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Study of the physico-chemical properties of vancomycin hydrochloride for determining its potential applications in formulation development

Harjas Saini, Vikrant Abbot, Gopal Singh Bisht & Poonam Sharma*

Department of Biotechnology & Bioinformatics, Jaypee University of Information Technology, Waknaghat, Solan, Himachal Pradesh- 173234, India

E-mail: poonam.sharma@juit.ac.in, drpoonamsharma@rediffmail.com

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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 specific conductivity, density, viscosity, velocity of sound and specific gravity are determined which further helps in 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 favourable and exothermic in nature. Along with this, thermo-acoustic parameters such as apparent molar volume and apparent molar compressibility have also been determined. These parameters reveal that electrostatic interactions are favourable at lower concentrations of vancomycin hydrochloride whereas hydrophobic interactions are dominant at higher concentrations. All these studies have proven useful in determining the types of interactions occurring in the system. Moreover, a stable concentration of vancomycin hydrochloride can also be determined which may aid in the topical formulation of this drug.

Keywords: Thermodynamics, Acoustic studies, Formulation, Micellization, Interaction studies

Physico-chemical properties refer to the physical and chemical interactions that lead to structural changes in atoms and molecules. These changes subsequently play a major role in affecting the drug kinetics. The study of physico-chemical properties of a drug substance in both, solid and liquid state, play a crucial role during drug delivery and pre-formulation studies[1]. Pre-formulation can be described as that part of research and development process where physical, chemical and mechanical properties of a drug along with its effects are characterized and are utilized to design a safe, stable and effective dosage form of the drug[2]. Some of the physico-chemical parameters that were studied includes: specific conductance, viscosity, density, velocity of sound and specific gravity, each playing a significant role in the pre-formulation studies.

Micelles are aggregates 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 amphiphilic 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[3,4]. The main forces of attraction result from the hydrophobic effects associated with non-polar tails and the main repelling forces are due to steric interactions and electrostatic interactions between polar heads[5]. 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[6,7]. Micelles are generally spherical shaped molecules ranging from 2 to 20 nm in size, depending on composition. Utilization of 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[8,9]. 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[10]. On plotting a graph between compounds 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 critical micelle concentration (CMC). At concentrations above CMC, solubility increases linearly with surfactant concentration, revealing that solubilisation is related to micellization[11].

CMC can therefore be defined as the minimum concentration at which micelle formation begins. CMC can be determined using conductivity[12], surface tension[13], capillary electrophoresis[14], scattering techniques[15], voltammetry[16], fluorescence and UV-visible spectroscopy[17,18]. 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.

Vancomycin hydrochloride is a glycopeptides antibiotic with molecular formula $C_{66}H_{75}Cl_2N_9O_{24}$.HCl. It is a white crystalline powder having molecular weight of 1485.723 g/mol. It is soluble in water, moderately soluble in ethanol and insoluble in higher alcohols, acetones and ethers. It has negligible oral bioavailability and is excreted out of the body as such without undergoing any metabolic process. The elimination time of drug from the body of an adult is 4 to 11 h and it has a half-life of 6 to 10 days in patients with impaired renal function. It is used to treat the severe infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). Vancomycin is administered orally to the patient in pseudo-membranous colitis caused by *C. difficile*, when the treatment is non-responsive to metronidazole. It is also used for treatment of gram positive bacterial infections and patients allergic to beta-lactam antimicrobials. Its use has also been found in treatment and prevention of endopthalmitis[19]. The structure of vancomycin hydrochloride is presented in Fig. 1.

