Preparation and evaluation of vancomycin polyelectrolyte complex nanoparticles

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Abstract

Background/Objectives: Nanoparticulate drug delivery has been a considerable research interest in the area of drug delivery using particulate delivery systems as carriers for bioactive agents. They show a promising approach to obtain desirable drug properties by altering the biopharmaceutical and pharmacokinetic properties of the molecule. Vancomycin is glycopeptide antibiotic with poor oral absorption.

Methods/Statistical analysis: In the present research work vancomycin nanoparticles were prepared by polyelectrolyte Complexation technique using two pairs of oppositely charged natural polymers neem gum and chitosan as one pair and hupu gum and chitosan as another pair. The vancomycin nanoparticles were characterized by using Scanning electron microscopy (SEM), particle size distribution by Zeta sizer. The nanoparticles were evaluated for their efficiency in drug diffusion, in vitro antimicrobial activity.

Results: Polyelectrolyte nanoparticles of vancomycin more effective when compared to pure drug.

Conclusion/Application: Among all formulations, the formulation in which was the drug to polymer ratio 2:3 (in the case of neem gum Nanoparticulate suspension) and 1:2 (in case of hupu gum precipitate Nanoparticles) were found to be effective.

Keywords: Nanoparticles, Vancomycin, Polyelectrolyte complexes, neem gum, hupu gum, chitosan.

1. Introduction

The recent past has witnessed the advancements of nano drug delivery technologies that can increase efficacy and safety, extend patent lives and provide competitive differentiation for biopharmaceuticals. The large size of most biopharmaceuticals, combined with their other molecular properties, lead to poor physical and chemical stability within the body and limited membrane permeability and severe toxicity when applied systematically [1]. Therefore researchers are developing a range of new delivery technologies and materials to enable these new drugs to be delivered intact to their target sites [2]. Nanoparticles are defined as particles with a diameter smaller than 100 nm and are increasingly used in different applications, majorly in drug carrier systems and to pass organ blood-brain barrier [3, 4]. The majority of drug products are in solids, so that nanoparticles are expected to have a broad impact on drug product development [5-8]. The fundamental properties for Nanoparticulate drug delivery systems are particle size, surface area, dispersion stability, magnetic and optical properties. As the particle size decreases, the number of molecules present on the particle surface increases and also the dissolution rate [5, 9]. They can show strong adhesion due to increased contact area for Vander Waals attraction [6]. Recently polyelectrolyte complexes have gained much attention in drug delivery because of their potential applications [10, 11]. Polyelectrolyte complexes (PECs) are the association complexes formed between oppositely charged particles (e.g. polymer-polymer, polymer-drug and polymer-drug-polymer) [12-14]. These are formed due to electrostatic interaction between oppositely charged polyions.

Vancomycin (VCM) is a glycopeptides antibiotic that is used for the treatment of infections caused by methicillin-resistant staphylococci [15, 16]. It acts by inhibiting bacterial cell wall synthesis at an earlier stage when compared to other beta-lacta antibiotic. It is usually given IV due to its minimal oral absorption [17]. It is a water soluble high molecular weight compound and hence poorly absorbable from the gastrointestinal tract [18]. The physicochemical properties that have been associated with poor membrane permeability of highly polar and macromolecular drugs are low octanol/aqueous partitioning, the presence of strongly charged functional groups, high molecular weight, a substantial number of hydrogen-bonding functional groups and high polar surface area [19, 20]. There are few reports showing the encapsulation of VCM in liposomes and microspheres may show a better bioavailability than the free drug [18-20]. Hence the present research work was planned to prepare and evaluate vancomycin nanoparticles by polyelectrolyte Complexation (PEC) technique using different natural polymers.

2. Materials and methods

Vancomycin was a gift sample from M/s. TherDose Pharma Private Limited, Hyderabad. Soya Lecithin is obtained from M/s. Hi-media laboratories, Mumbai. Chitosan is obtained from M/s. Qualigens fine chemicals, Mumbai. Glacial acetic acid and Nutrient agar are obtained from M/s. Merck specialities pvt. Ltd., Mumbai. Neem gum and Hupu gum are purchased from M/s. Girijan Corporation, Visakhapatnam. All other materials used in this study are of analytical grade.

