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Development of biodegradable scaffolds loaded with vancomycin micropartricles for the treatment of osteomyelitis

Souvik chakraborty*, Gowda D V, Vishal Gupta N

Department of Pharmaceutics, JSS College of Pharmacy, JSS Academy of Higher Education and Research, JSS Medical Institutions Campus, Shri Shivarathreeshwara Nagara, Mysuru-570015, Karnataka, India

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ABSTRACT

In this present research work, the development of biodegradable scaffolds loaded with Vancomycin micropartricles was carried out for the treatment of Osteomyelitis. Characterization Vancomycin Loaded microparticles and evaluation of the microparticles loaded scaffolds and also to carry out In-vitro release studies. Vancomycin hydrochloride microparticles were prepared with the help of double emulsion method. HPMC and Polaxomer 407 has been taken as the main polymers for the preparation of the microparticles. Chitosan was taken as the major polymer for the preparation of scaffolds for its greater biocompatibility and biodegradability. The preparation was done with the help of the solvent casting method. The formulation was taken for further characterization and evaluation studies. Fourier-Transform Infrared Spectroscopy and Differential scanning calorimetry were carried out for the pure vancomycin drug, and the chitosan polymer X-ray diffraction was carried out to check the crystallinity of the prepared scaffolds. The particle size, zeta potential and polydispersity index for vancomycin loaded microparticles were found to be 577.0±102.5 nm, 1624 mv and 0.254. The maximum and sustained release rate of the drug was found to be 95.6±0.478, at 16^{th} Hr. By taking all the reports, a conclusion can be drawn that, the formulated VLM biodegradable scaffolds will show burst release at the initial time of administration, which is essential for the wound healing activity and will be sustained throughout the process of treatment of osteomyelitis.

*Corresponding Author

Name: Souvik chakraborty
Phone: +91 8095031565
Email: souvik93pharmacist@gmail.com

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INTRODUCTION

Osteomyelitis

Osteomyelitis (OM) is defined as an infection caused by the bone.Symptoms may include pain in a specific bone area with certain indications such as redness, fever, and weakness (Baur *et al.*, 2015). The disease might occur in the long bones of the arms and legs for the children, while in adults, it majorly occurs in feet, spine, and hips (Wang *et al.*, 2017).

Causes for the disease

OM mainly caused due to bacterial infection; generally, it is called as a fungal infection (Baur *et al.*, 2015). It also may occur due to spreading from the blood or surrounding tissue (Wang *et al.*, 2017). Diabetes, intravenous administration of drugs, prior removal of the spleen and trauma to a particular area are a major risk to develop OM. Diagnosis

of OM can be suspected typically based on symptoms (Muthukumar *et al.*, 2013). This is then supported by blood tests, medical imaging, or bone biopsy. Depiction of a healthy bone and osteomyelitis defected bone is shown in Figure 1.

Staphylococcus aureus is the bacterial organism which is responsible for the formation of osteomyelitis. Bloodstream-sourced osteomyelitis is also caused by the above microbial agent, which is most frequently seen in children. In infants, Group B streptococci (most common microorganism; *S. aureus*) (Raucci *et al.*, 2010) are seen, and *Escherichia coli* are commonly isolated. In children at the age group of 1 to 16 years, *S. aureus*, *S. pyogenes*, and *Haemophilus influenzae* are usually seen for the cause of osteomyelitis. Gram-negative bacterias, including enteric bacteria, are the significant pathogens which are the major source for the disease in the patients who are subjected to intravenous administration of the drug and also to the splenectomized patients.

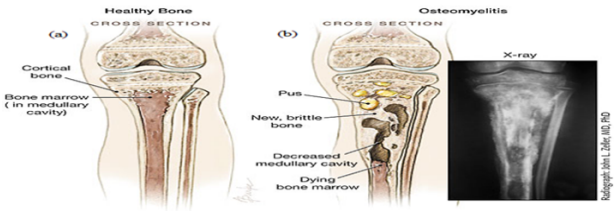

Figure 1: Depiction of two types of bones, (a) Healthy bone and (b) Osteomyelitis affected bone

Biodegradable scaffolds

Scaffolds are three-dimensional polymer matrix composed of drug implanted into biodegradable polymer fibers, used mainly for bone tissue regeneration. Biodegradable scaffolds are generally considered as indispensable elements for engineering living tissues as they are used as temporary templates with specific mechanical and biological properties similar to the native extracellular matrix (ECM). They allow modulating cell adhesion, invasion, proliferation, and differentiation; prior to the regeneration of biologically functional tissue or natural ECM. In the case of bone regeneration, a current challenge is to conceive new process strategies to fabricate composite or hybrid scaffolds able to provide three-dimensional templates and synthetic ECM environments (Wang *et al.*, 2007). In the case of bone, scaffold acts as the 3D support for tissue formation. It possesses peculiar morphological and functional properties which promote cell adhesion, differentiation and proliferation, and desirable mechanical integrity to maintain the predesigned tissue structure, non-cytotoxicity, and osteoconductivity.