Vancomycin has a property of forming aggregates as it is poly-cationic in nature. In this study, this aggregation property has been utilized to form micelles which helped us in determining critical micelle concentration and further thermodynamic parameters. These thermodynamic parameters

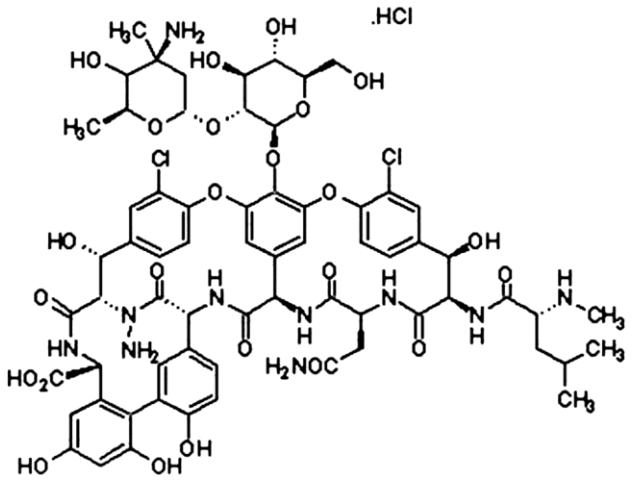

Fig. 1 — Molecular structure of vancomycin hydrochloride.

(change in entropy (ΔS^o_m), enthalpy (ΔH^o_m) and Gibb's free energy (ΔG^o_m)) provide the information regarding nature of reactions occurring within the system. The other parameters i.e. velocity of sound and density have been used to calculate acoustic parameters. The acoustic parameters have been utilized to study different interactions within the solute-solvent system. The knowledge of these interactions might prove helpful in designing an improved vancomycin containing topical formulations either alone or in combination with other antibiotics. The self-aggregation property of vancomycin hydrochloride can be utilized to entrap other drugs in its micellar structure so that a sustained drug release is achieved. The viscosity and specific gravity has been calculated in order to evaluate the physical changes in the solution when a solute is added in small concentrations. Overall, the present study is helpful in determining the nature of reactions occurring in the process as well as to study the types of interactions within the system so that this information can be utilized in developing an improved formulation of vancomycin hydrochloride.

Materials and methods

Chemicals

Vancomycin hydrochloride (CAS No. 1404-939) was obtained from HIMEDIA Laboratories Pvt. Ltd., India. The distilled water has been freshly prepared from Millipore Elix distillation assembly. The specific conductance of water is within the range of (1-3) x 10^{-7} S.cm^{-1} at 25 ^oC and pH has been found to be within the range of 6.5-7.0.

Methodology

Stock Preparation and pH Determination

A stock solution of vancomycin hydrochloride of 0.75 mM concentration 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.

Specific conductivity

The specific conductivity was measured with the help of CON-510 conductivity meter manufactured by Eutech Instruments. Standard protocol for 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 was determined.

Determination of CMC

CMC was determined from the plot of vancomycin 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.

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[20]:

$$\Delta H_m^\circ = -RT^2(2-\alpha)[d(lnX_{CMC})/dT$$

$$\Delta G_m^\circ = (2-\alpha)RT\,(lnX_{CMC})$$

$$\Delta S_m^\circ = (\Delta H_m^\circ - \Delta G_m^\circ)/T$$

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

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 ρ_s:

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

where, ρ_w is density of water at 25 °C = 0.9970 g/cm^3.

Ultrasonic velocity of sound

The velocity of sound was calculated from Digital Ultrasonic Pulse-Echo Velocity meter for liquids and solids (VCT-70A), supplied by Vi Microsystems Pvt. Ltd., Chennai. The velocity 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.

Determination of thermo-acoustic parameters

The following formulae were used to determine apparent molar volume and apparent molar adiabatic compressibility denoted by ϕ_v and ϕ, respectively[21]:

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

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

where, c is the concentration of surfactant, ρ is the density of solution, ρ_0 is the density of solvent system, M is the molecular weight of SDS (288.38 gmol-1), β and βo are the adiabatic compressibility of the solution and solvent respectively, calculated from the relation $\beta = 1/\rho\mu^2$.

Viscosity

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

assembled to a water thermostat to maintain a constant temperature of 25 °C. First distilled water was added from an opening upto a specified mark. 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. 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 η_s) of a given solution[22],

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

where, η_w is viscosity of water at 25 °C = 0.8903 cP.

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[23]:

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

Results and Discussion

Conductivity, micellization and thermodynamics

The specific conductivity increases with increase in concentration of vancomycin hydrochloride. The data obtained for specific conductivity is shown in Table 1.

