# STUDY OF THE PHYSICO-CHEMICAL PROPERTIES OF VANCOMYCIN HYDROCHLORIDE FOR APPLICATIONS IN THE FORMULATION DEVELOPMENT

A Thesis submitted in partial fulfilment of the requirement for the degree of

Bachelor's of Technology

in

**Biotechnology**

By

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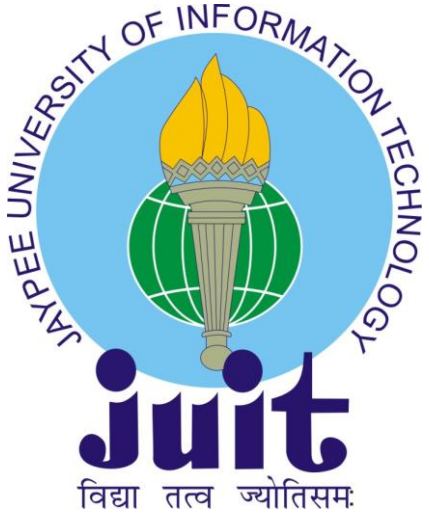
Under the guidance of

**Dr Gopal Singh Bisht**

(Associate Professor)

to




Department of Biotechnology and Bioinformatics

**JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY (HP)**

**MAY 2019**

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# DECLARATION

I hereby declare that the dissertation work which is being presented in the thesis, entitled "**Study of physico-chemical properties of Vancomycin Hydrochloride for applications in the formulation development**" for the partial fulfilment for the award of degree of Bachelor's of Technology in Biotechnology to Jaypee University of Information Technology, Solan (HP) is an authentic record of my own research work carried out during the academic year 2018-2019 under the guidance of Dr. Gopal Singh Bisht.

The matter embodied in this thesis is my original work and has not been submitted by me or any other person for the award of any degree in this or any other university/institute.

Place: Solan

Date: Harjas Saini

# CERTIFICATE

This is to certify that the work titled "**Study of physico-chemical properties of Vancomycin Hydrochloride for applications in the formulation development**" submitted by **Harjas Saini (151818)** for the partial fulfilment for the award of degree of Bachelor's of Technology in Biotechnology to Jaypee University of Information Technology, Solan (HP) is a bonafide research work under my guidance and supervision. No part of this thesis has been submitted for any other degree diploma in any university.

The assistance and help received during the course of investigation has been duly acknowledged.

Place: Solan

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I wouldn't have been able to go through this project without the help of any of those mentioned above. I express a deep sense of gratitude to all of them.

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# LIST OF SYMBOLS AND ACRONYMS

| | |
|---|---|
| R&D | Research and Development |
| ρ | Density |
| κ | Specific Conductivity |
| CMC | Critical Micelle Concentration |
| ϒ | Surface tension |
| η | Viscosity |
| μ | Ultrasonic Velocity of Sound |
| *x* | Specific gravity |
| MRSA | Methicillin-resistant *Staphylococcus aureus* |
| MIC | Minimum Inhibitory Concentration |
| RP-HPLC | Reverse-phase High Performance Liquid Chromatography |
| $\Delta H°_m$ | Standard enthalpy change |
| $\Delta G°_m$ | Standard Gibb's free energy change |
| $\Delta S°_m$ | Standard entropy change |
| $X_{CMC}$ | Molar fraction of CMC |
| $\phi_v$ | Apparent molar volume |
| $\phi_k$ | Apparent molar adiabatic compressibility |
| β | Adiabatic compressibility |

# ABSTRACT

The study of physico-chemical properties of vancomycin hydrochloride is an important parameter in the pre–formulation analysis of a drug. Pre-formulation study provides important information for formulation design or support the need for molecular modification. The main objective of pre-formulation is to develop a stable and an effective drug, with a safe dosage. Therefore properties like density, specific conductivity, surface tension, viscosity, velocity of sound and specific gravity are determined. Using these parameters further studies are carried out for the determination of critical micelle concentration (CMC). Other thermodynamic parameters are calculated from CMC like change in entropy, enthalpy and Gibb's free energy which indicated that formation of micelles is a favourable and exothermic in nature. Along with this, thermo-acoustic parameters are also determined that revealed that electrostatic interactions were favourable at lower concentrations and hydrophobic at higher concentrations. All this study may aid in development of the topical formulation of vancomycin hydrochloride.

## 1.1 ANTIBOTICS

Antibiotics originally used to be chemical substances synthesised by a microorganism that could kill or inhibit the growth of other microorganisms in a host organism, now the term can be applied for both naturally occurring and chemically synthesised substances and molecules in the laboratory. Most of these antitoxins are produced by either *Streptomyces* species (e.g. streptomycin, kanamycin, tetracycline, erythromycin, neomycin), *Bacillus* species (e.g. bacitracin, polymyxin), or fungi species (e.g. penicillin, cephalosporin). Antibiotics can either be broad-spectrum or narrow spectrum, which can act on both, Gram-positive and Gram-negative bacteria, or on either one of them respectively.

An ideal antibiotic should be non-toxic to the host, expressing selective toxicity. It should also be non-allergenic, have less probability of eliciting resistance, able to be maintained at therapeutic levels, soluble in body fluids, low cost and longer shelf life.

### 1.1.1 Modes of Action

There are different ways in which an antimicrobial drug can be used to kill foreign microorganisms. Some of them are as follows:

(i) **Inhibition of cell wall synthesis:** Antibiotics act upon vegetative cells causing osmotic pressure difference leading to cell death. Antimicrobial drugs that contain beta lactam rings inhibit the formation of peptidoglycan by irreversibly binding to the enzymes that crosslink NAM subunits. This causes interference of cross linkage of NAM subunits of bacterial cell wall subjecting microbes to environmental osmotic pressure. Antibiotic *vancomycin* interferes with alanine-alanine cross-link bridges between NAM subunits in most Gram-positive bacteria.

(ii) **Inhibition of protein synthesis:** Macrolides bind reversibly to the 50S subunit of the target microorganism, inhibiting protein elongation by enzyme peptidyltransferase. Aminoglycosides binds reversibly to the 30S subunit, blocking bacterial translation by distorting it in a way such that the anticodons of charged tRNAs cannot fail to align correctly with the codons of mRNA.

(iii) **Disruption of cytoplasmic membrane:** Polyene drugs result in pore formation on the plasma membrane of pathogens resulting in cell lysis. Azoles and Allylamines are antifungal drugs that inhibit the synthesis of ergosterol, a compound necessary for fungal membrane structure.

(iv) **Inhibition of DNA or RNA synthesis:** Antibiotics having narrow spectrum activity bind to the 30S subunit of bacterial ribosomes blocking the attachment of the 50S subunit to the initiation complex.

(v) **Inhibition of general metabolic pathway:** Some antibiotics interfere with the ETS of fungi and protozoa. Several heavy metals (e.g. As, Hg, Sb) inactivates enzymes by disrupting glucose uptake and tubulin polymerization. Para-aminobenzoic acid (PABA) is a precursor for synthesis of DNA and RNA. Sulfonamides and dapsone compete with PABA for the active site of the enzyme required in synthesising dihydrofolic acid, inhibiting the synthesis process. Another antibiotic binds to the enzyme involved in the conversion of dihydrofolic acid to tetrahydrofolic acid, which is a precursor for the synthesis of purines and pyrimidines.

(vi) **Inhibition of pathogen's attachment to or recognition of, host:** Antiviral agents targets host receptors interfering the binding of virus with the host cell. Some antibiotics even act to neutralize the acid released by phagolysosome within a macrophage reventing viral uncoating.

### 1.1.2 Antibiotic Resistance

Antibiotic resistance means that a specific antibiotic that formerly could kill a microorganism no longer has the potential to do so, as the microorganism develops resistance towards the antibiotic. Antibiotic resistance can also be transferred among bacteria on plasmids. Resistance maybe caused due to changes in selective permeability of cell walls and membranes, changes in sensitivity of affected enzymes, enzymatic alteration of the drug, or increased production of competitive substrate.