Advantages of Biodegradable Scaffolds

1. The main purpose of scaffolds includes retention and deliverance of cells and biochemical factors for cell attachment and migration. (O'brien, 2011).
2. They also facilitate vital cells nutrient diffusion and help in the modification of the behavior of cell phase. (Berezin *et al.*, 2015). Biodegradable Scaffolds serve as templates in guiding the development of new tissues.
3. These are mainly used in bone and cartilage regeneration.

Microparticles

Microparticles are enucleated phospholipid cell fragments, of diameter between 100 and 1000 nm (Parida *et al.*, 2013a) and also usually quantified by flow cytometry and use of antibodies markers of this cell type. The drug is gradually released on erosion and diffusion from the particles. (Ilium *et al.*, 1988; Komatsu *et al.*, 1983) Microparticles can be further categorized into 2 types, namely, microcapsules and microspheres. Double-emulsion technique is appropriate for the efficient incorporation of water-soluble peptides, proteins, and other macromolecules. This method can be used with both synthetic and natural polymers. In this technique, polymers are dissolved in an organic solvent and emulsified into an aqueous drug solution to form a w/o emulsion. (Parida *et al.*, 2013b) The primary emulsion is subjected then to the homogenization before addition to the aqueous solution of the polyvinyl alcohol (PVA). This results in the formation of multiple (w/o/w) dispersions. The organic phase acts as a barrier between the two aqueous compartments, avoiding the diffusion of the active material to the external aqueous phase. The emulsion is then subjected to solvent removal either by solvent evaporation or by solvent extraction. Finally, the microspheres are collected by filtration and are washed with demineralized water. Microparticles provide accurate delivery of potent drugs, reduce the concentration of drug at sites other than the target tissue and serve as effective delivery systems for insoluble (or) sparingly water-soluble active agents. Microparticles increased the relative bioavailability of drugs and show taste-masking property. (Chawla and Bansal, 2003; Liu *et al.*, 2011) The microparticles have great potential in reducing the dosage frequency and toxicity of various drugs. The preparative methods are simple and can be administered into the body through a hypodermic needle.

Vancomycin for the treatment of OM

Vancomycin is a well-known antibiotic that is commonly used to treat several bacterial infections. Vancomycin is generally recommended for complicated infections of the joints and bones, bloodstream, skin, Staphylococcus aureus-induced meningitis and endocarditis. Vancomycin remains the most commonly used agent for osteomyelitis despite high treatment failure and recurrence rates. (Jeffres, 2017). Use of vancomycin frequently requires one or more daily infusions, based on renal function, to achieve trough concentrations of 15–20 mg/L required for osteomyelitis.

Importance for the development of Biodegradable Scaffolds

The major reason for the development of Biodegradable Scaffolds is to provide the antibiotic drug to be administered into the area of abrasion caused due to the disease, with the help of biodegradable polymer which will provide the drug to sweep through the bone without having any penetration at the process of treatment. The formation of scaffolds are done in such a way that, the active drug will help to deactivate or to remove the bacteria from site of the defect in the bone and the polymer will help the drug to provide sustained release and will be attached to the bone in such a way that, it will form as a bone glue at the site of abrasion and will fulfill the cavity forms due to the disease. Scaffolds are actually the polymer matrix, which will attach to the bone and will repair and protect the defected bone for a long term and the polymer matrix will get solidify according to the body temperature.

In this study, the scaffolds are prepared with the help of Chitosan derivative polymer, which is biodegradable and also bio compatible in nature and provides the sustained release of the antibiotic drug and help in the treatment of the osteomyelitis.

MATERIALS AND METHODS

Materials

Vancomycin hydrochloride drug and Chitosan polymer was purchased from Antila Lifesciences Pvt.Ltd., Ahmedabad, India. Hydroxy-proply-methyl cellulose(HPMC), methylene chloride, sodium chloride, ethanol, hydrochloric acid, methanol and glacial acetic acid was purchased from Merck Life science Pvt.Ltd., Mumbai, India. Polyethylene Glycol 600 (PEG) was purchased from Ranbaxy Laboratory Ltd., S.A.S Nagar, India. Sodium Alginate (Sodium Polymannuronate) was purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Poloxamer 407 (PLURONIC® F-127) was purchased from Sigma Aldrich, St. Louis, US.