Table 1 — Specific conductivity for different concentrations of vancomycin hydrochloride

S. N.	Vancomycin conc. (mM)	κ (μs) ± S.D
1	0.05	12.7 ± 0.2
2	0.1	14.36 ± 0.14
3	0.15	21.2 ± 0.2
4	0.2	24.6 ± 0.1
5	0.25	31.5 ± 0.1
6	0.3	42.3 ± 0.2
7	0.35	44.5 ± 0.2
8	0.4	52.1 ± 0.1
9	0.45	56.2 ± 0.1
10	0.5	63.3 ± 0.2
11	0.55	68 ± 0.1
12	0.6	76 ± 0.2
13	0.65	82.5 ± 0.2
14	0.7	88.8 ± 0.1
15	0.75	99.2 ± 0.2

S.D. = Standard deviation

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[24]. The CMC value was determined from the plot between vancomycin concentration and specific conductivity (Fig. 2) which was found out to be 0.36 mM. 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 $\Delta G°_m$ are given in Table 2. All the values were found to be negative. This indicates that the formation of micelles in case of vancomycin hydrochloride is exothermic in nature, due to negative value of $\Delta H°_m$. This also indicates the dominance of London dispersion forces within the system during micelle formation process[25]. A negative change in $\Delta S°_m$ indicates that the entropy of vancomycin hydrochloride decreases on the formation of micelles. The negative value of $\Delta G°_m$ indicates that micelle formation is favourable in case of vancomycin hydrochloride and the reaction proceeds in forward direction.

Density, velocity of sound and acoustic studies

The values obtained for density were found to increase with increasing concentration, except at one

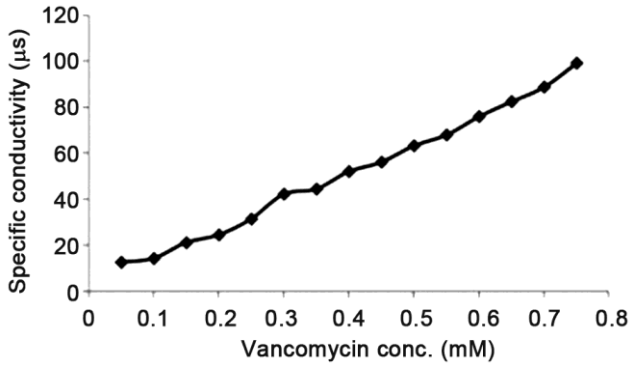

Fig. 2 — Plot between concentration of vancomycin hydrochloride and measured specific conductivities.

Table 2 — Thermodynamic parameters for different concentrations of vancomycin hydrochloride

Concentration	0.05 mM – 0.75 mM
Temperature	25 °C
CMC	0.36 mM
X_{CMC}	6.48052×10^{-6}
$\Delta H°_m$	-9.49875
$\Delta S°_m$	-0.31402
$\Delta G°_m$	-1.64813

point near 0.3 mM where a decrease in density was observed, indicating micelle formation. 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 to solubilisation of the solute in the solvent. 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. The values for density and ultrasonic sound velocity are presented in Tables 3 and 4, respectively. This data was further used to calculate thermo-acoustic parameters i.e. apparent molar volume (ϕ_v) and apparent molar compressibility (ϕ_k).

Table 3 — Density for different concentrations of vancomycin hydrochloride

S. N.	Vancomycin conc. (mM)	W_1 (g)	W_2 (g)	W_3 (g)	ρ_s (g/cm^3)
1	0.05	5.605	10.567	10.526	0.9888
2	0.1	5.605	10.567	10.55	0.9936
3	0.15	5.605	10.567	10.567	0.9970
4	0.2	5.605	10.567	10.589	1.0014
5	0.25	5.605	10.567	10.642	1.0121
6	0.3	5.605	10.567	10.56	0.9956
7	0.35	5.605	10.567	10.59	1.0016
8	0.4	5.605	10.567	10.623	1.0083
9	0.45	5.605	10.567	10.637	1.0111
10	0.5	5.605	10.567	10.649	1.0135
11	0.55	5.605	10.567	10.668	1.0173
12	0.6	5.605	10.567	10.674	1.0185
13	0.65	5.605	10.567	10.688	1.0213
14	0.7	5.605	10.567	10.693	1.0223
15	0.75	5.605	10.567	10.699	1.0235