The mechanisms of antibiotic resistance include:

(i) Enzymatic degradation of drug
(ii) Alteration in target site of the drug
(iii) Altering the cell's metabolic pathway
(iv) Rapid ejection of the drug; pumping drug out of the cell
(v) Inducing changes in the cell membrane prevention of penetration of drug into cell

Reasons behind antibiotic resistance are their misuse against resistant mutants, for example:

(i) Using weakened or outdated antibiotics
(ii) Using antibiotics for common cold and other inappropriate conditions
(iii) Using antibiotics in animal feed
(iv) Failure to complete prescribed regiment
(v) Using someone's leftover prescription

There are ways in which the process of drug resistance can be slowed down. Some of them are listed below:

(i) Limiting the use of antibiotics to unavoidable cases, preventing indiscriminate prescribing and uncontrolled use
(ii) Using sufficient concentrations of a drug for a sufficient time to kill only the sensitive cells and inhibit all others long enough for the body's defence system to destroy them
(iii) Using combinations of antibiotics, promoting synergism, resulting in increased efficacy that exceeds the efficacy of either drug alone
(iv) Developing new variations of pre-existing drugs [1].

### 1.1.3 Routes of Administration

There are two major routes of drug administration – *enteral* and *parenteral* routes. These routes greatly affect the bioavailability of the drug depending on the number of biological barriers it needs to cross or its exposure time to pumping and changing other metabolic mechanisms.

**A. *Enteral Route***

This route of administration involves drug absorption via gastrointestinal tract including oral, gastric and rectal administration.

(i) *Oral administration* is the most frequently used route due to its simplicity, convenience and ease of administration which improve patient compliance. This route is effective for drugs with varying $pK_a$ as the gut pH varies considerably throughout the GI tract, and for drugs with moderate to high oral bioavailablity. Drugs delivered by this route must be acid stable and not irritating to the GI tract.

(ii) *Rectal administration* via suppositories produces a systemic effect and is useful in patients which cannot take the drug orally. In this route of administration, the drug is absorbed through the rectal mucosa. Because of the anatomy of the rectum's venous drainage, almost 50% of the dose bypasses portal circulation, advantageous in case of drugs with low oral bioavailability. Drug absorption through this route is incomplete and erratic, due to variability in drug dissociation from the suppository. This route is also used for local topical effects.

(iii) *Sublingual or buccal administration* – under the tongue and in between the gum and cheek respectively, is advantageous for drugs having low oral bioavailability as venous drainage from the mouth bypasses the liver. Drugs must be lipophilic and rapidly absorbed. Buccal formulations can even provide long lasting effects.

**B. *Parenteral Route***

This route of administration refers to any route of administration that does not involve drug absorption via GI tract. This route of administration can be chosen for drugs with low oral bioavailability, patients who cannot take the drug orally, need for an immediate effect, or the desire to control the rate of absorption and duration of effect.

(i) *Intravenous (IV) administration* is the most reliable method of drug delivery to systemic circulation in the body as it dodges most efflux pumps, absorption barriers and metabolic mechanisms. The bioavailability of the drug is 100% through this route as the drug is administered directly into vascular space. This route is one of the most preferred routes for administration to achieve therapeutically effective drug concentrations in the bloodstream rapidly. The

drug must be in aqueous solution or very fine suspensions to avoid the possibility of embolism and precipitate formation, in case of using combinations of drugs.

(ii) *Intramuscular (IM) administration* of drugs in aqueous solutions results in rapid drug absorption, which is dependent on blood muscle flow, which is in turn influenced by other factors that control the blood flow to the muscle. A slower and more constant absorption and effect of the drug can also be achieved by altering the drug vehicle.

(iii) *Subcutaneous (SC) administration* is used for drugs having low oral bioavailability. The rate of absorption can be steered by using varying formulations of the drug. This route should be avoided for solutions irritating the tissue as it might result in necrosis and sloughing of the skin.

(iv) *Transdermal administration* is delivering a drug through the skin. The drug should be highly lipophilic. These drugs can be applied as ointments or other special formulations. This route of administration shows slow absorption but is conducive to produce long-lasting effects.

(v) *Inhalational administration* is the route of drug administration through the lungs where pulmonary alveoli provide large surface area and minimal barriers for diffusion. Lungs also receive total cardiac output as blood flow, thus making the drug absorption through this route very rapid and complete. The intended effect of the drug could be systemic or local. The drug administered through this route should be non-irritant, and gaseous or fine aerosols.

(vi) *Topical administration* involves application of the drug at the site of application primarily to elicit local effects and to avoid systemic effects. The drug should be less lipophilic to minimise systemic absorption. Drugs administered to the eye, nasal mucosa or skin are examples of topical route of administration.

(vii) *Intrathecal administration* provides the drug access to the cerebrospinal fluid of the spinal cord by penetrating through the subarachnoid space. This approach is used to bypass the blood-brain barrier. This route of drug administration is used to produce spinal anaesthesia and in pain management [2].

## 1.2 PHYSICO-CHEMICAL PROPERTIES

Physico-chemical properties refer to the physical and chemical interactions involved in the formation of or changes in the structure of atoms and molecules, and their interaction affecting the drug kinetics. The study of physic-chemical properties of a drug substance in both, solid and liquid state, play a crucial role during pre formulation studies and its delivery [3]. Pre-formulation can be described as that part of R&D processes where we can characterize physical, chemical and mechanical properties and effects of a new drug substance and can utilise these properties to design a safe, stable and effective dosage form of the drug. Some of the parameters that were studied includes: density, specific conductance, viscosity, surface tension, velocity of sound and specific gravity, each playing a significant role in the pre formulation studies.

### 1.2.1 Density

Density is an essential attribute of any substance, including drugs. Density is defined as the measure that compares the amount of matter an object has to its volume. In simpler terms, it can be said that density equals to mass per unit volume. It is denoted by the symbol $\rho$ and its SI unit is kilograms per cubic metre ($kg/m^3$). This parameter forms a prerequisite in the determination of other physicochemical parameters that are an important part of the pre formulation studies. It affects the performance and the function of the drug. It also helps analysing the safe dosage of the drug, without causing any side effects, or minimal, if any.

### 1.2.2 Specific Conductivity

Specific conductivity can be defined as the measure to determine the ability of a material, in solid or aqueous form to conduct electricity. The formula used to calculate specific conductivity manually is $\kappa = (l/A)\, L$, where $l$/A is referred to as the conductivity cell constant ($l$ is the distance between the electrodes and $A$ is the area of cross section of the electrode) and $L$ is the conductivity (reciprocal of resistance). The SI unit of specific conductivity is Siemens/metre (S/m) and is denoted by the symbol $\kappa$. Determining the specific conductivity of a drug can be used in the penetration of electrically charged drug molecules into surface tissues by a simple method called *iontophoresis* [4]. This parameter is also used to determine the critical micelle concentration (CMC) of a drug, which is another important pre formulation parameter.

### 1.2.3 Surface Tension

Surface tension is an intricate property of fluids, which helps in the understanding of biochemical reactions taking place not just in the solution but on the surface and interface [5]. Surface tension can be defined as the forces of attraction exerted upon the surface molecules of a fluid by the molecules underneath that tends to draw the surface molecules into the bulk resulting in the fluid assuming the shape with minimum surface area. Minimum surface area is required by a fluid to stay in the lowest energy state. It is represented by the symbol ϒ. The SI unit of surface tension is Newton/metre (N/m). Having knowledge about surface tension enables the formulation of a stable drug.

### 1.2.4 Viscosity

Quite a few pharmaceutical products or drugs are viscous due to either the presence of various hydrophilic polymers or other agents that aid in enhancing viscosity [6]. Viscosity is a physical property of fluid, whose magnitude expresses the internal friction in a fluid. In simpler terms, it is the resistance of a fluid to flow. Mathematically it can be expressed as force per unit area resisting uniform flow. Viscosity is denoted by η and the SI unit of viscosity is Newton second per square metre ($Ns/m^2$) which is also equivalent to Pascal second (Pa-s). Other very commonly used unit for viscosity is Poise, named after a French physician Jean Louis Marie Poiseuille (1799 – 1869). 1 Pa-s is equivalent to 10 Poise. Temperature also plays a role in influencing viscosity. It is observed that at lower temperatures the viscosity increases exponentially [7-10]. Identifying the viscosity of a drug plays an important role in designing the drug and determining its route of administration.