Methods

Analytical Method

Standard calibration curve of Vancomycin hydrochloride in 0.1N HCl: Preparation for Calibration Curve

Calibration curve of Vancomycin hydrochloride was prepared by taking 100mg of Drug and it dissolved in 100 ml phosphate buffer solution (pH 7.4) (PBS). The prepared solution was considered as a stock solution (Vancomycin of 1mg/ml). The stock solution was diluted to prepare a different working solution of 20, 40, 60, 80, 100, 120and 140 μg/ml, by taking 2, 4, 6, 8, 10, 12 and 14 mL in 100ml volumetric flask and adjusted with 0.1NHCl. The UV-Vis spectroscopy (Shimadzu-1800, Japan) was performed to determine the absorbance for an individual sample at 272nm .0.1HCl solution was taken as blank. (Cevher *et al.*, 2006; Bartolotta *et al.*, 2005)

Formulation of Biodegradable Scaffolds

Preparation of Vancomycin hydrochloride loaded microparticles

In this study, Vancomycin loaded microparticles(VLM) were prepared by Double emulsion method as depicted in Figure 2. In the first part, primary solutions were prepared by mixing 0.05g of the drug (Vancomycin hydrochloride), and specific quantities of other ingredients such as HPMC, PEG, Methylene Chloride and Sodium Alginate in a Millipore water. With the help of a glass rod, the mixture is stirred manually till a viscous solution is formed. Homogenization was carried out at the rate of 10000-15000 rpm for a duration of 5 min, with the help of Homogenizer(PT 1600 E Polytron, USA). The secondary solutions were also prepared by mixing two solutions. One solution contains Poloxamer 407 and another containing NaCl, which is solubilized in 5ml of Millipore water each. The emulsion was homogenized for 15 min at the rate of 10000 rpm. The secondary emulsion was kept in a beaker and stirring of the solution was carried out with the help of Magnetic stirrer, with the help of a magnetic bead into the solution, within a temperature of 60 to 70 °C, at 250-300 rpm speed. After this process, the primary solution was added to the secondary solution with the help of a 5ml syringe. The syringe was dipped into the primary solution, and the desired volume was taken out and mixed drop by drop to the secondary solution, under stirring.

The microparticles were washed with Millipore water and kept for drying in a hot air oven at the temperature of 60°C. The micro-particles were stored at

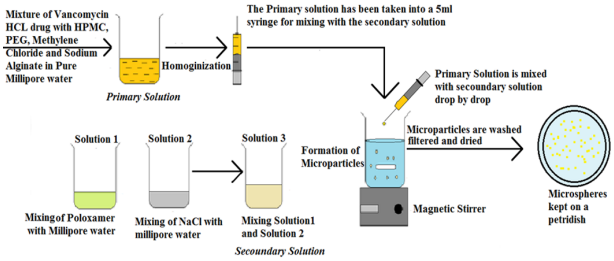

Figure 2: Preparation of VLM by Double Emulsion method

room temperature, avoiding any contamination, by keeping it into a cellophane pouch.

Different formulations were prepared for the preparation of the primary solution and secondary solution taking specific quantity The quantities for secondary are given in Tables 1, 2 and 3. (Dorati *et al.*, 2016).

Preparation of Biodegradable Scaffolds

The Biodegradable preparation scaffolds have been carried out by Solvent casting method, shown in Figure 3. Briefly, 50 mg, 100 mg and 150 mg of the VLM (F1 formulation)were weighed and dispersed in 10 ml of Millipore water with the help of magnetic stirrer at the temperature of 30°C, individually. 50mg of Chitosan was dissolved in 10 ml of glacial acetic acid and mixed with 20 ml of Millipore water. For the development of biodegradable scaffolds, VLM solutions (containing different concentration) were poured into the chitosan solution, separately and were kept for stirring on a magnetic stirrer at 350 rpm speed at the temperature of 60°C for 2 Hr. Development of chitosan biodegradable scaffolds containing a different ratio of VLM has been listed in Table 4.

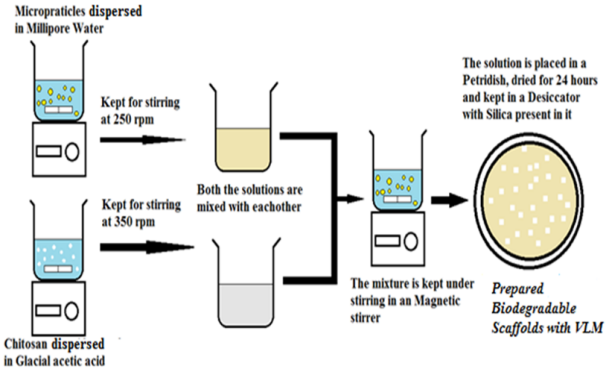

Figure 3: Preparation of VLM Biodegradable Scaffolds by Solvent Casting method

The above Figure 4 shows the final formulation of VLM Biodegradable Scaffolds, which is taken for further evaluation studies. (Murthy *et al.*, 2017).