Standard deviation (S.D.) = ±0.1%

Table 4 — Ultrasonic velocity of sound for different concentrations of vancomycin hydrochloride

S. N.	Vancomycin conc. (mM)	μ (m/s) ± S.D.
1	0.05	1428.65 ± 0.03
2	0.1	1443.97 ± 0.02
3	0.15	1445.9 ± 0.03
4	0.2	1453.05 ± 0.03
5	0.25	1453.71 ± 0.02
6	0.3	1447.26 ± 0.02
7	0.35	1448.5 ± 0.01
8	0.4	1448.5 ± 0
9	0.45	1449.8 ± 0.03
10	0.5	1449.15 ± 0.02
11	0.55	1450.45 ± 0.02
12	0.6	1453.05 ± 0.01
13	0.65	1455.67 ± 0.04
14	0.7	1467.57 ± 0.03
15	0.75	1468.24 ± 0.01

S.D. = Standard deviation

The values obtained for thermo-acoustic parameters ϕ_v and ϕ_k were found to decrease with increase in concentration but increased at CMC value, thus confirming micellization (Table 5). The increase in 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, the ionic interactions between drug and solvent increases, leading to enhancement in incompressibility of system[26]. The graphical representation between vancomycin hydrochloride concentrations and thermo-acoustic parameters is shown in Fig. 3.

Viscosity and specific gravity studies

Viscosity was found to increase with increase in vancomycin concentration except at the point where a

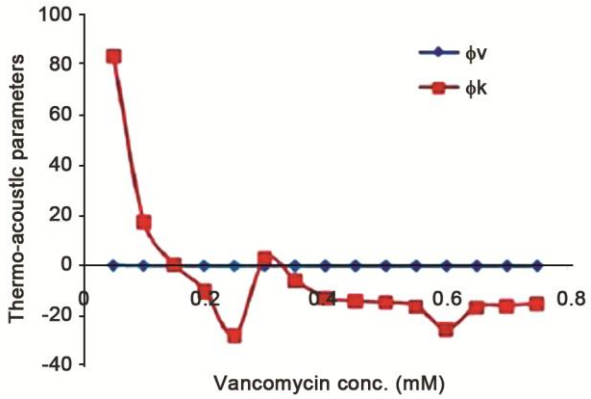

Fig. 3 — Plot between concentrations of vancomycin hydrochloride and calculated thermo-acoustic parameters.

Table 5 — Thermo-acoustic parameters for different concentrations of vancomycin hydrochloride

S. N.	Vancomycin conc. (mM)	β (10^{-10})	β_0 (10^{-10})	ϕ_v (m^{3}mol^{-1})	ϕ_k (m^{3}mol^{-1}TPa^{-1})
1	0.05	4.96	4.48	0.1686359	83.56157
2	0.1	4.83	4.48	0.03640415	17.5731
3	0.15	4.75	4.48	0.00148947	0.707579
4	0.2	4.73	4.48	-0.0206522	-9.75874
5	0.25	4.73	4.48	-0.058273	-27.5411
6	0.3	4.8	4.48	0.0062266	2.985915
7	0.35	4.76	4.48	-0.0117357	-5.58431
8	0.4	4.73	4.48	-0.0265106	-12.5318
9	0.45	4.71	4.48	-0.0295269	-13.8939
10	0.5	4.67	4.48	-0.0311464	-14.5557
11	0.55	4.56	4.48	-0.0349203	-15.9235
12	0.6	7.44	4.48	-0.0338303	-25.1531
13	0.65	4.62	4.48	-0.0352788	-16.3016
14	0.7	4.65	4.48	-0.0340356	-15.8249
15	0.75	4.54	4.48	-0.0332005	-15.0609