2.1 Neem gum chitosan vancomycin (NCV) polyelectrolyte nanoparticles

Chitosan solution was prepared by dissolving accurately weighed quantity of chitosan in 2% v/v aqueous acetic acid solution to get 0.5% w/v solution. Neem gum (42.5 mg) was dissolved in 10 ml of 0.1N hydrochloric acid. Accurately weighed 100 mg of vancomycin was added to neem gum solution while mixing using mechanical stirrer (M/s. Remi, medium duty mechanical stirrer). 1.5 ml of chitosan solution (equivalent to 7.5 mg) was added to drug and neem gum mixture while stirring to obtain drug to polymer weight ratio of 1:0.5. The stirring continued for 30 minutes. The colloidal dispersion was collected and stored until further use. Different formulations were prepared as shown in Table 1 to obtain drug to polymer ratios of 1:1, 1:1.5 and 1:2.

Table 1: Formula of vancomycin polyelectrolyte nanoparticles

Ingredients	Quantity Required (mg)							
	F1	F2	F3	F4	F5	F6	F7	F8
Vancomycin	100	100	100	100	100	100	100	100
Chitosan	7.5	15	22.5	30	7.5	15	22.5	30
Neem Gum	42.5	85	127.5	170	-	-	-	-
Hupu gum	-	-	-	-	42.5	85	127.5	170
0.1N HCl	q.s.	q.s.	q.s.	q.s.	-	-	-	-
Phosphate buffer pH 7.4	-	-	-	-	q.s.	q.s.	q.s.	q.s.

2.2. Hupu gum chitosan vancomycin (HCV) polyelectrolyte nanoparticles

The preparation with Hupu gum (42.5 mg) is same as stated above (in section 2.1)

2.3. Characterization and Evaluation

The prepared formulation are characterized to ensure their predictable in vitro and in-vivo performances. The nanoparticle formulation produced by different polymer ratios may have different physicochemical characteristics. These differences do have an impact on their behavior in vivo and in vitro. The characterization parameters for the purpose of evaluation could be classified into three broad categories, which include physical, chemical and microbiological parameters. Some of the parameters characterized in product development are size distribution, surface topology, diffusion rate profile and minimum inhibitory concentration.

2.3.1. *Scanning electron Microscopy (SEM)*: SEM was conducted to characterize the surface morphology of the prepared formulation (colloidal solution and precipitate). One drop of formulation was mounted on a clear-glass

stub, air-dried, coated with Polaron E 5100 sputter coater (Polaron, Watford, United Kingdom), and visualized under a scanning electron microscope (Leo-435 VP; Leo, Cambridge, United Kingdom).

2.3.2. *Particle size determination*: The mean particle size was obtained by particle size analyzer (Malvern). The instrument measures the particle size based on the laser diffraction theory. The apparatus consists of a He-Ne laser beam of 632.8 nm focused with a minimum power of 5 mW using a Fourier lens to a point at the center of multielement detector and a sample holding unit (Su cell). The sample was stirred using a stirrer before determining the vesicle size. The nanosuspension was diluted about 100 times in the deionized water. Diluted nanosuspension was added to sample dispersion unit containing stirrer and stirred at high speed in order to reduce interparticles aggregation and laser beam was focused.

2.3.3. *Fourier Transform Infrared Spectroscopy (FTIR)*: To investigate any possible interaction between the drug and the utilized polymers under investigation FT-IR spectrophotometer method was used. The IR Spectra of pure drug (Vancomycin) and the combination of drug with polymers were carried out by using FT-IR spectrophotometer on Spectrum II Perkin Elmer. The pellets were prepared on KBr press. Sample preparation includes grinding a small quantity of the sample with a purified salt usually potassium bromide finely to remove scattering effects from large particles. The powder mixture was crushed in a mechanical die press to form a translucent pellet through which the beam of the spectrometer can pass. The pressed sample was carefully removed from the die and was placed in the FTIR sample holder. The IR spectrum was recorded from 4000 cm-1 to 400 cm-1. The resultant spectra were compared for any spectral changes

2.3.4. *Drug loading efficiency*: To evaluate the loading capability of nanoparticles 5 ml of the nanoparticle dispersion and 5 ml of distilled water is subjected for centrifugation at 18000 rpm using Remi R-8C BL centrifuges for 1 hour. The supernatant clear solution was collected separately. The free drug present in the supernatant was estimated after suitable dilution at 280 nm by using UV Visible Spectroscopy method. For total drug content the nanoparticle dispersion was shaken thoroughly and subjected to sonication for complete dissolution of drug and estimated after suitable dilution at 280 nm by using UV Visible Spectroscopy method.

