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TRESEARCH

MATHEMATICAL CORRELATION OF IC_{50} AND MIC OF HYDROPHOBIC ANTIBIOTICS FOLLOWING COMPETITIVE INHIBITION IN THE DISK DIFFUSION METHOD

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ABSTRACT

Objective: This study explores the mathematical link between binding affinity, drug resistance, IC50, MIC, and Zone of Inhibition (ZOI) in the disc diffusion method for antimicrobial susceptibility testing. We consider ZOI (x) as a response to antibiotic concentration, showing that x is proportional to –log (MIC) and correlated with the binding free energy (exp ΔG). Our findings establish a clear relationship between binding affinity, MIC, ZOI, and antibiotic concentration (c) for hydrophobic antibiotics under competitive inhibition in the disk diffusion method, the value of half Maximal inhibitory concentration IC_{50} is equal to the twice of minimum inhibitory concentration to the multiple of exponent of fraction of Zone of Inhibition 'x' and dissipative term "V" by twice the diffusion term. It will Contributing to a better understanding of resistance mechanisms.

Methods: The mathematical derivations are been validated by the published data available from the journal such as MIC, diffusion constant (D), ZOI (x) and IC50 values and Dissipative Value (V) has been calculated and calculated, IC50 values are compared with reported values.

Result: The data had been validated for ΔG, Dissipative Value (V), IC_{50} values from predictive to reported it had been observed that while calculating IC_{50} values of resistance and non-resistance susceptibility, the MIC value for resistance group is more hence the value of IC_{50} will depreciates and depend more on values of ZOI (x) and V the dissipative term Table 1 revels while analysing the ratio between resistance and non-resistance MIC and IC_{50} shows direct relationship it can be cross verified by reported and calculated values, Levofloxacin IC_{50} values ranges from 9.81- 3.2 µg/ml while calculated value is 10-1.6 µg/ml, binding energy ΔG reported is -6.57 and calculated is -7.72 to 7.82 K Cal/mol (Table 1) similarly with ciprofloxacin the value of IC_{50} for non-resistant to resistant is 3.5- 10 µg/ml where Mic is 0.001-0.5 µg/ml, binding energy reported -7.13 and calculated was 8.37 Kcal/mol. In the case of Tetracycline, the value of reported IC_{50} was 35- 47 µg/ml and calculated is 54-60 µg/ml and value of reported and calculated ΔG is -12.48 and -7.62 to 7.07K Cal/mol respectively. The reason behind difference of ΔG is, large value of V 4.16 and 2.23 for tetracycline.

Significance: The equations and derivation give better understanding between ZOI (x) and Dissipative Value (V) we can predict the resistance and factors effecting resistance which give low value of ZOI (x) against same concentration of antibiotics against non-resistance bacteria

KEYWORDS: MIC, Zone of Inhibition, Binding affinity, antibiotic resistance, IC_{50}.Competative Inhibition. Disk diffusion.

1. INTRODUCTION

Agar plate diffusion is a conventional method for determining the minimum inhibitory concentration (MIC) in semisolid media(Wiegand., 2008). Using the disc diffusion method, the antibiotic is diffused into the bacterially loaded agarose medium[2], preventing bacterial growth surrounding the source and generating a clean zone free of bacterial lawns(Jones., 1983) There is a clear correlation between the antibiotic concentration and the diameter of these zones(Abdelaziz, 2021). Plotting the inhibition zone radii (x mm squared values) against the logarithm of antibiotic concentration yields a graph. The value of MIC at the squared size of these zoi is provided by the linear regression's intercept(Bonev, 2008). Suggested by James and Boyan (2008)

1.1 HYDROPHOBIC COMPETITIVE INHIBITION

The antibiotics e.g. Tetracycline[6] and Gentamycin(Kwiecień, 2022) are relatively hydrophobic in nature and an alternate model of diffusion through the agar medium more slowly than predicted by the free diffusion(Bonev, 2008). Boneav Hooper proposed an alternative model of diffusion in which part of the drug may interact with the diffusion matrix or be lost through another dissipative mechanism so they carried a new term known as a dissipative mechanism The dissipative term was introduced in the diffusion equation

$$D\ \frac{d^2c(x,t)}{dx^2} + V\ \frac{d^2c(x,t)}{dx} - \frac{d^2c(x,t)}{dt} = 0 \ldots\ldots\ldots eq(1)$$

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Where V=coefficient characterizing the dissipation rate. Assuming that concentration distribution, which is the function of time and distance express

ed as the product of two functions each depends on 'x' and 't' only rearranging the above equation (1) which leads to two ordinary differential equations one describing the time dependence of concentration and other its variation in space.

