

Study Self-association, Optical Transition Properties and Thermodynamic Properties of Neomycin Sulfate Using UV-Visible Spectroscopy

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Abstract In this paper UV-Vis spectroscopy was used to study the self-association, optical transition probabilities (molar decadeic absorption coefficient, integrated absorption cross-section, transition dipole moment and oscillator strength of the spectra) and thermodynamic properties of Neomycin sulfate in aqueous solution at room temperature (295K). The optical transition probabilities were calculated by integrated absorption coefficient techniques in the wave number range 27173.9 - 37650.6 cm^{-1} . The dimerization constant of Neomycin Sulfate ($1.062 \times 10^5 \text{ M}^{-1}$) was analyzed using dimer model by nonlinear curve fitting to the experimental values. In addition the thermodynamic parameters (Gibbs free energy, enthalpy and entropy) of dimerization reactions for the self-association of the compound were investigated using Vant's Hoff equation at the temperature range (295 –312)K. Thus, the change of enthalpy calculated for Neomycin Sulfate is (3.765 ± 0.666) $\Delta H/\text{kJ.mole}^{-1}$. The values of change in enthalpy and entropy indicate that the hydrophobic interaction play the major role in the interaction between the molecules of Neomycin sulfate.

Keywords Neomycin Sulfate, Optical Transition Probabilities, UV-Vis spectroscopy, Thermodynamic Properties, Self-Association

1. Introduction

Neomycin Sulfate is a water soluble hydrophilic aminoglycoside antibiotic and calcium channel protein inhibitor that binds to prokaryotic ribosomes inhibiting translation, and is highly effective against Gram-positive and Gram-negative bacteria (Waksman, 1950; Xue et al., 2010). It is commonly used for the prevention of bacterial contamination of cell cultures and for treatment in various eye, skin and gastrointestinal infections (Zejc & Gorczyca, 2002; Patel, Dave, 2015; Darwhekar, Jain, Choudhary, 2012). Neomycin sulfate inhibits DNase I induced DNA degradation (Xue et al., 2010).

Investigating the self-association, optical transition and thermodynamic properties of such compound are very useful in optics, biotechnology, medical imaging, medicine, drug delivery, structural materials (Sunil Kumar, Yousaf Khan, Verma, Chakarvarti, 2008). Determination of these properties are important to characterize and understanding the biological binding system, to know interaction strength

in liquid solution and electron transition of the molecules, and interpretation of absorption spectral of the compound (Belay, 2013; MacRae, 1957; Milonni and Eberly, 1988). The knowledge of the optical properties may also applicable for experimental work (in absorption, emission and dispersion), and in characterize and stringent test of atomic and molecular structure calculations of the compound in theoretical work (Belay, 2013; Bayliss, 1950; MacRae, 1957; Milonni and Eberly, 1988). Different spectroscopic techniques were developed for investigating the optical transition properties of a compound. In this study, the simplest and more powerful spectroscopic technique in measuring the intensity of absorption light called integrated absorption technique were used using UV/Vis spectroscopy (Belay et al., 2010; Belay, 2013; Niazi et al., 2006). The technique is highly sensitive, rapid and easily implemented. Therefore, the objective of this study is to investigate the Self-association, optical transition probability and thermodynamic parameters of Neomycin Sulfate, since these properties are not yet investigated in this regard.

2. Materials and Methods

The Perkin-Elmer Lambda 19 UV-Vis Spectrophotometry with double monochromator and 1 cm quartz cuvette was used

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for the electronic absorption measurements of the investigated solution of Neomycin Sulfate at room temperature (293K). The analyzed spectra were obtained by subtracting the spectrum of pure solvent (water) from that of the solution containing Neomycin Sulfate.

Neomycin Sulfate (NOMS, Fig. 1) was bought from Sigma-Aldrich and used for measurements without any further purification. The experimental method implemented to study of self-association of the compound was similar to the method applied by Belay (2012), for self and hetero association of caffeic acid. The self - association of Neomycin Sulfate was studied over the concentration range of $(3.1 - 1.65) \times 10^{-2} M$. The absorbance as a function of concentration has been measured at absorption maxima 304.8 nm to obtain the greatest accuracy of detection. For numerical analysis the dimer model equation were used by fitting to the experimental data by non-linear curve fitting based on Levenberg-Marquardt algorithm using origin 8 software. The molar extinction coefficients and equilibrium constant were used as searching parameters, in order to achieve minimum discrepancy between the experimental data and theoretical model.