### 1.2.5 Ultrasonic Velocity of Sound

The velocity of sound is the distance travelled per unit time by a sound wave as it propagates through a medium. It tells us about the flow properties of the drug. Knowledge regarding ultrasonic velocity and related thermo-acoustic parameters gives data regarding molecular interactions, which is further helpful in solution processing technology. It is represented by the symbol $\mu$ and its SI unit is metres per second (m/s). The ultrasonic velocity of sound in a fluid is related to the forces binding atoms or molecules [11].

### 1.2.6 Specific Gravity

Specific gravity is the ratio of the density of a substance to the density of a reference substance at a given temperature. It is denoted by *x* and is unit-less. It aids in the manufacturing processes and is a measure to check if the drug is correctly manufactured or not.

### 1.2.7 Micellization

Micelles are an aggregate of surfactant molecules dispersed in liquid colloids. The aggregate in an aqueous solution is formed by hydrophilic head (polar) regions in contact with the surrounding solvent, guiding and segregating the hydrophobic tail (non polar) regions in the micelle centre. The compounds that form micelles are typically ampiphilic in nature, indicating that they are not only soluble in protic solvents (like water) but also in aprotic solvents in the form of reverse micelles [12, 13]. The main forces of attraction result from the hydrophobic effects associated with non polar tails and the main repelling forces are a result of steric interactions and electrostatic interactions between polar heads [14]. Micelles help in the delivery of macromolecules by providing the sustained and controlled use of macromolecules, providing physical and chemical stability of the encapsulated molecules, improving drug pharmacokinetics, and improving drug bioavailability [15, 16]. Micelles are generally spherical shaped molecules ranging from 2 to 20 nm in size, depending on composition. Utilizing micelles as drug carriers has some advantages over other alternatives like soluble polymers and liposomes, as they help minimising drug loss and degradation, prevent harmful side effects and increase drug bioavailability [17, 18]. Solubilization can be defined as a spontaneous process of dissolving a substance by a reversible interaction of micelles with water to result in the formation of a thermodynamically stable isotropic solution with reduced thermodynamic activity of solubilised materials [19]. On plotting a graph between a compound that exhibits poor solubility versus surfactant concentration, it can be observed that the solubility is very low until the surfactant concentration reaches a critical point referred to as the CMC. At concentrations above CMC, solubility increases linearly with surfactant concentration, revealing that solubilisation is related to micellization [20].

Critical micelle concentration can therefore be defined as the minimum concentration at which micelle formation begins. CMC can be determined using conductivity [21], surface tension [22], capillary electrophoresis [23], scattering techniques [24], voltammetry [25],

fluorescence spectroscopy and UV-Vis [26, 27]. Keeping the above statements in perspective, it can be addressed that CMC of a surfactant is of vital significance in determining various other parameters that find a use in the pharmaceutical industry. Therefore micellar solutions can be used as one of the mediums to attain a desired functionality depending on temperature, pH, concentration and presence of other molecules.

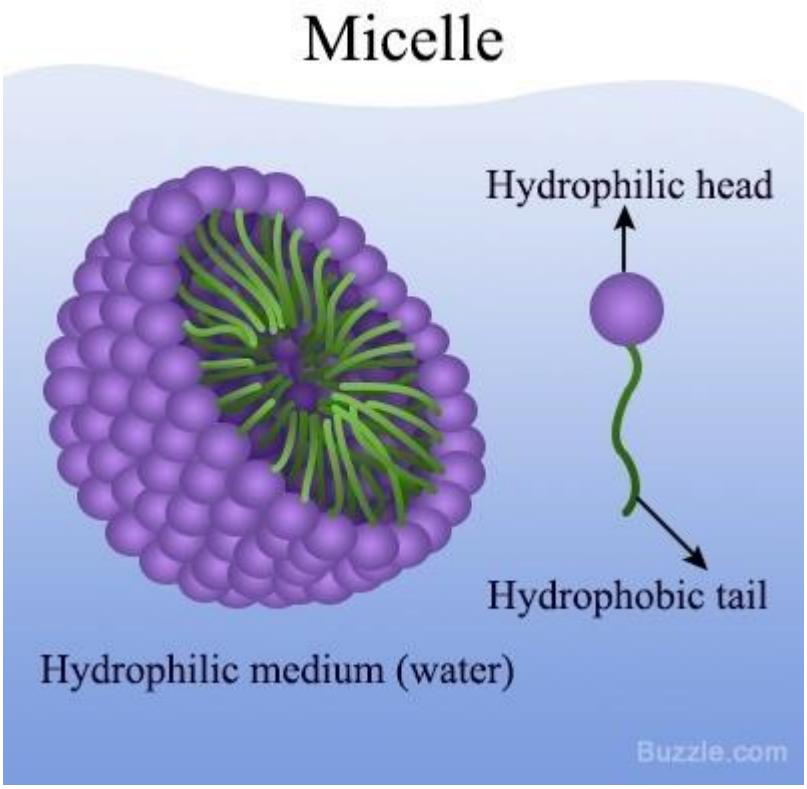


**Figure 1:** Representation of a micellar structure

### 1.2.8 Thermodynamic and Thermo-acoustic Parameters

#### *A. Thermodynamic Parameters*

Thermodynamic parameters include change in enthalpy (ΔH), change in Gibb's free energy (ΔG) and change in entropy (ΔS). These parameters are useful in telling the fate of a reaction and how scientists can achieve a desired rate of reaction by certain alterations or changing the concentrations.

(i) Enthalpy: A thermodynamic quantity which is equivalent to the total heat content of a system. Mathematically, H = U + PV (where H denotes enthalpy, U represents internal energy of the system and P, V represents pressure and volume respectively).

(ii) Gibb's free energy: A thermodynamic quantity which is equivalent to the enthalpy of a system or process minus the product of entropy and absolute temperature, i.e. $\Delta G = \Delta H - \Delta ST$ (where $\Delta S$ denotes change in entropy).

(iii) Entropy: A thermodynamic quantity which is regarded as the unavailability of a system's thermal energy for conversion into mechanical work. In other words, it is the degree of randomness of constituent atoms or molecules in a system.

***B. Thermo-acoustic Parameters***

Thermo-acoustics is the interactions between temperature, density and pressure variations of acoustic waves. The knowledge of thermo-acoustic parameters is significant in understanding the physico-chemical pattern and molecular arrangement in various fluids [11]. Apparent molar volume and thermal adiabatic compression are included under these parameters. These parameters are determined using other parameters like velocity of sound and viscosity. Apparent molar volume is the volume occupied by one mole of a liquid in a solution. This helps in studying the interactions between a drug and solvent and in identifying hydrophobic and hydrophilic interactions. Thermal adiabatic compression also referred to as apparent molar compressibility is used to measure the compactness of a system and helps determine the transport properties of drugs.

## 2. AIM

The aim of the project was to study the physico-chemical properties of the antibiotic vancomycin hydrochloride.

Physico-chemical properties are important parameters in the pre-formulation analysis of a drug. Pre-formulation study provides important information for formulation design or support the need for molecular modification. The main objective of pre-formulation is to develop a stable and an effective drug, with a safe dosage.

The specific objectives of our study were:

- To measure the following parameters:
    - (i) Density
    - (ii) Specific Conductivity
    - (iii) Surface Tension
    - (iv) Viscosity
    - (v) Ultrasonic Velocity of Sound
    - (vi) Specific Gravity
- To determine CMC from specific conductivity and surface tension
- Utilising these parameters to determine other thermodynamic and thermo-acoustic parameters namely:
    - (i) Change in enthalpy, change in Gibb's free energy and change in entropy
    - (ii) Apparent molar volume and thermal adiabatic compression.

These parameters can further be utilised in drug formulation of vancomycin hydrochloride.