The general form of the equation comprising the space part of the solution shows diffusion as absorption-dominated and exponentially decaying which can expressed in the case of semi-infinity media

$$In(MIC) = In(c) - (2D)^{-1}\,(V \pm \sqrt{(V^2 - 4D)}\,)x \ldots\ldots\ldots eq(2)$$

The equation and **Figure 1** describe an exponential reduction in the amount of antibiotic available for diffusion. This reduction may be attributed to antibiotic binding to the agarose matrix [8], as well as degradation during the diffusion process. When the dissipative term dominates ($V^2 >> 4D$), the solution exhibits an exponential decrease in antibiotic concentration with increasing distance. Conversely, when $V^2 = 4D$, the equation yields a critical solution that can be transformed from the vanishing velocity (V = 0) case into the free diffusion solution through the method of separation of variables.

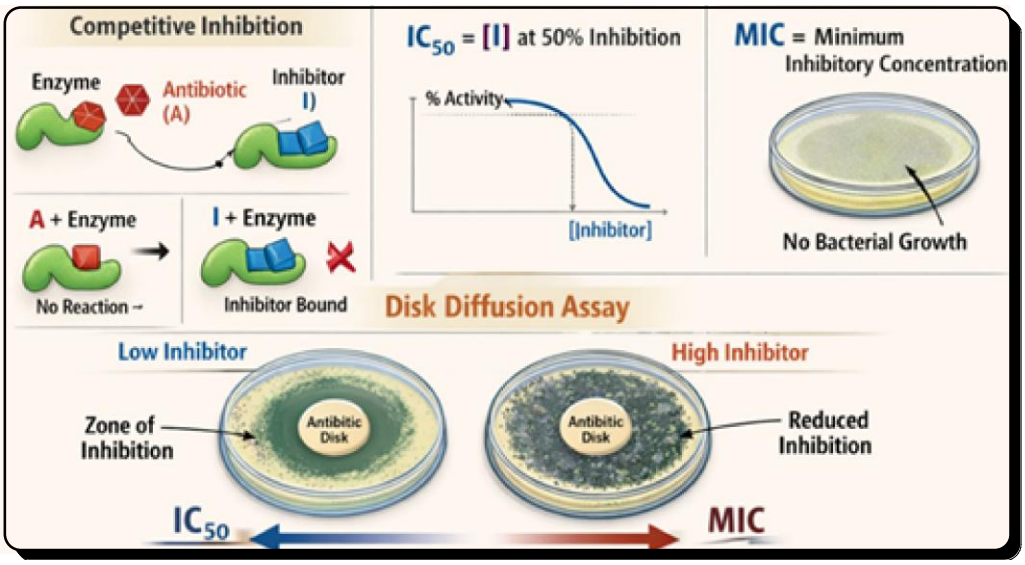

Figure 1: Exponential reduction in the amount of antibiotic available for diffusion.

For competitive inhibition, the reaction is as follows for inhibitor [I]

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$E + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EI$$

Figure 2. Reaction mechanism of competitive inhibition.

The equation [3] gives the initial rate of reaction

$$v_0 = \frac{V[S]}{k_m\left(1 + \frac{[I]}{k_i}\right) + [s]} \ldots\ldots\ldots eq(3)$$

The Michaelis-Menten constant is denoted by Km[9]. substrate concentration[s], where [I] is the inhibitor concentration and Vmax is the system's maximal velocity at maximum (saturating) substrate concentrations. restructuring the above equation (3) for the inhibitor concentration [I] in the above equation

$$[I] = -\left(\frac{k_i(Kmv_0 + [s](v_0 - V_{max}))}{v_0}\right) \ldots\ldots\ldots\ldots eq(4)$$

Ki= the inhibitor's dissociation constant k-3/k3

To calculate the competitive inhibitor's concentration the fraction $fv_o$of velocity vo, where 0 <fvo<1. The dissociation constant KD of an inhibitor that binds competitively can be understood as the Ki value [10].