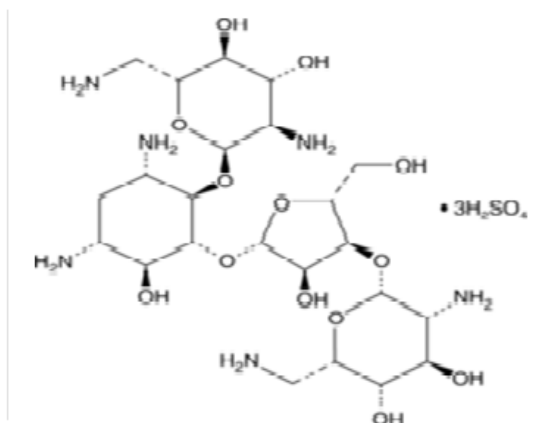


Figure 1. Chemical Structure of Neomycin Sulfate

The optical transition probabilities (transition dipole moment, oscillator strength and integrated absorption cross-section) for pure compound of NOMS were studied at (6.29) pH values of the medium. The optical transition probabilities were measured by integrating the absorption coefficient and molar decadic absorption coefficients in the wave number regions of $27173.9 - 37650.6 \text{ cm}^{-1}$. Usually the UV-Vis spectrometer measures the concentration in terms of absorbance versus wavelength; this was recalculated into absorption coefficient or molar decadic absorption coefficient versus wave number using Origin 8 software and finally the average of the optical transition probabilities in a concentration range $(1.65 - 2.71) \times 10^{-2}$ were recorded.

Similarly, the thermodynamic parameters such as enthalpy, Gibbs free energy and entropy of the self-association NOMS were studied at the temperature range (295 –312)K. These parameters have been determined using the model of Vant's Hoff's equation by linear curve fitting for the experimental

data with that of the experimental values.

3. Results and Discussion

Self-Association of Neomycin Sulfate

The experimental spectra of NOMS was studied in a concentration range of $(3.1 - 1.65) \times 10^{-2} M$ using a bi-distilled water solvent at room Temperature [Fig. 2], and its quantitative analysis was carried out using the concentration dependent molar extinction coefficient of the drug molecule on the monomer form at absorption maximum of 304.8 nm. For the case of numerical analysis dimer model was derived by considering the following molecular equilibrium in solutions (Belay, 2012; Belay, 2010).

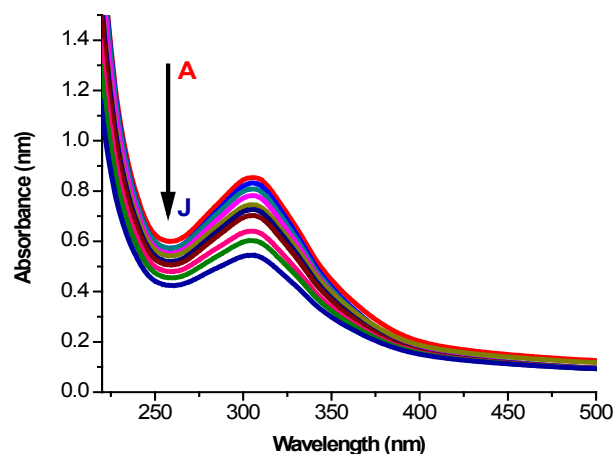
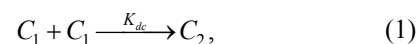