## 3.1 DRUG REVIEW

**Generic name:** Vancomycin hydrochloride

**Class:** Glycopeptide antibiotic

**Molecular formula:** $C_{66}H_{75}Cl_2N_9O_{24}.HCl$

**Molecular weight:** 1485.723 g/mol

**Molecular structure:**

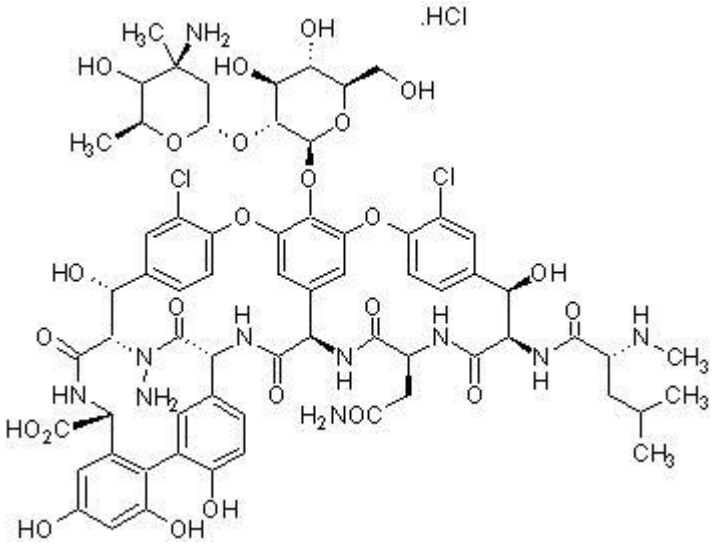


**Figure 2:** Molecular structure of vancomycin hydrochloride

**Description:** White crystalline structure

**Solubility:**

- Soluble in water at room temperature (>100mg/mL).
- Moderately soluble in dilute ethanol.
- Insoluble in higher alcohols, acetones and ether.

**Pharmacokinetic data:**

- Bioavailability: Negligible (by mouth)
- Metabolism: Excreted unchanged
- Elimination: 4 hours to 11 hours (in adults)
- Half-life: 6 days to 10 days (adults, impaired renal function)

- Excretion: Urine (IV), feces (oral)

**Uses:**

- Treatment of serious infections caused by MRSA
- When the treatment is non-responsive to metronidazole in Pseudomembranous colitis caused by *C. difficle*, vancomycin is administered orally to the patient.
- For treatment of Gram positive bacterial infections and patients allergic to beta-lactam antimicrobials.
- Treatment and prevention of endopthalmitis [28].

**Side effects:**

- Local pain
- Rashes
- Damage to kidney and nephrotoxicity
- Ototoxicity [29].

### 3.2 REVIEW OF LITERATURE

Extensive literature review was studied to gain more knowledge about the vancomycin and vancomycin hydrochloride and an insight into the work done by various scientists or researchers on the same, and its pre-formulation and formulations.

**Geraci J.E., Hermans P.E. (1953):** Vancomycin is a narrow spectrum antibiotic that possesses anti-staphylococcal properties. It was introduced in 1956 seeing its efficacy in opposition towards resistant Staphylococcus strains that produce penicillinase. Vancomycin proved to be a very effective alternative for penicillins and cephalosporins to treat Staphylococcal infections. Later vancomycin was used frequently for treating:

(i) MRSA infections

(ii) Streptococcal endocarditis in combination with aminoglycosides in patients that are intolerant towards penicillins or ampicillins

(iii) Prosthetic devices associated infections caused due to organisms with multiple antibiotic resistance

(iv) Antibiotic induced enterocolitis associated with *Clostridium difficle* [30].

**Griffith R.S. (1981):** Vancomycin was discovered to be produced by microorganisms present in the jungles of Borneo. It exhibited antistaphylococcal activity. The drug proved to be very beneficial during the golden plague of 1950s where hospitalized patients were infected because of antibiotic-resistant strains of *Staphylococcus aureus*. The administration of this newly discovered drug was found to be safe. Vancomycin is now also being used in patients undergoing renal dialysis for prophylaxis; to treat antibiotic-induced enterocolitis; in patients suffering from cancer for sterilization purposes of the intestinal tract in combination with an aminoglycoside [31].

**Bingen E. *et al.* 2006:** Vancomycin is used in treating infections owing to methicillin-resistant *Staphylococcus aureus* in both, community and nosocomial-aquired infections. It is a time-dependent or concentration-independent antibiotic. The therapeutic range was assessed through serum concentrations of 5-10 mg/l. The emergence of resistance can however be associated with prolonged exposure to the serum concentration that is closer to the MIC. Higher vancomycin concentrations of 15-20 mg/l can be used to treat severe Staphylococcus infections or in cases where there is poor vancomycin penetration. There are guidelines that also recommend using serum concentrations that are 5-10 times that of MIC, due to the great variability of vancomycin MIC(s) of susceptible Staphylococcus strains [32].

**Cunha B.A., Ristuccia A.M. (1983):** Vancomycin is bactericidal that interferes with the peptidoglycan synthesis of multiplying organisms. It is usually supplied in the hydrochloride salt form and is made available in 500 mg ampuls. The administration of vancomycin is usually intravenous or oral. The intravenous route of administration of vancomycin is done rather slowly (over 30-60 minutes) and in an adequate volume of about 100-250 ml of 5% dextrose injections. The usual adult dosage of the drug is either 500 mg every 6 hours or 1 g every 12 hours. Serum vancomycin kinetics was studied and could be best explained on the foundation of two- or three- open compartment model. The mean concentrations of vancomycin in the presence of pleural fluid, ascetic fluid, pericardial fluid, synovial fluid, bile and inflamed meninges are approximated to be 15% of serum concentrations. To prevent any infections that can be caused by Gram-positive cocci, vancomycin is best used prophylactically. Vancomycin serves as an ideal drug for prophylaxis used in prosthetic implant surgery due to its activity against MRSA and long serum half-life. This drug has also been used to prevent and treat patients of haemodialysis. It can also be used in association

with various other antibiotics to treat different bacterial infections. Vancomycin is considered to be one of the most potent antistaphylococcal drug available [33].

**Huvelle S. *et al.* 2016:** Preparation of oral solutions of vancomycin hydrochloride helps in improving time management and cost factor of drug delivery. This study aimed to explore long-term stability of the drug – brand Vancocin and generic Vancomycin, maintained at 5 ℃ ± 3 ℃. Five vials of each oral solution of concentration 1.25 g/100 mL of both Vancocin and Vancomycin were stored for 57 days. Concentrations were then measured by HPLC – diode array detection. pH determination and visual inspections by microscopy and spectrophotometry were determined during the storage period itself. Throughout the study period there was no change in colour or precipitation observed. The lower confidence limit of concentrations for these two solution samples remained superior to 90% of initial concentration as recommended by U.S. FDA for 57 day storage period for Vancocin (106.74%) and Vancomycin (102.73%). The solutions prepared either from the brand or the generic vancomycin hydrochloride were found to be chemically stable for over a month and could also be prepared in advance [34].

**Mohammed M.I. *et al.* 2016:** This study was aimed to evaluate the transdermal delivery of vancomycin hydrochloride by using the combination of ethosomes as encapsulating vesicles, and iontophoresis. Ethosomes were prepared keeping electrochemical stability in account. Anodal iontophoresis of positively charged vesicles and free drug solution along with cathodal iontophoresis of negatively charged vesicles were conducted. The effects of concentration of drug, density, ionic strength and current modes were studied. *In vivo* study was carried out by inducing mediastinitis in rats using MRSA as infected pathogen. One group of rats was treated by injecting the drug intramuscular whereas the other group received treatment of vancomycin through iontophoretic delivery of optimized ethosomal formulation, and then mean bacterial count was compared between the groups. Ethosomes revealed effective electrochemical stability, cathodal iontophoresis revealed maximum transdermal flux (550 µg/cm$^2$/h) when compared to free drug solutions and other ethosomal formulations, transdermal flux was reduced by changing mode of current from continuous to ON/OFF, by reducing current density and using normal saline as drug solvent. Flux on the other hand was potentiated by increasing drug concentration to 75 mg/mL from 25 mg/mL. Studies have shown that there was a significant difference of bactericidal count between treated and untreated groups, whereas there was no difference between intramuscular treatment of

vancomycin and its iontophoretic delivery encapsulated in ethosomal formula. The combination of iontophoresis and ethosomes has proven to be successful in transdermal delivery of vancomycin hydrochloride [35].