$$\%\text{Encapsulation efficency} = \{1 - (\text{unencapsulated drug/Total drug})\} \times 100$$

2.3.5. *In vitro diffusion Studies*: In vitro diffusion studies were done by using Franz diffusion cell. The capacity of the receptor compartment was 20 ml. The area of the donor compartment exposed to receptor compartment was 1.41cm2. Dialysis membrane was soaked overnight in phosphate buffer pH 7 and 0.1N HCl to conduct diffusion studies for the nanopaticle precipitate and colloidal nanoparticle solution respectively. 10 ml of prepared formulations were taken and placed in the donor cell. Dialysis membrane was placed in between donor cell and receptor cell. In receptor cell approximately 20 ml of phosphate buffer pH 7 and 0.1N HCl was taken respectively until it touches the dialysis membrane. The temperature of the receptor phase was maintained at 37 ± 0.5 0C and the receptor compartment was stirred with magnetic stirrer to maintain homogeneous condition. The aliquots of 3 ml were withdrawn at the time interval of (30 min, 60 min, 2hrs, 3hrs, 4hrs, 5hrs, 6hrs, 7hrs, 8hrs and so on up to 48hrs) and replaced with equal volume of dissolution medium. The samples were analysed at 280nm in a UV-Visible spectrophotometer and amount of drug release at various time intervals were calculated.

2.3.6. *Microbiological assay*: Staphylococcus aureus *was chosen as test micro organism for microbiological assay of Vancomycin drug.*

2.3.7. *Preparation of culture*: The working culture was prepared from the stock culture by inoculating the slant into nutrient broth and incubated for 48hrs.

2.3.8. *Preparation of plates with nutrient agar*: The petri plates were prepared by pour plate technique with the working culture and nutrient agar. With help of sterilized cylinders cut holes on the agar plates and fill those cavities

with prepared standard and sample solution. Subject the petri plates to diffusion by keeping them at 4°C for 20 minutes and then they are transferred to incubator (without disturbing the plates) at a temperature of 37°C for 48 hours. The diameter for zone of inhibition was measured using antibiotic zone reader around the cavities.

3. Results and discussion

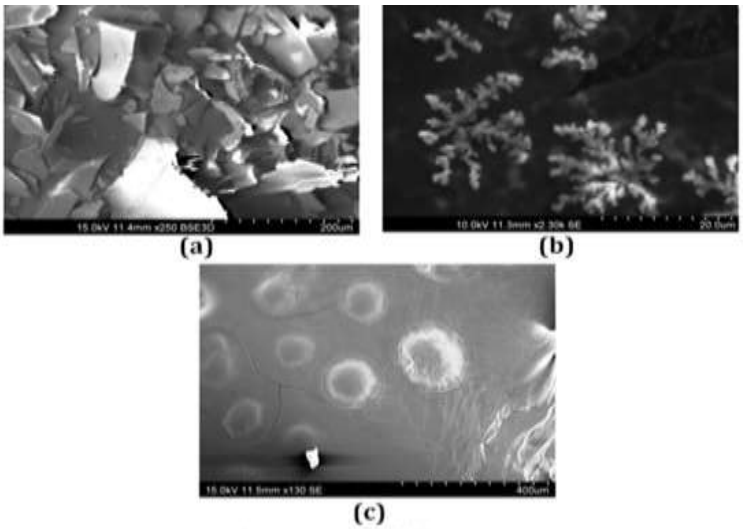
3.1. PEC Nanoparticles

The PEC nanoparticles were prepared by using Neem gum with chitosan and Hupu gum with chitosan. In case of Neem gum and chitosan, we obtained clear, colloidal dispersion of PEC complexes. In case of Hupu gum and chitosan, we obtained opaque colloidal dispersion of PEC complexes. The dispersions are uniform and were further characterized for various properties.

3.2. SEM observation

The surface morphology of colloidal solution containing nanoparticles formulations i.e. F-1, Precipitate containing Nanoparticles i.e. F-5 and Vancomycin pure drug were studied by SEM (Figures 1). Surface morphology of neem gum chitosan PEC nanoparticles showed branched structures where as the hupu gum and chitosan PEC nanoparticles showed spherical shape with encapsulated drug molecules.