Figure 2. Absorbance of Neomycin Sulfate A – J of concentration $(3.1 - 1.65) \times 10^{-2} M$



where C_1 and C_2 and K_{dc} are monomers, dimers and the equilibrium dimerization constant of the drug respectively. The mass conservation law of the overall concentration of the dissolved molecules in solution is,

$$[C_0] = [C_1] + 2[C_2], \quad (2)$$

where $[C_0]$ is the total concentration and

$$[C_2] = K_{dc} [C_1]^2.$$

The contribution of the monomer and dimer to the molar extinction coefficient, of the solution is commonly considered to be additive, and

$$\varepsilon = \varepsilon_m f_m + \varepsilon_d f_d, \quad (3)$$

where $\varepsilon_m, \varepsilon_d, f_m$ and f_d are molar monomer extinction coefficients, molar dimer extinction coefficients, equilibrium mole fraction of the molecules in the monomer concentration and equilibrium mole fraction of the molecules in the dimer concentration. And also,

$$f_m = \frac{[C_1]}{[C_0]},$$

$$f_d = 2K_{dc} \frac{[C_1]^2}{[C_0]},$$

The dimer model for this can be obtained using Eq. 2 and Eq. 3,

$$\varepsilon = \varepsilon_d + (\varepsilon_d - \varepsilon_m) \frac{1 - \sqrt{8[C_0]K_{dc} + 1}}{4[C_0]K_{dc}}. \quad (4)$$

The unknown parameters ε_m , ε_d and K_{dc} of Eq. 4 can be obtained by fitting this dimer model equation to the experimental data shown in Fig. 3 by Nonlinear curve fitting based on the Levenberg-Marquardt algorithm using origin 8 software. They are serving as search parameters being adjusted in order to achieve the minimum discrepancy between the experimental data and the theoretical value of Eq. 4. The values of molar extinction coefficients of Neomycin Sulfate at the maximum wavelength decrease as the concentration increases as shown in Fig. 3. The calculated association constants K_{dc} obtained at the wavelength of 304.8 nm is $1.06 \times 10^5 M^{-1}$. And also the calculated values of the monomer and dimer extinction coefficients are $1.239 \times 10^3 M^{-1}$ and $12.02 M^{-1}.cm^{-1}$ respectively. The obtained values are quite reasonable and comparable with the results obtained by (Xue et al., 2010) for intercalator-neomycin conjugates, with dimerization constant $(2.4 \pm 0.1) \times 10^5 M^{-1}$ at pH of 5.5.

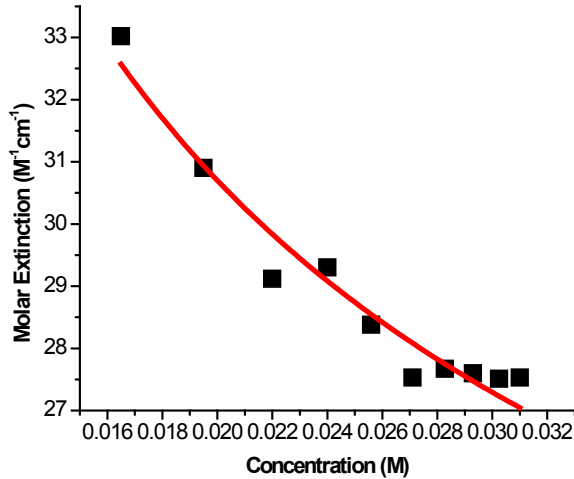


Figure 3. Molar extinction Coefficient vs Concentration of NOMS Absorption Maxima of 304.8 nm

Fig. 4, shows the mole fraction of monomer and dimer versus concentration of Neomycin sulfate drug molecules under the peak of 304.8 nm. The graphs show increase and decrease in the mole fraction of dimer and monomer as the concentrations of the drugs increases. The results indicated dimerization is favored at high concentration of the drugs.

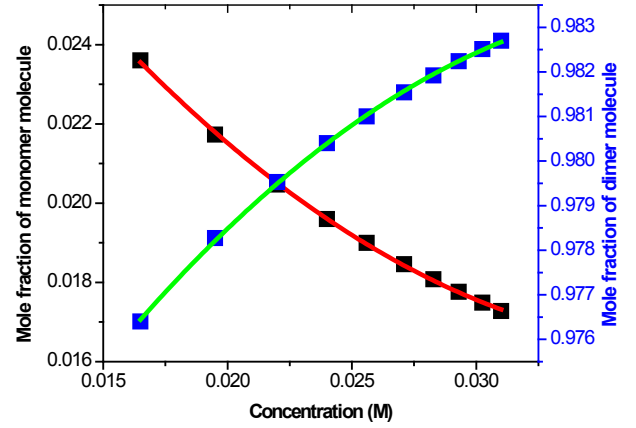


Figure 4. The Mole Fraction of Monomer and Dimer versus Total Concentration of Neomycin Sulfate under the peak of 304.8 nm