**Serri A**. ***et al.*** **2017:** A simple, rapid and selective Reverse phase – High Performance Liquid Chromatography (RP-HPLC) method was developed to determine vancomycin hydrochloride. Capital C8-Optimal column with mobile phase consisting of citrate buffer (pH 4), methanol and acetonitrile in the ratio of 85:5:10 by volume was used to achieve separation. An isocratic HPLC system with flow rate of 1 mL/min wasused to pump the mobile phase and analyte was quantified by measuring its peak areas at 280 nm. Cephalexin monohydrate was used as an internal standard (IS). The retention times for vancomycin hydrochloride and cephalexin were determined to be 4.30 and 7.50, respectively. The authenticity and reliability of the proposed HPLC protocol was affirmed with respect to ranges, linearity, specificity, precision, accuracy and detection limit. For concentration ranges of 1-100 µg/mL calibration curve was found to be linear with correlation coefficient of 0.9999. The proposed HPLC method proved to be selective and stability-indicating by the analyte's resolution from forced degradation products. The affirmed method was successfully applied to analyse vancomycin hydrochloride in pharmaceutically approved dosage forms [36].

**Lee C.K., Su W.D. (1999):** This research studied the partitioning behaviour of vancomycin in temperature-induced non-ionic surfactant two-phase system. N-Decyltetra (ethylene oxide) was used as the non-ionic surfactant. Inspite of vancomycin having partiality towards micelle-rich top phase under most experimental conditions, at pH 4, vancomycin preferred to stay in the micelle-poor bottom phase.D-Alanyl-D alanine modified cholesterol was employed as an affinity co-surfactant in the extraction system to increase the partition coefficient of vancomycin, which did increase significantly from 0.87 to 15.98. Vancomycin was found to be self-aggregating and could form a micelle. Its CMC was determined to be 0,4 mM at pH 4 and 1.2 mM at pH 7 as per the dye-binding method. This study also demonstrated the direct recovery of vancomycin from fermentation broth by utilising affinity-extraction technique [37].

## 4.1 MATERIALS

**Test substance:** Vancomycin hydrochloride (CAS No. 1404-939) was obtained from HIMEDIA Laboratories Pvt Ltd, India.

**Table 1:** Glasswares and other laboratory materials used

| S.No. | Glasswares and other laboratory materials used |
|---|---|
| 1. | Beakers – 25, 50 and 100 mL |
| 2. | Conical flask – 100 mL |
| 3 | Measuring Cylinders – 10, 100 mL |
| 4. | Pipettes |
| 5. | Specific gravity bottle |
| 6. | Tarson tubes |
| 7. | Ice bucket |
| 8. | Aluminium foil |
| 9. | Stopwatch |
| 10. | 70% v/v ethanol |
| 11. | Distilled water |

**Table 2:** Apparatus and Equipments Used

| S. No. | Apparatus and Equipments | Source/Company |
|---|---|---|
| 1. | Weighing Balance | Citizon (Model: CG 203) |
| 2. | Water Bath | Relitech |
| 3. | pH meter | ELICO India (Model: LI 127) |
| 4. | Conductivity Meter | Eutech Instruments |
| 5. | Ostwald's Stalagmometer | HARCO |
| 6. | Ubbelohde Viscometer | HARCO |
| 7. | Digital Ultrasonic Velocity Meter | Vi Microsystems Pvt Ltd, Chennai (Model: VCT-70A) |

## 4.2 METHODOLOGY

### 4.2.1 Stock Preparation and pH Determination

A stock solution of vancomycin hydrochloride concentrated at 0.75 mM was prepared by dissolving 0.111 g of the peptide in 100 ml distilled water and was shaken vigorously. Another solution of vancomycin hydrochloride was prepared in the same way but of a different concentration to determine pH. 0.010 g of vancomycin hydrochloride was prepared in 10 mL distilled water, and pH meter was determined to be 3.4. Dilutions were made ranging from 0.05 mM to 0.75 mM, each measuring 25 mL. All the parameters were studied at a uniform temperature of 25°C.

**Table 3:** Dilutions Prepared of Vancomycin Hydrochloride from Stock of 0.75 mM

| S. No. | Concentration | Stock Volume | Distilled Water Volume |
|---|---|---|---|
| 1. | 0.05 mM | 1.7 mL | 23.3 mL |
| 2. | 0.10 mM | 3.3 mL | 21.7 mL |
| 3. | 0.15 mM | 5.0 mL | 20.0 mL |
| 4. | 0.20 mM | 6.7 mL | 18.3 mL |
| 5. | 0.25 mM | 8.3 mL | 16.7 mL |
| 6. | 0.30 mM | 10.0 mL | 15.0 mL |
| 7. | 0.35 mM | 11.7 mL | 13.3 mL |
| 8. | 0.40 mM | 13.3 mL | 11.7 mL |
| 9. | 0.45 mM | 15.0 mL | 10.0 mL |
| 10. | 0.50 mM | 16.7 mL | 8.3 mL |
| 11. | 0.55 mM | 18.3 mL | 6.7 mL |
| 12. | 0.60 mM | 20.0 mL | 5.0 mL |
| 13. | 0.65 mM | 21.7 mL | 3.3 mL |
| 14. | 0.70 mM | 23.3 mL | 1.7 mL |
| 15. | 0.75 mM | 25.0 mL | 0 |

### 4.2.2 Density

A specific gravity bottle and weighing balance was used to calculate the density of different dilutions of the stock solution. The empty specific gravity bottle weighed 5.605 g. The weight

of specific gravity bottle filled with distilled water, up to the brim, was noted. Then, the weight of the specific gravity bottle filled with different dilutions of vancomycin hydrochloride, up to the brim, was noted. Assigning the notations $w_1$, $w_2$ and $w_3$ to the weight of empty specific gravity bottle, specific gravity bottle containing distilled water and specific gravity bottle containing vancomycin hydrochloride solution respectively, the following formula can be used to calculate the density of different dilutions of vancomycin hydrochloride, denoted by $\rho_s$:-

$$\rho_s = \frac{w_3 - w_1}{w_2 - w_1} \times \rho_w$$

where $\rho_w$ is density of water at 25°C = 0.9970 g/cm$^3$.

### 4.2.3 Specific Conductivity

The standard protocol of measuring conductivity suggests dipping the probe of the conductivity meter up to a specific given mark, in a beaker containing the solution whose conductivity is to be determined. Using this protocol, the specific conductivity of different dilutions of vancomycin hydrochloride solution were determined.

### 4.2.4 Surface Tension

Stalagmometer with an attached rubber tube and a screw pinch cock was used to determine the surface tension of a solution. The following steps were performed to determine the surface tension:

(i) Dipped the end of the stalagmometer in a beaker containing distilled water and sucked water from the rubber tube end until it reaches a defined mark on the stalagmometer. Tightened the screw.

(ii) Clamped the stalagmometer and loosened the screw pinch cock allowing 15-20 drops to fall per minute.

(iii) Counted the number of drops that fell when the meniscus crosses the upper defined mark and reaches the lower defined mark.

(iv) Repeated the process for different dilutions of vancomycin hydrochloride solution.

Assigning the notations $n_w$ and $n_s$ to drops of water and drops of solution respectively, the following formula can be used to calculate surface tension (denoted by $\gamma_s$) of a given solution:-

$$\gamma_s = \frac{\rho_s \times n_w}{\rho_w \times n_s} \times \gamma_w$$

where $\gamma_w$ is surface tension of water at 25°C = 71.99 dyne/cm.

### 4.2.5 Viscosity

A calibrated jacketed ubbelohde type viscometer based on capillary method was used to measure the viscosity of the test solution. The following steps were performed to measure viscosity:

(i) The viscometer was assembled to a water thermostat to maintain a constant temperature of 25 °C.

(ii) First distilled water was added from an opening upto a specified mark.

(iii) Since water and test solution were both colourless, time was noted when the lower meniscus of the solution started flowing from the mark above till its upper meniscus reached the mark specified below.

(iv) The above two steps were repeated for varying concentrations of vancomycin hydrochloride solution.