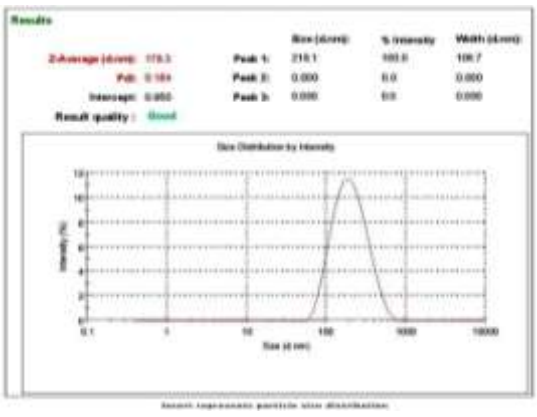
Figure 1: SEM analysis of (a) Vancomycin pure drug (b)Neem gum chitosan vancomycin nanoparticles (c) Hupu gum chitosan vancomycin nanoparticles



3.3.Particle size distribution analysis

The particle size distribution analysis was performed by using particle size analyzer (Malvern) and the results in Figure 2 showed that the average particle size of the nanoparticles for F1 was 178.3 d.nm. The particle size distribution curve indicated that they are in uniform size.

Figure 2: Zeta sizer report for the NCV nanoparticles



3.4.Fourier transform infrared radiation (FTIR) spectroscopy

The FTIR spectra for vancomycin and vancomycin nanoparticles precipitate are shown in Figure 3. Vancomycin FTIR (shown in table 2) show that the characteristic peaks were at 3387.38, 2935.1, 1632.81, 1093.52 and 687.81cm-1. The FTIR for prepared nanoparticle showed all major characteristic peaks at 3889.52 cm-1, 2926.28 cm-1, 1650.83 cm-1, 1020.1 cm-1 and 612.19 cm-1 with minor shift. Hence the results indicated that there is no chemical interaction between the selected polymer and the

Figure 3: FTIR of (a) Vancomycin pure drug (b) Hupu gum chitosan vancomycin (HCV) nanoparticles

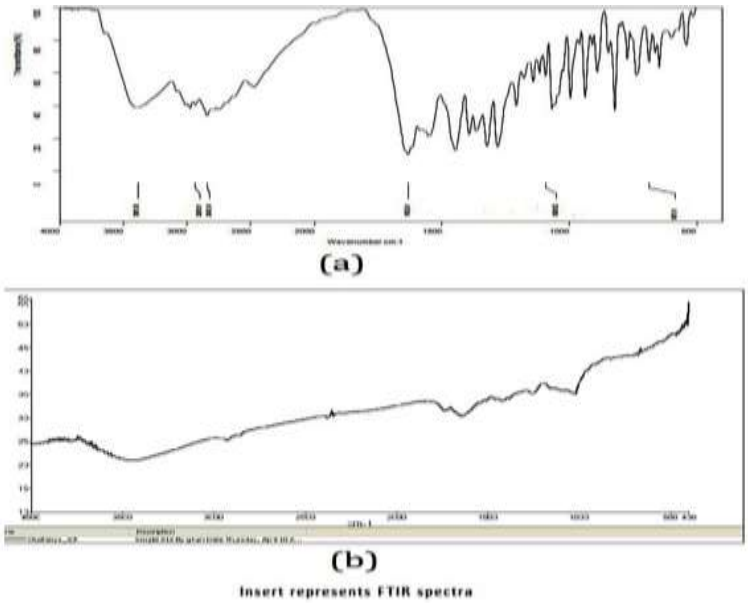


Table 2: FTIR bands of vancomycin and vancomycin nanoparticles

Functional Group	IR band of Vancomycin cm^{-1}	IR band of Vancomycin nanoparticles cm^{-1}
COOH	3387.38	3889.52
R-CH_2-CH_3	2935.51	2926.28
R-CO-NH_2	1632.81	1650.83
R-O-R	1093.52	1020.1
R-NH2	687.81	612.19
*as revealed by 'FTIR studies'		

3.5. Drug loading efficiency

The drug encapsulation was calculated among all formulations and results of Neem gum Chitosan Vancomycin (NCV) nanoparticle formulation showed drug content of 73.1%, 74.2, 78.5%, and 75.6% for formulations-1, 2, 3 and 4 respectively whereas among the Hupu gum Chitosan Vancomycin (HCV) nanoparticle formulations the % drug content was found to be 74.6%, 72.7%, 71.5% and 76.5% for formulation-5, 6, 7 and 8 respectively.