Optical transition properties of NOMS

The average optical transition probabilities of NOMS in a concentration range $(1.65 - 2.71) \times 10^{-2}$ were obtained from the absorption spectra to characterize the strength of the electron transition and to interpret the absorption spectra. The molar decadic absorption coefficient which represents the ability of a molecule to absorb light in a given solvent at a given wavelength was calculated using Beer-Lambert's law (Liptay, 1969; Belay, 2013) and integrated absorption coefficient is the sum of absorption coefficient for all frequencies. It is independent of line function which may be varying due to pressure, temperature, interaction of solute and solvent (Milonni and Eberly, 1988; Michale, 1999). The integrated absorption coefficient (α_i) in the certain frequency (ν) ranges can be expressed by,

$$\alpha_i = \int \alpha d\nu \quad (5)$$

From Eq. (5) the integrated absorption cross-section (σ_i) is given by (Milonni and Eberly, 1988);

$$\sigma_i = \frac{1}{N} \int \alpha d\nu \quad (6)$$

Where, α absorption coefficient and N is number density of the molecules.

Oscillator strength which represents the average number of electrons per atom that can be excited by the incident radiation is important parameter for providing the relative strength of electron transition and can be related as below with the molar decadic absorption coefficient (ε) as a function of frequency (Georgakopoulos et al., 2004);

$$f = 4.32 \times 10^{-9} \frac{mol.cm^2}{L} \int \varepsilon(\nu) d\nu \quad (7)$$

The transition dipole moment (μ_{km}) is a vector that depends on both ground state and excited state and couple the transition to the electric field of light and it is related with molar decadic absorption coefficient as (Liptay, 1969; Michale, 1999).

$$S \frac{|\mu_{km}|^2}{3} = \int \frac{\varepsilon(v)dv}{v} \quad (8)$$

Where $S = 2.9352 \times 10^{60} C^{-2} mol^{-1}$. Using the above equations the average value of optical transition properties of NOMS in a concentration range $(1.65 - 2.71) \times 10^{-2}$ calculated in bi-distilled water are presented in Table 1. The peak in the visible region of NOMS is due to $\pi \rightarrow \pi^*$ electronic transitions of chromophore groups (Bakhshiev, 1961; Firth et al., 1983).

Thermodynamic Properties of the Self-Association of Neomycin Sulfate

Heating the aqueous solution of Neomycin Sulfate shows that the absorption spectra of the molecules are strongly dependent on the temperature in the range of (295–312)K. The equilibrium constants of the drug molecules at the above mentioned temperature were calculated at peak of wavelengths of the self-association using Eq.(3) respectively.

Fig. (5), shows the variation of $\ln K$ versus $f(\frac{1}{T})$. The magnitude of the enthalpy was estimated from the slope of the approximating line according to Vant's Hoff's equation (Belay, 2010):

$$\frac{d \ln(K_{dc})}{f(\frac{1}{T})} = -\frac{\Delta H}{R}, \quad (9)$$

where ΔH is the molar enthalpy change, $R = 8.31 J.mol^{-1} K^{-1}$ is the universal gas constant and T the temperature in Kelvin. The entropy was derived from Gibbs free energy and enthalpy. The relation between Gibbs free energy and entropy can be expressed as

$$\Delta G = -RT \ln(K_{dc}), \quad (10)$$

$$\Delta S = -\frac{\Delta G - \Delta H}{T}. \quad (11)$$

Finally, the Vant's Hoff's equation can be given by

$$\ln K = \frac{\Delta S}{R} - \frac{\Delta H}{R} \frac{1}{T}. \quad (12)$$

Plots of $\ln K$ versus T^{-1} gives a straight line, whose slope and intercept can be used to determine ΔS and ΔH and Gibb's free energy can be determined at a specific temperature using Eq. (11). In order to characterize the force between NOMS molecules, thermodynamic parameters on the given temperatures were analyzed using Vant's Hoff's equation. The thermodynamic parameters, Gibb's free energy change (ΔG), enthalpy change (ΔH) and entropy change (ΔS) are important for confirming the binding mode. The calculated value for the Gibbs free energy, enthalpy, and entropy of the drug molecules at a temperature (295–312)K are given in table (2). Since $\Delta H > 0$ and $\Delta S > 0$, hydrophobic interactions are dominant for the interaction of NOMS molecules (Liu et al., 2014). Moreover, the negative value for the Gibb's free energy indicates that the absorption process of the drug is continuous. Also, the positive value of