Characterization and evaluation of

Table 2: Preparation of Biodegradable Scaffolds

Formulation	VLM (mg)	Chitosan (mg)
BS1	50	50
BS2	100	50
BS3	150	50

Figure 4: Prepared VLM Biodegradable scaffolds from Vancomycin loaded micro-particles, with Chitosan in glacial acetic acid

VLM/Biodegradable Scaffolds

Fourier transform infrared spectroscopy(FT-IR)

FT-IR spectroscopy(Shimadzu-1800, Japan) was used to determine compatibility between pure drug and the physical mixture. A physical mixture of the polymer and the drug was mixed with potassium bromide (KBr), which is anhydrous in nature, in the ratio of 1:4. Around 100mg is mixed properly by means of mortar and pestle to prepare a compressed transparent and clear KBr pellet by via KBr press(Techno Search Instruments, India) which is set to 15 tons of pressure. Individually each of the pellets was taken for scanning between the wave region of 4000 to 400 cm^{-1}. Compatibility of the polymer and the drug was investigated with measuring the IR spectra of both drug and the polymer. The physical mixture, as well as the polymer, detects the shift, appearance or disappearance of the peaks (Clarke, 1995).

Differential scanning calorimetry (DSC)

Differential scanning calorimeter(Shimadzu DSC-60, Japan) was used to perform thermal analysis. In order to confirm the compatibility of thermal behavior of naive drug and physical mixture of the drug & the polymer DSC is used. Around 5mg of the sample was weighed and kept in a non-hermetically sealed aluminum pans and crimped. Heating is done to the samples from 0^oC to 350^oC at the rate of 10^oCmin^{-1}.

Table 1: Preparation of Primary Solution and secondary solution

Pre-Formulation	HPMC (g)	PEG (g)	Methylene Chloride (ml)	Sodium Alginate (mg)	Poloxamer 407 (g)	NaCl (g)
F1	0.1	1.5	0.5	1	0.5	1
F2	0.2	1.5	0.5	1.5	1	1
F3	0.3	1.5	0.5	2	1.5	1
F4	0.4	1.5	0.5	2.5	2	1
F5	0.5	1.5	0.5	3	2.5	1

During the measurement, nitrogen will be continuously purged at the flow rate of 40ml min^{-1} (Dash *et al.*, 2019).

Particle size and polydispersity index analysis

The particle size and polydispersity index of formulations (F1, F2, F3, F4, and F5) was determined by Zeta Sizer (Malvern Instruments, UK). The samples for particle size determination were prepared by dispersing different VLM formulations in Millipore water, individually followed by stirring with the help of magnetic stirrer. The samples were weighed and dissolved in Millipore water, and size measurement was conducted at 25 °C.

X-Ray Diffraction (XRD)

The physical changes in the drug during the preparation of the formulation were evaluated by X-Ray diffraction(XRD) by using X-ray diffractometer (Bruker, AXS/8, Berlin, Germany). Using CuK (α radiation, 40 mA of current and 45 kV voltage), the study was carried out for the scaffolds, and they were analyzed in the diffraction angle of 2θ 10^o-80^o. Size taken was 0.02^o at 2θ. The analysis of samples were carried out at 4^o/min radiation scanning speed.

Scanning electron microscopy (SEM)

Formulated biodegradable scaffolds were placed on brass studs, and they were coated with gold with the help of ion coater. Surface morphology was observed with a scanning electron microscope (SEM)(JSM-IT300, Japan), working at an accelerating voltage of 5–20 kV (Varma *et al.*, 2014).

Estimation of Drug content

1g of VLM-Biodegradable Scaffold formulation was taken into 50ml of the volumetric flask, and it is diluted with 20ml of methanol. 5ml of the solution was diluted using 25ml of methanol, and 100% dilution was made. It was subjected to analysis by UV-visible spectrophotometer(Shimadzu-1800, Japan). Drug content was determined at 272 nm (Muthukumar *et al.*, 2013).