3.6. In vitro diffusion studies

I n vitro diffusion studies have been performed for all the prepared nanoparticles. The diffusion studies were also performed for pure drug for comparison (Figure 4, 5 and 6). The drug release for the pure drug was found to be 46.3±1.22%. The drug release for formulations F1-F8 was uniform and extended for a period of 48 hours. The percentage drug release was in the range of 55.96±1.26% to 74.85±1.15% for colloidal solution containing nanoparticles and in a range of 68.72±1.35% to 95.33±1.45% for colloidal precipitate containing nanoparticles. Among all the Colloidal solution formulations, F3 showed maximum drug release of 74.85±1.15% in 48 hours and among all the colloidal precipitate formulations; F8 showed the maximum drug release of 95.33±1.45%.

Figure 4: percentage drug release plats of F_1,F_2,F_3 and F_4

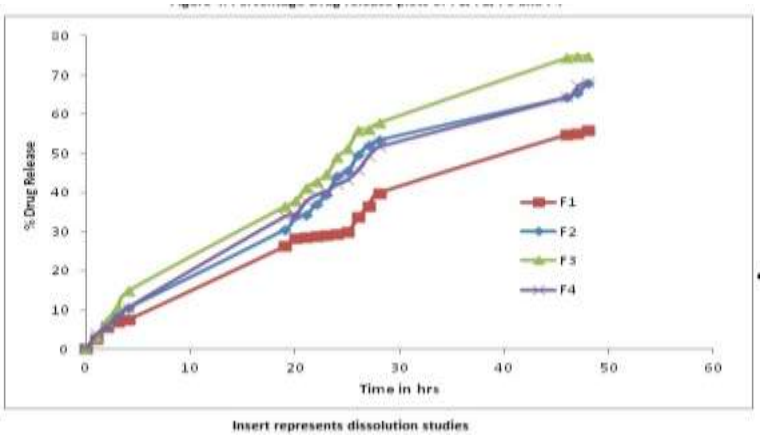


Figure 5: Percentage drug release plots of F_5,F_6,F_7 and F_8

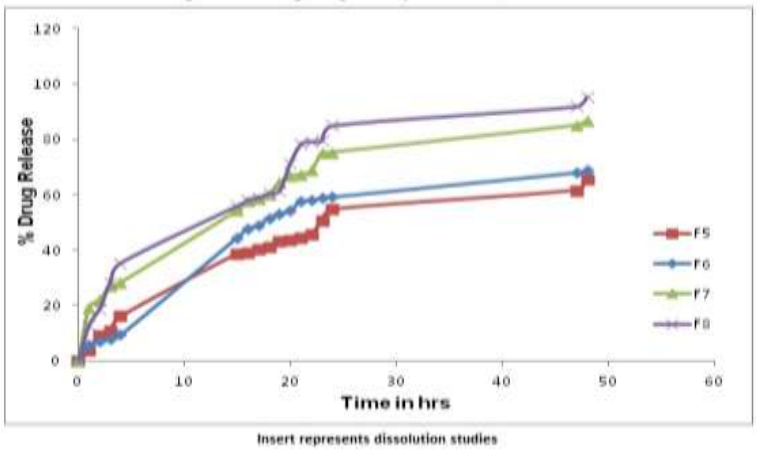
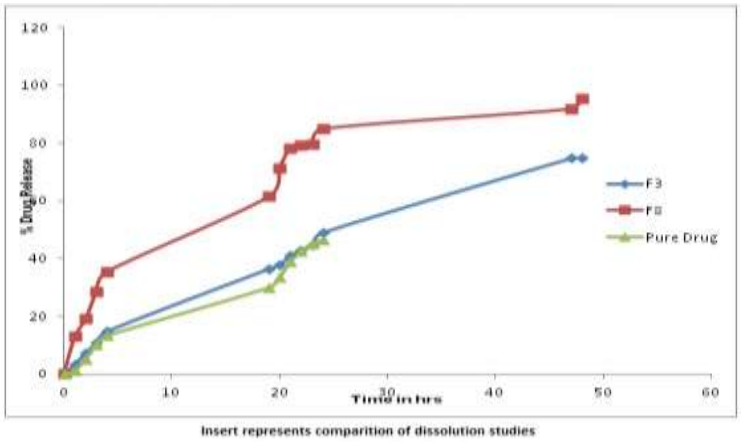