Assigning the notations $t_w$ and $t_s$ to times observed for water and solution respectively, the following formula can be used to calculate viscosity (denoted by $\eta_s$) of a given solution:-

$$\eta_s = \frac{\rho_s \, X \, t_s}{\rho_w \, X \, t_w} \, X \, \eta_w$$

where $\eta_w$ is viscosity of water at 25 °C = 0.8903 cP.

### 4.2.6 Ultrasonic Velocity of Sound

The velocity of sound was measured by filling the transducer of the apparatus with water and closing it with a cap. The transducer cable was then attached to the transducer at one end and velocity meter at the other. Then the velocity meter was turned on and automatic settings would display the velocity on its screen. The same was carried out for different test dilutions. It is denoted by μ.

### 4.2.7 Specific Gravity

Specific gravity was obtained by dividing the density of the test solution by the density of water at same temperature. It is denoted by *x* and calculated by the given formula:-

$$x = \frac{\rho_s}{\rho_w}$$

### 4.2.8 Determination of CMC

CMC was determined from the plot of concentration *vs* conductivity, obtained by measuring specific conductivity from conductivity meter. The deviation in the graph indicates the CMC of vancomycin hydrochloride solution. Two tangents were drawn and the intersection point between two straight lines was considered as the CMC value.

### 4.2.9 Determination of Thermodynamic Parameters

The $X_{CMC}$ data was used to determine the thermodynamic parameters. The values of standard enthalpy change ($\Delta H°_m$), standard entropy change ($\Delta S°_m$) and standard Gibb's free energy change ($\Delta G°_m$) were calculated using the following equations [38]:

$$\Delta H°_m = -RT^2(2-\alpha)[d(\ln X_{CMC})/dT]$$

$$\Delta G°_m = (2-\alpha)RT\ (\ln X_{CMC})$$

$$\Delta S°_m = (\Delta H°_m - \Delta G°_m) / T$$

The $d(\ln X_{CMC})/dT$ was the slope of the straight line obtained by plotting $\ln X_{CMC}$ against temperature.

### 4.2.10 Determination of Thermo-acoustic Parameters

The following formulae were used to determine apparent molar volume and apparent molar adiabatic compressibility denoted by $\phi_v$ and $\phi_k$, respectively [39]:

$$\phi_v = \frac{1000}{c}\left\{\frac{\rho_0 - \rho}{\rho_0}\right\} + \frac{M}{\rho_0}$$

$$\phi_k = \frac{1000(\beta - \beta_0)}{c.\rho_0} + \beta.\phi_v$$

where c = concentration of the test solution

$\rho$ = density of solution

$\rho_0$ = density of solvent (water)

M = molecular weight of the drug

$\beta$ = adiabatic compressibility of solution

$\beta_0$ = adiabatic compressibility of solvent (water).

$\beta$ can be calculated by using the relation $\beta = 1/ \rho\mu^2$.

## 5.1 RESULTS

### 5.1.1 Density

**Table 4:** Density for different concentrations of vancomycin hydrochloride

| S. No. | Concentration *(mM)* | $w_e$ *(g)* | $w_w$ *(g)* | $w_s$ *(g)* | $\rho_s$ *(g/cm$^3$)* |
|---|---|---|---|---|---|
| 1. | 0.05 | 5.605 | 10.567 | 10.526 | 0.988762 |
| 2. | 0.1 | 5.605 | 10.567 | 10.55 | 0.9935842 |
| 3. | 0.15 | 5.605 | 10.567 | 10.567 | 0.997 |
| 4. | 0.2 | 5.605 | 10.567 | 10.589 | 1.0014204 |
| 5. | 0.25 | 5.605 | 10.567 | 10.642 | 1.0120695 |
| 6. | 0.3 | 5.605 | 10.567 | 10.56 | 0.9955935 |
| 7. | 0.35 | 5.605 | 10.567 | 10.59 | 1.0016213 |
| 8. | 0.4 | 5.605 | 10.567 | 10.623 | 1.0082519 |
| 9. | 0.45 | 5.605 | 10.567 | 10.637 | 1.0110649 |
| 10. | 0.5 | 5.605 | 10.567 | 10.649 | 1.013476 |
| 11. | 0.55 | 5.605 | 10.567 | 10.668 | 1.0172936 |
| 12. | 0.6 | 5.605 | 10.567 | 10.674 | 1.0184992 |
| 13. | 0.65 | 5.605 | 10.567 | 10.688 | 1.0213122 |
| 14. | 0.7 | 5.605 | 10.567 | 10.693 | 1.0223168 |
| 15. | 0.75 | 5.605 | 10.567 | 10.699 | 1.0235224 |

### 5.1.2 Specific Conductivity

**Table 5:** Specific conductivity for different concentrations of vancomycin hydrochloride

| S. No. | Concentration *(mM)* | $\kappa$ *(μs)* |
|---|---|---|
| 1. | 0.05 | 12.7 |
| 2. | 0.1 | 14.36 |
| 3. | 0.15 | 21.2 |
| 4. | 0.2 | 24.6 |
| 5. | 0.25 | 31.5 |
| 6. | 0.3 | 42.3 |

| | | |
|---|---|---|
| 7. | 0.35 | 44.5 |
| 8. | 0.4 | 52.1 |
| 9. | 0.45 | 56.2 |
| 10. | 0.5 | 63.3 |
| 11. | 0.55 | 68 |
| 12. | 0.6 | 76 |
| 13. | 0.65 | 82.5 |
| 14. | 0.7 | 88.8 |
| 15. | 0.75 | 99.2 |

### 5.1.3 Surface Tension

**Table 6:** Surface tension for different concentrations of vancomycin hydrochloride

| S. No. | Concentration *(mM)* | $n_w$ | $n_s$ | $\rho_s$ *(g/cm$^3$)* | $\gamma_s$*(dyne/cm)* |
|---|---|---|---|---|---|
| 1. | 0.05 | 37 | 42 | 0.988762 | 62.89574 |
| 2. | 0.1 | 37 | 41 | 0.9935842 | 64.74401 |
| 3. | 0.15 | 37 | 39 | 0.997 | 68.29821 |
| 4. | 0.2 | 37 | 38 | 1.0014204 | 70.40631 |
| 5. | 0.25 | 37 | 38 | 1.0120695 | 71.15501 |
| 6. | 0.3 | 37 | 43 | 0.9955935 | 61.8575 |
| 7. | 0.35 | 37 | 38 | 1.0016213 | 70.42044 |
| 8. | 0.4 | 37 | 38 | 1.0082519 | 70.88661 |
| 9. | 0.45 | 37 | 37 | 1.0110649 | 73.00558 |
| 10. | 0.5 | 37 | 37 | 1.013476 | 73.17968 |
| 11. | 0.55 | 37 | 36 | 1.0172936 | 75.49576 |
| 12. | 0.6 | 37 | 36 | 1.0184992 | 75.58523 |
| 13. | 0.65 | 37 | 36 | 1.0213122 | 75.79399 |
| 14. | 0.7 | 37 | 36 | 1.0223168 | 75.86854 |
| 15. | 0.75 | 37 | 35 | 1.0235224 | 78.12824 |

### 5.1.4 Viscosity

**Table 7:** Viscosity for different concentrations of vancomycin hydrochloride

| S. No. | Concentration *(mM)* | $t_w$ *(s)* | $t_s$ *(s)* | $\rho_s$ *(g/cm$^3$)* | $\eta_s$ *(cP)* |
|---|---|---|---|---|---|
| 1. | 0.05 | 175 | 175 | 0.988762 | 0.882944 |
| 2. | 0.1 | 175 | 178 | 0.9935842 | 0.90246 |
| 3. | 0.15 | 175 | 180 | 0.997 | 0.915737 |
| 4. | 0.2 | 175 | 180 | 1.0014204 | 0.919797 |
| 5. | 0.25 | 175 | 183 | 1.0120695 | 0.945071 |
| 6. | 0.3 | 175 | 185 | 0.9955935 | 0.939847 |
| 7. | 0.35 | 175 | 182 | 1.0016213 | 0.930204 |
| 8. | 0.4 | 175 | 183 | 1.0082519 | 0.941506 |
| 9. | 0.45 | 175 | 185 | 1.0110649 | 0.954452 |
| 10. | 0.5 | 175 | 185 | 1.013476 | 0.956728 |
| 11. | 0.55 | 175 | 187 | 1.0172936 | 0.970714 |
| 12. | 0.6 | 175 | 188 | 1.0184992 | 0.977061 |
| 13. | 0.65 | 175 | 190 | 1.0213122 | 0.990183 |
| 14. | 0.7 | 175 | 193 | 1.0223168 | 1.006806 |
| 15. | 0.75 | 175 | 195 | 1.0235224 | 1.018439 |

