture plates (Greiner CELLSTAR 96-well plates, Merck, South Africa) at a concentration of 2 × 10^4 cells/well. Different concentrations of VCM-PEG-PCL-PEG conjugated nanoparticles (20, 40, 60, 80, and 100 μg/mL) were introduced into the wells seeded with cells. Positive control wells (with the culture medium comprising only cells) and negative control wells (with the culture medium without cells) were also included. The sample was incubated 48 h, after which the sample-laden medium was substituted by 100 μL of fresh culture medium and 20 μL of MTT solution (5 mg/mL in PBS) in each well. An aliquot (85 μL) from the wells were removed, and 50 μL (DMSO) from each well was added and thoroughly mixed together with the pipette and incubated at 37 °C for 10 min. In the microplate reader, cell viability was determined at an absorbance of 540 nm (Spectrostar Nano, Germany) to determine the amount of viable cells. It is based on the selective ability of viable cells to reduce the tetrazolium component of MTT into purple-colored formazan crystals.31 The percentage cell viability was calculated using eq 3.

\[
\%\text{cell viability} = \frac{(A540 \text{ nm treated cells})}{(A540 \text{ nm untreated cells})} \times 100 \quad (3)
\]

4.10. In Vitro Antibacterial Activity. The minimum inhibitory level (MIC) of the drug-loaded with nanoparticles was measured using the 96-well plate technique recorded with minor alteration.5,32 MIC values were determined against methicillin-resistant Staphylococcus aureus Rosenbach ATCC BAA-1683 (MRSA) for powdered VCM and VCM loaded formulations (VCM-PEG-PCL-PEG conjugates). The cultures of bacteria were grown for 18 h in a shaking incubator set at 100 rpm in nutrient broth (Biolab, South Africa) at 37 °C. The bacterial cultures were adjusted with sterile distilled water to achieve a concentration equivalent to a 0.5 McFarland standard using a DEN-1B McFarland densitometer (Latvia). In order to achieve a final concentration of 5 × 10^4 colony-forming units (CFU)/mL, bacterial cultures were further diluted (1:150) with sterile distilled water. Stock solutions of VCM and VCM-PEG-PCL-PEG conjugates were prepared by dissolving in PBS by sonication and sterilized through a 0.2 μm syringe filter. Using 96-well plates, a serial dilution of VCM and VCM-PEG-PCL-PEG conjugates were prepared in Mueller-Hinton broth 2 (MHB). These were then further incubated with the diluted cultures in a shaking incubator set at 100 rpm at 37 °C for 18 h. After completion of incubation,
10 μL of both VCM and VCM-PEG-PCL-PEG conjugates was spotted onto the MHA plates and incubated for further 24 h at 37 °C. This procedure was repeated every day for 3 days, and all MIC studies were performed three times. A blank PEG-PCL-PEG conjugate formulation was utilized as a negative control, while powder VCM was used as a positive control.

4.11. Bactericidal Time-Kill Kinetics. Time-kill assays were conducted in accordance with the CLSI guideline M26-A. An overnight MRSA culture in MHB was diluted to 5 × 10^6 CFU/mL with phosphate buffer. Powder VCM and VCM-PEG-PCL-PEG conjugates were added at concentrations equivalent to five times of the MIC result.

To this bacterial broth, sterile water was added along with the test samples to serve as a negative control. For bacterial cell viability, it was continuously monitored for 24 h. Samples were removed at specific time intervals, serially diluted in PBS, and plated in triplicate on MHA plates. It was incubated overnight at 37 °C. After completion of incubation, the growth of bacteria was evaluated, and the colonies were counted on the plates. The colony-forming units (CFUs) were counted and converted to log_{10} values and plotted in a graph.

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Notes
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