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Substituting $In(c)$ in equation (2) with $[I] = \left(\frac{1}{fv_0} - 1\right) k_i \left(1 + \frac{[s]}{k_m}\right)$ of equation (4). Here C the concentration of an antibiotic is considered as the concentration of a competitive inhibitor [I] that yields a fraction fv_0 of velocity v_0 where $0 < f\ v_0 < 1$, the formula is deriv ed from the competitive inhibition model. The formula with respect to [I] we get eq (5)

Antibiotic concentration C = Inhibitor concentration I (assumption)

$$C = \left(\frac{1}{fv_0} - 1\right) k_i \left(1 + \frac{[s]}{k_m}\right)$$

$$ln(MIC) = In\left(\left(\frac{1}{fv_0} - 1\right) k_i \left(1 + \frac{[s]}{k_m}\right)\right) - (2D)^{-1}\left(V \pm \sqrt{(V^2 - 4D)}\right) x \ldots\ldots\ldots eq(5a)$$

1.2 THE DISSIPATIVE TERM

The equation describes an exponential reduction in the amount of antibiotic available for diffusion which might be due to the binding of antibiotic to the agarose matrix, degradation as another mechanism. when the dissipative term dominates $V^2 >> 4D$.

The solution exhibits an exponential decrease in concentration with distance If $V^2 = 4D$ equation gives to solution that can be converted from Vanishing V to a solution obtained from the free diffusion equation by separating of variable (Bonev., 2008).

Rearranging the above equation (5) for V dissipative term

$$V = \frac{[\frac{2D}{x} - (ln\ ln\left(\left(\frac{1}{fv_0} - 1\right) k_i \left(1 + \frac{[s]}{k_m}\right)\right) - ln\ ln\ (MIC)\ ]^2 + 4D}{[\frac{2D}{x} - (ln\ ln\left(\left(\frac{1}{fv_0} - 1\right) k_i \left(1 + \frac{[s]}{k_m}\right)\right) - ln\ ln\ (MIC)\ ]}$$

On simplifying the above equation $\because \left(\frac{1}{fv_0} - 1\right) k_i \left(1 + \frac{[s]}{k_m}\right) = c$

$$V = \frac{[\frac{2D}{x} - (ln\ ln\ c\ - ln\ ln\ (MIC)\ ]^2 + 4D}{[\frac{2D}{x} - (ln\ ln\ c\ - ln\ ln\ (MIC)\ ]}$$

On simplifying the equation 2 we get

$$V = \frac{D}{x} ln\left(\frac{c}{MIC}\right) + \frac{x}{ln\left(\frac{c}{MIC}\right)} \ldots\ldots\ldots eq(5b)$$

1.3 ZONE OF INHIBITION 'X'

rearranging the above equation (5) with respect to the "x" zone of inhibition

$$x = \frac{2D[ln\ ln\left(\frac{1}{fv_0} - 1\right) k_i \left(1 + \frac{[s]}{k_m}\right) - ln\ ln\ MIC]}{\left(V \pm \sqrt{(V^2 - 4D)}\right)} \ldots\ldots eq(6)$$

The zoi is directly proportional to D diffusion coefficient if D increases x increases(Akanbi., 2017), the ln MIC increases, which decreases the overall expression within the bracket, hence zoi decreases with increasing MIC. The inhibition constant's value Ki directly affects the minimum inhibitory concentration (MIC); a smaller ki indicates a better binding affinity[12], which implies a lesser amount of ligand (inhibitor) is required to impede the enzyme's activity, lowering the MIC value. A high Ki value indicates a lower binding affinity, which raises the MIC value equation (7). Under competitive inhibition, substrate and inhibitors vie for the same active site[12]. As such zoi is directly proportional to Ki but inhibition constant influences the MIC value so overall the resultant effect dominates, and the incremental value of ki was compensated by the MIC value in both ways. zoi is inversely related to the term $\left(V \pm \sqrt{(V^2 - 4D)}\right)$ in the denominator. If this term V dissipative coefficient increases, x decreases. The square root term can significantly affect this relationship depending on whether we add or subtract the square root of $\left(V \pm \sqrt{(V^2 - 4D)}\right)$.

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$$ki = \frac{ln\ ln\ (MIC)\ + \frac{1}{2D}\left(V \pm \sqrt{(V^2 - 4D)}\right)x}{ln\left(\frac{1}{fv_0} - 1\right)\left(1 + \frac{[s]}{k_m}\right)} ..eq(7)$$

As ln (MIC) increases, leading to a larger numerator, which increases ki Conversely, a lower MIC decreases ki. ki is directly proportional to the logarithm of the MIC.

The value of ki is inversely proportional to $ln\left(\frac{1}{fv_0} - 1\right)\left(1 + \frac{[s]}{k_m}\right)$, where$fv_0 = \frac{v_0}{v_i}$, v_0 is the velocity of enzymatic reaction without inhibitor and v_i velocity of reaction in the presence of inhibitor. If the equilibrium shifts in favour of v_i it will decrease the value of ki and hence the binding affinity of the inhibitor with the substrate increases[13]. In competitive inhibition, the value of k_m gets increased in the presence of an inhibitor while v_{max} remains the same it will reduce the ratio$\frac{[s]}{k_m}$ and the value of ki decreased.