Percentage Yield

Determination of percentage yield of the VLM-biodegradable Scaffolds was carried out by weighing the scaffolds and the initial ingredients such as weight of the scaffolds, the weight of the pure drug and the weight of Chitosan polymer in Equation (1).

$$\%yield = \frac{wt.\ of\ Scaffolds}{(wt.\ of\ pure\ drug\ +\ wt.\ of\ C.P)} \times 100 \quad (1)$$

Porosity

Determination of porosity for the prepared formulated scaffolds was done with the means of the liquid displacement method. For easy penetration of drug via the pores of the samples, ethanol has been taken as the displacement liquid (Ramanathan *et al.*, 2014). Known weights of the sample was taken and added in a known volume of ethanol. The scaffolds were removed and added to the solvent repeatedly until there is a formation of bubbles. The total volume of ethanol after adding and removing the scaffolds samples was recorded. Percentage of porosity was obtained using Equation (2).

$$Porosity\ (\%) = (V1 - V3/V2 - V3) \times 100 \quad (2)$$

where *V1*= sample deep in a known volume of ethanol

V2= total volume ethanol-impregnated in sample

V3= volume remained in the beaker after removal of the sample

Tensile Strength

Dumb-bell shaped of the composite scaffolds ($100 \times 16mm^2$) were taken, and load-elongation measurement has been done at 5mm/min crosshead speed, at a temperature of 25^oCand relative humidity of 65%, correspondingly. Tensile testing machine (SIMPLETECH Instruments)was used for the measurement of the percentage of the elongation at the breaking point.

In vitro drug release study

Release of Vancomycin from the VLM biodegradable scaffolds was determined by introducing the scaffolds into a model setup of Franz-type diffusion cell at the temperature of 37±0.5. The formulations BS1, BS2, and BS3, as shown in Table 4, has been taken for the release study. PBS(pH 7.4)was kept in the receiver compartment of the apparatus. At specific intervals (0,1,2,4,6,8,12,16 and 24 Hr), 1ml of buffer solution was taken out from the receiver compartment, and the same quantity was added to maintain the volume. The whole process was carried out in a magnetic stirrer. The Vancomycin content was determined by measuring the absorbance via UV-Vis spectrophotometry technique at 272nm (Shimadzu UV 1800 ver. 2.43). With the help of Equation (3), the percentage release of vancomycin from the scaffolds was determined using was determined.

$$\%E = Qp/Qt \times 100 \quad (3)$$

Where E is the percentage of drug release from VLM, Qp the quantity of drug release and Qt the total quantity of Vancomycin loaded in the scaffold (Ramanathan *et al.*, 2014).

RESULTS AND DISCUSSION

Standard Calibration Curve

The standard plot data for Vancomycin drug in 0.1N HCl is shown in Table 5, and the Calibration curve is shown in Figure 5. The y value was found to be 0.006.

Table 3: Standard Plot data for the Vancomycin

Serial No.	Concentration (μm/ml)	Abs at 272nm
1	0	0
2	20	0.135±0.246
3	40	0.261±0.362
4	60	0.391±0.430
5	80	0.514±0.660
6	100	0.632±0.721
7	120	0.756±0.111
8	140	0.869±0.114

FT-IR Analysis

FT-IR of Vancomycin Pure Drug

Figures 6 and 7 shows the FTIR spectra of Vancomycin hydrochloride drug. The spectra show broadband at 3460.45 cm^{-1}, 3250.18 cm^{-1}, 3016.23 cm^{-1}, 1663.05 cm^{-1}. The data from the study co-relates with the peaks of standard

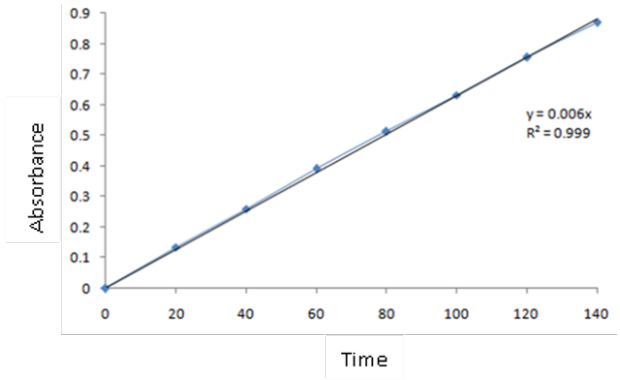

Figure 5: Standard Calibration curve of Vancomycin in 0.1 N HCl

Vancomycin hydrochloride drug, as mentioned in Table 6.