Standard deviation (S.D.) = ±0.1%

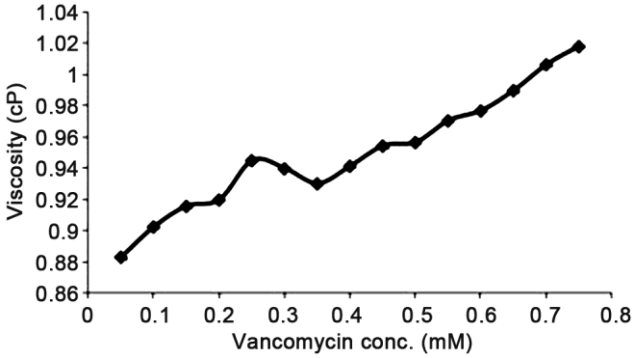

Fig. 4 — Plot between concentration of vancomycin hydrochloride and determined viscosities.

Table 6 — Viscosity for different concentrations of vancomycin hydrochloride

S. N.	Vancomycin conc. (mM)	t_w (s)	t_s (s)	ρ_s (g/cm^3)	η_s (cP)
1	0.05	175	175	0.9888	0.8829
2	0.1	175	178	0.9936	0.9025
3	0.15	175	180	0.9970	0.9157
4	0.2	175	180	1.0014	0.9198
5	0.25	175	183	1.0121	0.9451
6	0.3	175	185	0.9956	0.9398
7	0.35	175	182	1.0016	0.9302
8	0.4	175	183	1.0083	0.9415
9	0.45	175	185	1.0111	0.9545
10	0.5	175	185	1.0135	0.9567
11	0.55	175	187	1.0173	0.9707
12	0.6	175	188	1.0185	0.9771
13	0.65	175	190	1.0213	0.9902
14	0.7	175	193	1.0223	1.0068
15	0.75	175	195	1.0235	1.0184

Standard deviation (S.D.) = ±0.1%

Table 7 — Specific gravity (x) for different concentrations of vancomycin hydrochloride

S. N.	Vancomycin conc. (mM)	x ±S.D
1	0.05	0.9917 ± 0.0002
2	0.1	0.9966 ± 0.0002
3	0.15	1 ± 0.0001
4	0.2	1.0044 ± 0.0002
5	0.25	1.0151 ± 0.0003
6	0.3	0.9986 ± 0.0002
7	0.35	1.0046 ± 0.0001
8	0.4	1.0113 ± 0.0001
9	0.45	1.0141 ± 0.0002
10	0.5	1.0165 ± 0.0003
11	0.55	1.0204 ± 0.0002
12	0.6	1.0216 ± 0.0002
13	0.65	1.0244 ± 0.0003
14	0.7	1.0254 ± 0.0001
15	0.75	1.0266 ± 0.0002

S.D. = Standard deviation

drop in density was also observed. As represented in Fig. 4, a graph between observed viscosity and drug concentration was plotted which almost gives a straight line except for one point where micellization occurred. The values of viscosity have been presented in Table 6. The increase in viscosity with vancomycin concentration can be accounted due to the fact that on addition of vancomycin, suspended particles are formed in the solution due to aggregates forming nature of vancomycin, thus affecting the homogeneity of the system[27]. The values of specific gravity were obtained in accordance with the values of density and showed a similar pattern (Table 7).

Conclusions

The physico-chemical studies were performed to determine the thermodynamic and thermo-acoustic properties of well-known and widely used antibiotic vancomycin hydrochloride. The specific conductivity was observed to increase with increasing drug concentration, 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 exothermic and spontaneous nature of our studied system indicates that vancomycin exhibits the property of penetrating into the cell membrane when used in micellar form. Moreover, the acoustic studies reveal that increasing the amount of vancomycin in the system favours hydrophobic interactions which might further aid in cell permeation process. The viscosity and specific gravity studies will be helpful in reducing the cost of drug development by making the translation of drug candidates into marketed products much easier.In conclusion, these interactions are favourable for the system to be utilised in pharmaceutical industries for formulation development.

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