Substituting the value of Ki= $e^{\frac{\Delta G}{RT}}$ in equation(Hata., 2021) (5)

$$MIC = EXP[In\left(\left(\frac{1}{fv_0} - 1\right)e^{\frac{\Delta G}{RT}}\left(1 + \frac{[s]}{k_m}\right)\right) - \frac{1}{2D}\left(V \pm \sqrt{(V^2 - 4D)}\right)x] ..eq(8)$$

On simplifying the equation (8)

Where Exp is exponential $e^{m-n} = e^m . e^{-n}$

$$MIC = \left(\frac{1}{fv_0} - 1\right)\left(1 + \frac{[s]}{k_m}\right)e^{\frac{\Delta G}{RT}}e^{-\frac{1}{2D}\left(V \pm \sqrt{(V^2 - 4D)}\right)x} ..eq(9)$$

Hence

As zoi ‘x’ increases, the expression $-\frac{1}{2D}\left(V \pm \sqrt{(V^2 - 4D)}\right)x$ becomes more negative in exponential function therefore MIC decreases as x increases. MIC is inversely related to x. If MIC increases, the drug becomes less effective, requiring a higher concentration to inhibit microbial growth. MIC is directly related to ΔG. As ΔG have to increase to compensate for the change brought by MIC, if there is no sufficient increase in ΔG the value of zoi x will decrease, resulting in resistance(Clarelli., 2020).

1.4 DERIVATION OF BINDING ENERGY

$$\Delta G = -RTln\{\frac{ln\ ln\ (MIC)\ + \frac{1}{2D}\left(V \pm \sqrt{(V^2 - 4D)}\right)x}{ln\left(\frac{1}{fv_0} - 1\right)\left(1 + \frac{[s]}{k_m}\right)}\} ..eq(10a)$$

Since ln (C) = ln (I)

The equation can be written as

$\Delta G = -RTln\left[\frac{ln(MIC) + \frac{1}{2D}\left(V \pm \sqrt{V^2 - 4D}\right)x}{ln(c)}\right]$ replacing $ln\left(\frac{1}{fv_0} - 1\right)\left(1 + \frac{[s]}{k_m}\right)$ with ln(C)

Here R= gas constant 1.985 x 10^{-3} kcal/mol k

T= Temperature 298.15K

$$\Delta G = -RTln\{\frac{(MIC)\ + \left(V \pm \sqrt{(V^2 - 4D)}\right)x)}{2D(k_m + [s])ln\left(\frac{1 - fv_0}{fv_0}\right)}\} ..eq(10b)$$

If the velocity of reaction with inhibitor v_i is half of the velocity without inhibitor v_0 then the value of $fv_0 = \frac{v_0}{v_i} where\ v_i = \frac{1}{2}v_0, fv_0 = 2$

eq (10b) can be written as

$\Delta G = -RTln\{\frac{(MIC) + \left(V \pm \sqrt{(V^2 - 4D)}\right)x)}{2D(k_m + [s])ln\left(\frac{1-2}{2}\right)}\} ..eq(10c)$

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$$\Delta G = -RTln\left\{\frac{(MIC) + \left(V \pm \sqrt{(V^2 - 4D)}\right)x)}{2D(k_m + [s])ln\left(-\frac{1}{2}\right)}\right\}..eq(11a)$$

The value of ln-1/2= 1.571i (iπ/2) where i is imaginary number

$$\Delta G = -RTln\left\{\frac{(MIC) + \left(V \pm \sqrt{(V^2 - 4D)}\right)x)}{\frac{2D(k_m + [s])i\pi}{2}}\right\}..(eq11b)$$

$$\Delta G = -RTln\left\{\frac{(MIC) + \left(V \pm \sqrt{(V^2 - 4D)}\right)x)}{D(k_m + [s])i\pi}\right\}..(11c)$$

$$\Delta G = -RTln\left\{\frac{(MIC) + \left(V \pm \sqrt{(V^2 - 4D)}\right)x)}{2D(k_m + [s])1.571\, i}\right\}..eq(11d)$$

$$x = \frac{2D[ln\ ln\left(\frac{1}{fv_0} - 1\right)e^{\frac{\Delta G}{RT}}\left(1 + \frac{[s]}{k_m}\right) - ln\ ln\ MIC]}{\left(V \pm \sqrt{(V^2 - 4D)}\right)}..eq(12)$$