Table 4: FT-IR spectra data of pure Vancomycin hydrochloride drug

Functional group	Standard wave number (cm^{-1})	The peak observed in the Vancomycin Hydrochloride drug (cm^{-1})
N-H Stretching	3400	3460.45
O-H Stretching	3200	3250.18
C-H Stretching	3000	3016.23
C=O	1650	1663.05

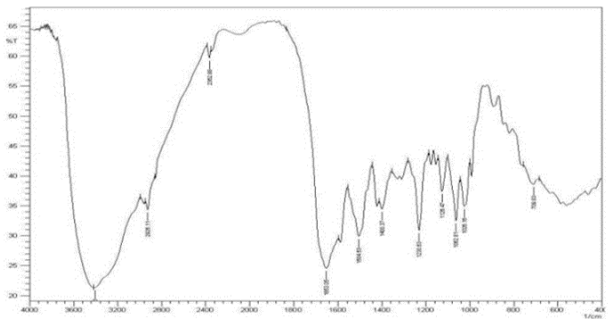

Figure 6: FTIR spectra of the Vancomycin hydrochloride drug

FT-IR of Vancomycin and the Chitosan polymer mixture

FTIR spectra of VLM Biodegradable Scaffolds is depicted in Figure 8. The spectra show broadband at 3504.22 cm^{-1}, 3200.22 cm^{-1}, 3000.19 cm^{-1} and 1799.55 cm^{-1}. The data from the study co-relates

with the peaks of standard Chitosan mixed with Vancomycin drug, as mentioned in Table 7.

Table 5: FT-IR spectra data of Vancomycin and Chitosan polymer mixture

Functional Group	Standard wave number (cm^{-1})	The peak observed in VLM Biodegradable Scaffolds (cm^{-1})
N-H Stretching	3500	3504.22
O-H Stretching	3200	3200.22
Ar-H Stretching	3000	3000.19
C=O Stretching	1750	1799.55

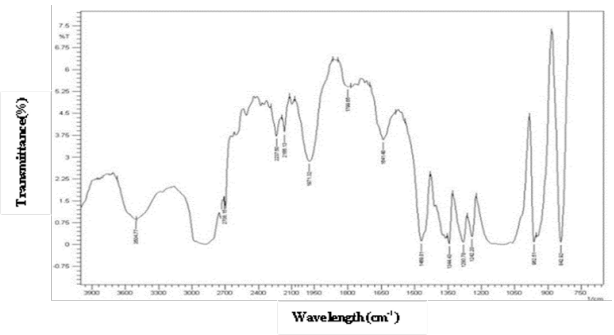

Figure 7: FTIR spectra of Vancomycin drug and Chitosan Polymer mixture

Differential Scanning Calorimetry (DSC)

DSC thermograms of the Vancomycin drug and the physical mixture of Vancomycin drug and the Chitosan Polymer are given in Figures 9 and 10 respectively.

The DSC graph shows that the optimum melting point of the Vancomycin drug is between 150-200°C, where in the below graph shows that, most of the vancomycin drug melted around 175°C.

In the DSC Graph, T_9 (glass transition) value should be found around 135°C, according to the literature. In Figures 10 and 11, the DSC graph shows shifts in the melting point of the Vancomycin Drug to 250°C, due to the presence of the Chitosan Polymer used.

Mean particle size and PDI of the VLM formulation (F1) was found 1624 nm and 0.245, correspondingly. Also, lower PDI of F1 revealed uniformity in particle distribution. Similarly, mean size and PDI of the other formulations (F2, F3, F4 and F5) were found as 853.9 nm with the PDI of 0.877, 1051 nm

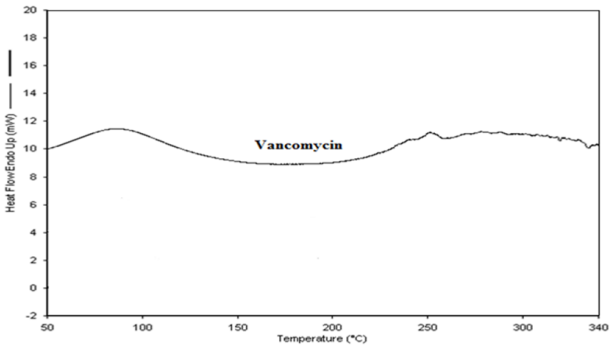

Figure 8: DSC curve of the Vancomycin Drug

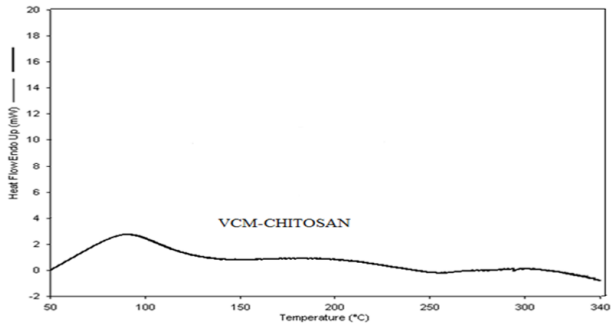

Figure 9: DSC curve of Vancomycin drug and the Chitosan Polymer mixture Particle Size and Polydispersity Index(PDI) analysis of VLM

with the PDI of 0.610, 587.3 nm with the PDI of 0.722 and 1501 nm with the PDI of 0.838, respectively shown in Table 8. The results of particles size of all the formulations have been observed that the F1 formulation was shown as higher particle size in micrometer range with lower PDI value compared to others.