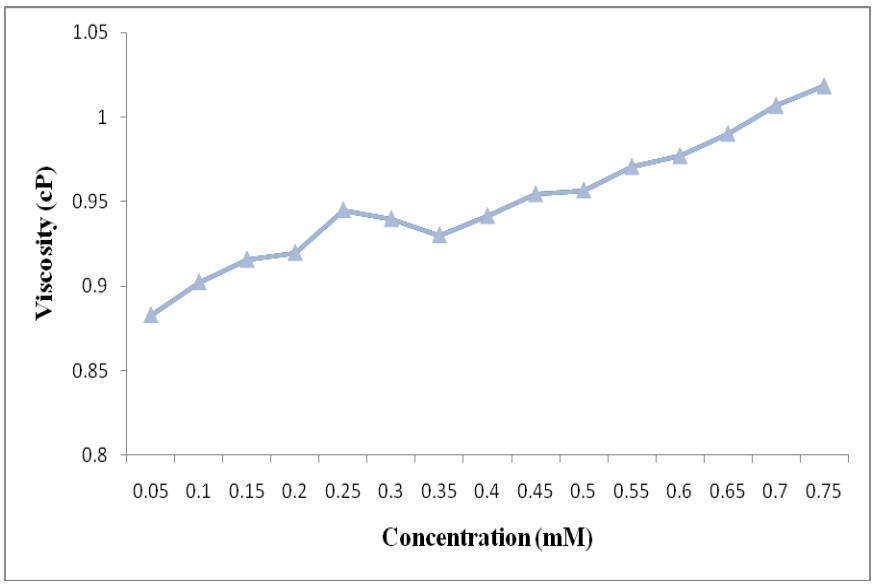


**Figure 3:** Plot between concentration of vancomycin hydrochloride and viscosities determined

### 5.1.5 Velocity of Sound

**Table 8:** Ultrasonic velocity of sound for different concentrations of vancomycin hydrochloride

| S. No. | Concentration *(mM)* | μ *(m/s)* |
|---|---|---|
| 1. | 0.05 | 1428.65 |
| 2. | 0.1 | 1443.97 |
| 3. | 0.15 | 1445.9 |
| 4. | 0.2 | 1453.05 |
| 5. | 0.25 | 1453.71 |
| 6. | 0.3 | 1447.26 |
| 7. | 0.35 | 1448.5 |
| 8. | 0.4 | 1448.5 |
| 9. | 0.45 | 1449.8 |
| 10. | 0.5 | 1449.15 |
| 11. | 0.55 | 1450.45 |
| 12. | 0.6 | 1453.05 |
| 13. | 0.65 | 1455.67 |
| 14. | 0.7 | 1467.57 |
| 15. | 0.75 | 1468.24 |

### 5.1.6 Specific Gravity

**Table 9:** Specific gravity for different concentrations of vancomycin hydrochloride

| S. No. | Concentration *(mM)* | *X* |
|---|---|---|
| 1. | 0.05 | 0.991737203 |
| 2. | 0.1 | 0.996573962 |
| 3. | 0.15 | 1 |
| 4. | 0.2 | 1.004433696 |
| 5. | 0.25 | 1.015114873 |

| | | |
|---|---|---|
| 6. | 0.3 | 0.998589279 |
| 7. | 0.35 | 1.004635228 |
| 8. | 0.4 | 1.011285772 |
| 9. | 0.45 | 1.014107215 |
| 10. | 0.5 | 1.016525595 |
| 11. | 0.55 | 1.020354696 |
| 12. | 0.6 | 1.021563886 |
| 13. | 0.65 | 1.024385328 |
| 14. | 0.7 | 1.025392987 |
| 15. | 0.75 | 1.026602177 |

### 5.1.7 Determination of CMC

The CMC from the plot of concentration *vs* conductivity was determined to be 0.36 mM.

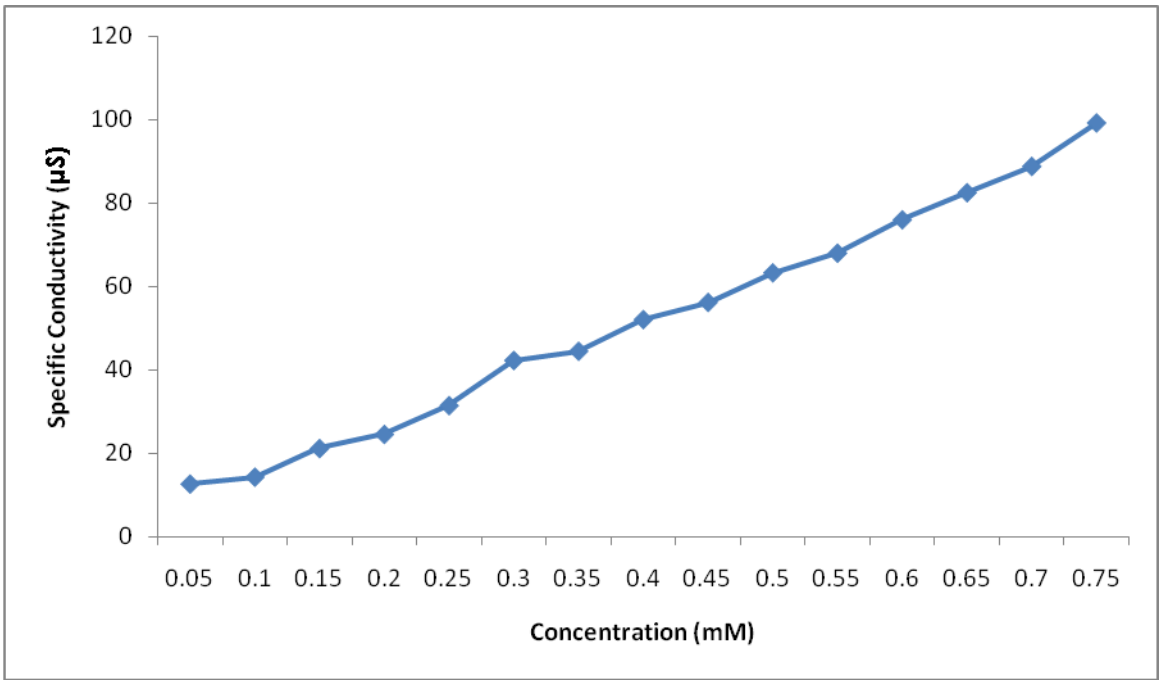


**Figure 4:** Plot between concentration of vancomycin hydrochloride and specific conductivities measured

### 5.1.8 Determination of Thermodynamic Parameters

**Table 10:** Thermodynamic parameters for different concentrations of vancomycin hydrochloride

| **Concentration** | 0.05 mM – 0.75 mM |
|---|---|
| **Temperature** | 25 ° C |
| **CMC** | 0.36 mM |
| $\mathbf{X_{CMC}}$ | 6.48052E-06 |
| $\mathbf{\Delta H°_m}$ | -9.49875 |
| $\mathbf{\Delta S°_m}$ | -0.31402 |
| $\mathbf{\Delta G°_m}$ | -1.64813 |