entropy confirms the increasing randomness of the solution interface during the absorption process of the drug molecules (Liu et al., 2014).

The obtained thermodynamic parameters for NOMS are quite reasonable and comparable with the result binding of neomycin with BSA obtained by (Keswani et al., 2010), the results are, $\Delta H = -(30.54 \pm 1.67) kJ.mol^{-1}$ and $\Delta S = 0.04393 kJ.K^{-1}.mol^{-1}$.

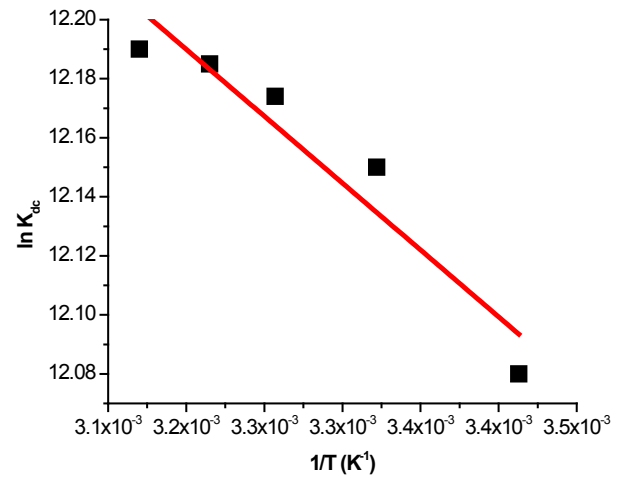


Figure 5. $\ln K_{dc}$ Vs $\frac{1}{T}$ of NOMS at Concentration $4.4 \times 10^{-2} M$

Table 1. Calculated result of the optical transition Properties of NOMS in a concentration range $(1.65 - 2.71) \times 10^{-2} mole.L^{-1}$

Parameters	Values
$\lambda_{max} / \varepsilon_{max} (nm / m^2.mol^{-1})$	304.8 / (2.971 ± 0.196)
α_t / m^{-2}	$(5.477 \pm 0.599) \times 10^5$
$\sigma_t / m.molecule^{-1}$	$(4.0975 \pm 0.31) \times 10^{-20}$
f	0.1065 ± 0.008
$\mu_{km} / C.m$	$(8.79 \pm 0.33) \times 10^{-31}$

Table 2. Calculated result of the thermodynamic Parameters of NOMS in a temperature range (295–312)K

Parameters	Values
$\Delta G / kJ.mole^{-1}$	$-(31.48 \pm 0.007)$
$\Delta H / kJ.mole^{-1}$	3.76 ± 0.66
$\Delta S / kJ.mole^{-1}.K^{-1}$	0.113 ± 0.00216

4. Conclusions

The findings of this research indicate that the molecule of Neomycin Sulfate interacts with itself due to hydrophobic

interaction. The calculated parameters for the self-association and thermodynamic properties of the compound are interpreting the study of kinetic chemical reaction system of the compound, and also the thermodynamic parameters which are derived from the temperature dependency of the dimerization constant, have given insight in to the forces that maintain the dimer structures in the solution. The other, optical transition probabilities are useful for understanding the nature and strength of its molecular interaction in liquid solutions, in order to characterize the electron transition probabilities and interpret the absorption spectra of the compound, for direct experimentally application in the emission, absorption and dispersion, and in providing stringent test of atomic and molecular structure calculation in theoretical works. Thus, knowledge of the mechanism of self-association of NOMS is useful in order to design the advanced and controllable carriers of drugs and food components. Therefore, the investigated results have wider applications in pharmaceutical and food companies in terms of economic and scientific utility.

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