Figure 6: comparison of percentage drug release for F_3 and F_8 with pure drug

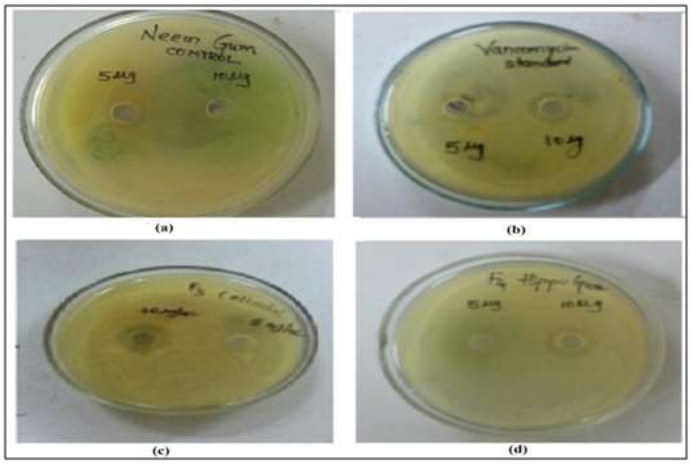


3.7.Microbiological assay

From the Table 3 and Figure 7, it is evident that the formulation F-3, F-4, F-8 at 10µg/ml concentration had 94.74% of zone of inhibition. The results also showed that there was a steady release of the drug and is capable of inhibiting the microorganism *Staphylococcus aureus* even at the end of 72 hours, whereas in the case of pure drug the microorganism is almost resistant by that time. Test of significance difference between pure drug with F3 and pure drug with F8 were calculated using t-test. The results (as shown in Table 4) suggested that calculated |t| for pure drug with F3 is 24.858 and

similarly pure drug with F8 is 21.596; both values are greater than tabulated value $|t_{0.05,4}|$ i.e. 2.132. This indicates there is significant difference between pure drug and F3, pure drug and F8. Whereas for F3 with F8 calculated |t| is 1.164 which is lower than the tabulated value $|t_{0.05,4}|$ i.e. 2.132. This indicates both F3 and F8 do not have any significant difference between them. Hence the results clearly indicated that the Vancomycin PEC nanoparticles are more efficient when compared with pure drug.

Figure 7: Zone of inhibition and MIC of (a) neem gum control (b) Vancomycin pure drug (c) Formulation F_3 (d) Formulation F_8



Insert represents Microbiological assay studies

Table 3: Minimum inhibitory concentration of pure vancomycin drug, control and formulation

Formulation	Concentration	Log Concentration	Inhibition diameter (mm)	% Inhibition
F_1	5	0.70	0	0
	10	1.00	10	52.63
F_2	5	0.70	0	0
	10	1.00	12	63.16
F_3	5	0.70	10	52.63
	10	1.00	18	94.74
F_4	5	0.70	14	73.68
	10	1.00	18	94.74
F_5	5	0.70	10	52.63
	10	1.00	14	73.68
F_6	5	0.70	12	63.16
	10	1.00	14	73.68
F_7	5	0.70	13	68.42
	10	1.00	16	84.21
F_8	5	0.70	14	73.68
	10	1.00	18	94.74
VANCOMYCIN	5	0.70	14	53.85
	10	1.00	19	73.08
	15	1.18	23	88.46
	20	1.30	26	100
NEEM GUM	5	0.69	0	0
	10	1.00	0	0
*as revealed by 'microbiological assay studies'				

Table 4: Test of Significance difference (t-test) between pure drug, F3 and F8

Comparison	Calculated t-Value at 10µg/ml	Tabulate, t value at 0.05,4	Inference
Pure drug with F3	24.858	2.132	Significantly different
Pure drug with F8	21.596	2.132	Significantly different
F8 with F3	1.164	2.132	Significantly not different
**as revealed by 'statistical analysis'*			

4. Conclusion

Vancomycin nanoparticles were prepared with different natural polymers – Neem gum and Hupu gum using polyelectrolyte complex formation. Vancomycin drug can be formulated as nanoparticulate formulation. Various polymers are used for entrapping the hydrophilic Vancomycin drug to form a Vancomycin nanoparticulate formulation with less particle size and improved efficiency in release and microbiological assay when compared to pure drug. Among the eight formulations, the formulation in which was the drug to polymer ratio is 2:3 (in the case of neem gum Nanoparticulate suspension) and 1:2 (in case of hupu gum precipitate Nanoparticles) was found to be effective.

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