Table 6: Cumulative results for the Particle size and PDI of the prepared VLM formulations

	Size(nm)	PDI
F1	1624	0.254
F2	853.9	0.877
F3	1051	0.610
F4	587.3	0.722
F5	1501	0.838

Drug Content

The prepared Vancomycin hydrochloride loaded microparticles(VLM) was analyzed for drug content, and the data is shown in Table 7. The obtained data of drug content formulations were ranging from 94.45±0.062 to 98.15±0.051. The highest drug content was found in Formulation F1 about 98.15 %, and the lowest drug content was found in Formulation F4 94.45%.

Percentage Yield

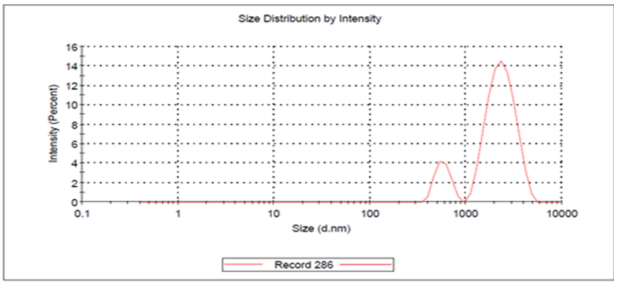

Figure 10: Particle Size analysis of the VLM formulation F1

Table 7: Drug Content data of the Prepared VLM

Formulations	% Drug content
F1	98.15±0.051
F2	95.72±0.045
F3	94.80±0.056
F4	94.45±0.062
F5	96.88±0.066

The prepared Vancomycin hydrochloride loaded microparticles(VLM) was analyzed for Percentage yield. The obtained data of Percentage yield of formulations were ranging from 52.23±0.042 to 68.45±0.056. The highest drug content was found in Formulation F1 about 68.45%, and the lowest drug content was found in Formulation F4 52.23%. The data is shown in Table 8.

Table 8: Percentage yield data of the prepared VLM

Formulations	% Yield
F1	68.45±0.056
F2	66.45±0.051
F3	62.18±0.048
F4	52.23±0.042
F5	59.48±0.054

Scanning Electron Microscopy (SEM)

SEM images of VLM biodegradable scaffolds (BS2) were observed at 10μm and 3μm magnificence, respectively shown in Figures 12 and 13. The images showed distinguish properties of the Vancomycin loaded formulation with the mixture of the Chitosan Polymer. The red highlighted circles in the images suggested that the Chitosan Polymer has coated over the VLM and the yellow highlighted circles showed the crystalline structure of the VLM Biodegradable Scaffolds.

PXRD analysis was performed to evaluate the crystallinity of the formulation shown in Figure 14. The PXRD result of biodegradable scaffold BS2 suggested that the crystallinity nature of the powder of scaffold of chitosan with the modification with VLM formulation F1. All major characteristics peaks appear the crystallinity in the VLM biodegradable formulation, while the sharp peak at 22.96 and 31.77 2θ showed the presence of the vancomycin in the formulation BS2.