### 5.1.9 Determination of Thermo-Acoustic Parameters

**Table 11:** Thermo-acoustic parameters for different concentrations of vancomycin hydrochloride

| **S. No.** | **Concentration** ***(mM)*** | **β** | **$\beta_0$** | **$\phi_v$** | **$\phi_k$** |
|---|---|---|---|---|---|
| 1. | 0.05 | 4.96E-10 | 4.48E-10 | 0.1686359 | 83.56157 |
| 2. | 0.1 | 4.83E-10 | 4.48E-10 | 0.03640415 | 17.5731 |
| 3. | 0.15 | 4.75E-10 | 4.48E-10 | 0.00148947 | 0.707579 |
| 4. | 0.2 | 4.73E-10 | 4.48E-10 | -0.0206522 | -9.75874 |
| 5. | 0.25 | 4.73E-10 | 4.48E-10 | -0.058273 | -27.5411 |
| 6. | 0.3 | 4.8E-10 | 4.48E-10 | 0.0062266 | 2.985915 |
| 7. | 0.35 | 4.76E-10 | 4.48E-10 | -0.0117357 | -5.58431 |
| 8. | 0.4 | 4.73E-10 | 4.48E-10 | -0.0265106 | -12.5318 |
| 9. | 0.45 | 4.71E-10 | 4.48E-10 | -0.0295269 | -13.8939 |
| 10. | 0.5 | 4.67E-10 | 4.48E-10 | -0.0311464 | -14.5557 |
| 11. | 0.55 | 4.56E-10 | 4.48E-10 | -0.0349203 | -15.9235 |
| 12. | 0.6 | 7.44E-10 | 4.48E-10 | -0.0338303 | -25.1531 |

| | | | | | |
|---|---|---|---|---|---|
| 13. | 0.65 | 4.62E-10 | 4.48E-10 | -0.0352788 | -16.3016 |
| 14. | 0.7 | 4.65E-10 | 4.48E-10 | -0.0340356 | -15.8249 |
| 15. | 0.75 | 4.54E-10 | 4.48E-10 | -0.0332005 | -15.0609 |

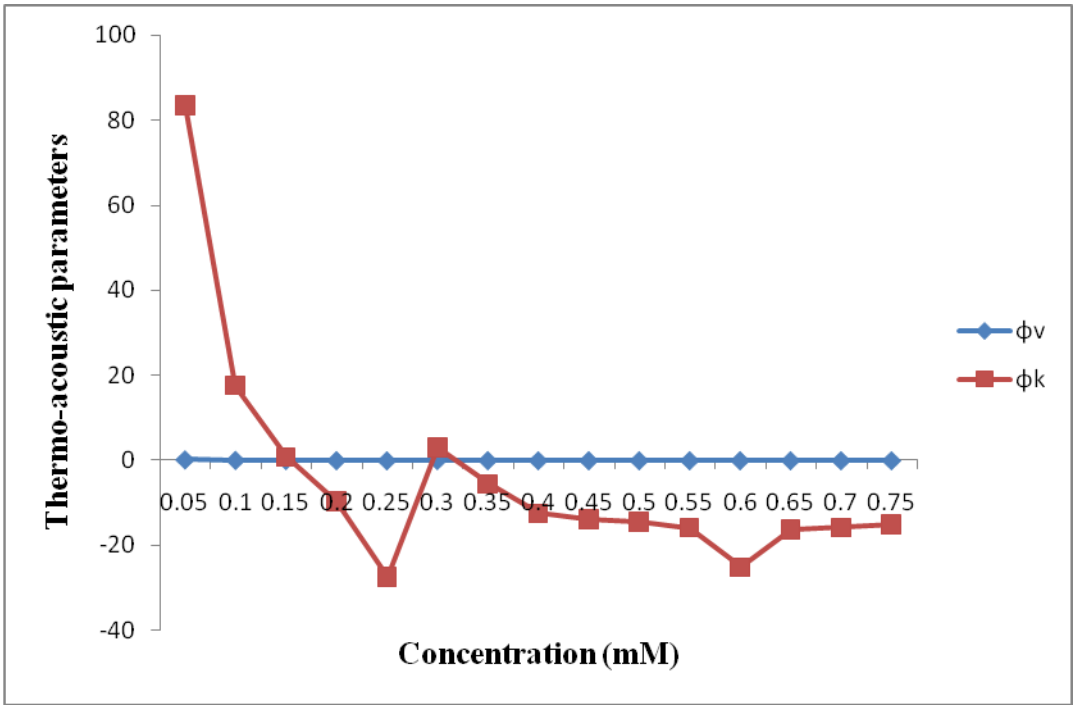


**Figure 5:** Plot between concentrations of vancomycin hydrochloride and thermo-acoustic parameters calculated

## 5.2 DISCUSSION

The values obtained for density were increasing with increasing concentration, except at one point near 0.3 mM where there was a decrease in the obtained value. [Table 4]. This trend occurs due to addition of more solute (i.e. the drug) in solvent (i.e. distilled water), and the drop in the density was due solubilisation of the solute in the solvent.

The specific conductivity increases with increase in concentration of vancomycin hydrochloride [Table 5]. As there is more amount of solute in the same volume of solvent in higher concentrations, the ionisation process increases, leading to the presence of more ions in the solution which aid in conducting electricity.

The values obtained for surface tension were not in accordance with the values that were expected. The surface tension of the solution was found to increase with increase in

concentration [Table 6], but as per earlier studies surface tension has only decreased with increase in concentration. The addition of surfactants causes the surface tension of a solution to decrease, aiding in the formation of micelles. So it is safe to say that promising results were not obtained for surface tension.

Viscosity was found to increase with increase in concentration except at the point where a drop in density was also observed [Table 7]. A graph was plotted of viscosity observed against concentration which almost gives a straight line [Figure 3].

Similarly, the ultrasonic velocity of sound consistently increases with increase in concentration, except at the similar concentration where there was a slight decline in the values of density and viscosity [Table 8].

The values of specific gravity were obtained in accordance with the values of density and showed a similar pattern [Table 9].

The CMC value was determined from the plot between concentration and specific conductivity which was found out to be 0.36 mM [Figure 4]. This concentration is the concentration where a shift in density, viscosity, velocity of sound and specific gravity was observed. Therefore there is a change in physico-chemical properties associated with the CMC.

The values obtained for all three thermodynamic parameters $\Delta H°_m$, $\Delta S°_m$ and $\Delta G°_m$ were found to be negative [Table 10]. This indicates that the formation of micelles in case of vancomycin hydrochloride is exothermic in nature, due to negative value of $\Delta H°_m$. A negative change in $\Delta S°_m$ indicates that the entropy of vancomycin hydrochloride decreases on the formation of micelles. And the negative value of $\Delta G°_m$ indicates that micelle formation is favourable in case of vancomycin hydrochloride and the reaction will proceed in the forward direction.

The values obtained for thermo-acoustic parameters $\phi_v$ and $\phi_k$ were found to decrease with increase in concentration, but increased at CMC [Table 11]. The increase in determined values indicates an increase in electrostatic interactions, and a decrease in the determined values indicates an increase in hydrophobic interactions. This means that with increasing concentration of vancomycin hydrochloride, hydrophobic interactions are much more stable

and favourable, whereas electrostatic interactions are more profound and favourable at lower concentrations of vancomycin hydrochloride.

Since our studies have helped determine the physico-chemical properties of vancomycin hydrochloride at various concentrations, and revealed the changes in the properties at CMC, we can identify our study to be of significance that might further help in the preparing better formulations of vancomycin hydrochloride.

## 6.1 Conclusion

The physico-chemical studies were performed to determine the thermodynamic and thermo-acoustic properties of well-known and widely used antibiotic vancomycin hydrochloride. The CMC was observed to increase with increasing specific conductivity, indicating the impact of solute-solvent interactions on micellization. The study performed suggested that the process of micellization of vancomycin hydrochloride is exothermic in nature due to the negative value of $\Delta H°_m$. The negative values of $\Delta S°_m$ and $\Delta G°_m$ reveals that the entropy of the system decreases upon micellization and the reaction is favoured to move in the forward direction, respectively. Thermo-acoustic data that was used to determine the interactions within the system revealed that electrostatic interactions dominated the overall system at lower concentrations of vancomycin hydrochloride, and on increasing its concentration, hydrophobic interactions started to dominate the system. The results obtained from the studies that were carried out were in accordance with the literature studied. In conclusion, these interactions are favourable for the system to be utilised in pharmaceutical industries for formulation development.

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