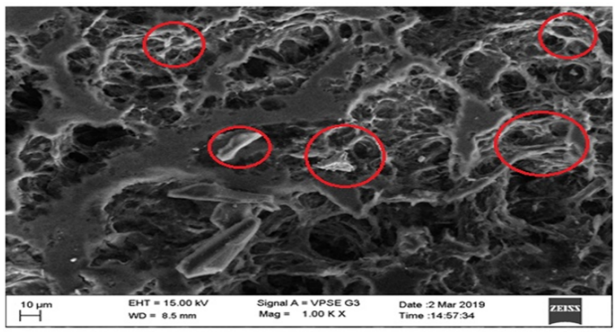

Figure 11: SEM image of VLM Biodegradable Scaffolds at a magnification of 10μm

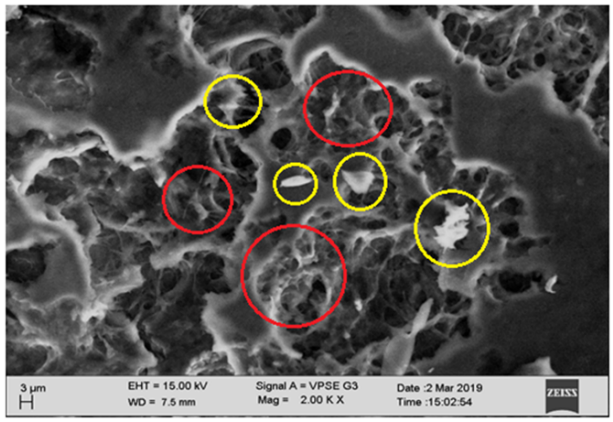

Figure 12: SEM image of VLM Biodegradable Scaffolds at a magnification of 3μm. Powder X-Ray Diffraction(PXRD)

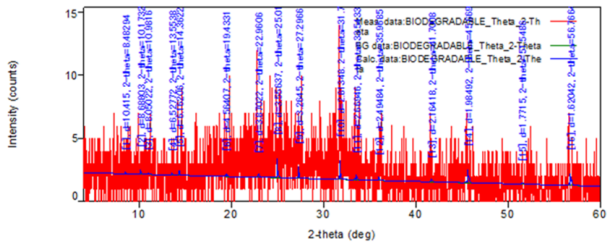

Figure 13: XRD of the prepared VLM Biodegradable Scaffolds

Porosity

For enhancing the collagen synthesis and migration of cells, which is required for the wound healing process, the oxygen permeability for the formulated scaffolds should be more. Porosity helps in exchanging of oxygen and nutrients. The porosity of the VLM Biodegradable scaffold was found to be 75%, by the

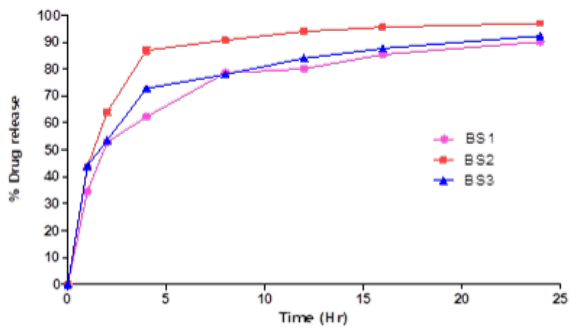

Figure 14: In vitro drug release from the VLM Biodegradable Scaffolds

liquid displacement method.

Tensile Strength

Scaffold having good mechanical property is considered by the physician to be taken care on the wound surface. The tensile strength of Vancomycin loaded microparticles biodegradable Scaffolds was found to be 7.85±0.58, and the elongation break was at 18.45±0.74.

In-vitro drug release study

The release study of drug Vancomycin from the Chitosan Biodegradable scaffolds is shown in Figure 14. The formulations BS1, BS2, and BS3 showed an initial burst release at 1 Hr. around 34.6%, 43.5 %, and 44.0 %, respectively. VLM scaffolds attain initial burst release, which caused because of non-entrapment of the drug within the microparticles. Formulation BS2 has been showed faster release as 97.1% at 24 Hr compare to the BS1 (90.2%) and BS3 (92.3%). High drug content must be required at the site of infection to inhibits the wound healing, and the BS2 formulation has been suggested that approximately 97.1 % drug release.

CONCLUSION

In this present study reveals that Biodegradable Scaffolds were successfully prepared by Solvent Casting Method. Chitosan has been taken as a suitable polymer for its higher biocompatibility and its biodegradability. Pre-formulation studies were carried out in order to establish the compatibility between the drug and the polymer by FT-IR spectroscopy. The study also revealed that the drug and the polymer were compatible. Developed VLM formulation F1 was highly effective with the mean particle size 1624 nm. Additionally, the electron micrographs (SEM) showed that the VLM formulation F1 was covered by the chitosan polymer as a biodegradable scaffold. Biodegradable scaffold BS2 has been suggested higher *in-vitro* drug release as 97.1% over the period of 24 Hr. The PXRD result of BS2 formulation exhibited crystalline nature of our developed final formulation. The drug content of the prepared biodegradable scaffolds was found to be 94.45 to 98.15%. The percentage yield of the prepared VLM was found between the range of 52.23 to 68.45. The porosity of the prepared VLM Biodegradable scaffold was found to be 75%. The tensile strength of VLM biodegradable Scaffolds was found to be 7.85, and the elongation break was at 18.45. Hence, it can be concluded that the chitosan polymer biodegradable scaffold of vancomycin loaded microparticles could be an effective delivery system for the treatment of osteomyelitis.

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