An increase in ΔG will increase the zoi value, more the binding energy of the inhibitor, the larger the area cleared by the antibiotic. zoi is directly related to the negative log of MIC, inflating values of MIC leads to depreciation zoi x (Yang., 2021). The zoi is inversely proportional to $\left(V \pm \sqrt{(V^2 - 4D)}\right)$ if the dissipative term dominates 'V' the zoi decreases. Substituting the value of by $Ki = \frac{IC50}{1+\frac{[s]}{Km}}$ Cheng–Prusoff equation[17] applicable for competitive inhibition in equation (5)

$$ln(MIC) = In\left(\left(\frac{1}{fv_0} - 1\right)\frac{IC50}{1 + \frac{[s]}{Km}}\left(1 + \frac{[s]}{k_m}\right)\right) - (2D)^{-1}\left(V \pm \sqrt{(V^2 - 4D)}\right)x..eq(13)$$

$1 + \frac{[s]}{Km}$ is cancelled in equation (13)

$$ln(MIC) = In\left(\left(\frac{1}{fv_0} - 1\right)IC50\right) - (2D)^{-1}\left(V \pm \sqrt{(V^2 - 4D)}\right)x \ldots\ldots..eq(14)$$

Minimum inhibitory concentration is directly proportional to IC_{50}, the more potent the drug will be potent less the value of IC_{50}, the lower the value of MIC. equation (14)

Rearranging the above equation (13) for the "x" Zone of inhibition

$$x = \frac{2D(log\ log\ (MIC)\ - log\ log\ \left(\frac{1}{fvo} - 1\right)\ IC50)}{\left(V \pm \sqrt{(V^2 - 4D)}\right)} \ldots\ldots..eq(15)$$

As IC_{50}decrease it means a more potent inhibitor and more will be the value of zoi.

Rearranging the above equation (14) for MIC minimum inhibitory concentration we get

$$MIC = \frac{(fv_0 - 1)IC50\ e^{\frac{-(x(V \pm \sqrt{(V^2 - 4D)})}{2D}}}{fvo} \ldots\ldots..eq(16)$$

1.5 RELATIONSHIP BETWEEN MIC AND IC_{50}

The MIC value is dependent on the negative exponent of the zoi value as MIC increase the value of x decreases exponentially (Veiga., 2019). MIC is inversely related to fvo it is the ratio of $\frac{v_0}{v_i}$, v_0 is the velocity of the reaction without an inhibitor. v_i is the velocity of reaction with inhibitor. At IC_{50} concentration the velocity of product formation is reduced to half of the velocity observed without inhibitor present, $v_i = 1/2v_0$ substituting the value of v_i in the above equation (16) becomes

$$MIC = \frac{IC50\ e^{\frac{-(x(V \pm \sqrt{(V^2 - 4D)})}{2D}}}{2} \ldots\ldots..eq(17)$$

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Equation (17) can be stated as

$$MIC = \frac{(fv_0 - 1)IC50 \;\; 2.71828^{\frac{(0.5x(V\pm\sqrt{(V^2-4D)})}{D}}}{fvo} \ldots\ldots..eq(18)$$

Driving the equation (18) for IC50

$$IC50 = \frac{fvo\ MIC\ e^{\frac{-(x(V\pm\sqrt{(V^2-4D)})}{2D}}}{(fv_0 - 1)} \ldots\ldots..eq(19)$$

here value of e=2.7182 Euler's number

Equation (19) can stated as

$$IC50 = \frac{fvo\ MIC\ 2.71828^{\frac{(0.5x(V\pm\sqrt{(V^2-4D)})}{D}}}{(fv_0 - 1)} \ldots\ldots..eq(20)$$

(where 2 in denominator is placed 0.5 in numerator)

IC_{50} is a measure of potency and it is directly related to minimum inhibitory concentration, at IC_{50} Concentration the value of $v_i=1/2v_{0,}$ substituting the value in equation (20)

$$IC50 = 2\ MIC\ 2.71828^{\frac{(0.5x(V\pm\sqrt{(V^2-4D)})}{D}} \ldots\ldots..eq21$$

or

$$IC50 = 2MIC\ e^{\frac{-(x(V\pm\sqrt{(V^2-4D)})}{2D}} \ldots\ldots..eq(22)$$

Table 1: Validation of calculated values from reported values

Sno	**Drug**	**Microorganism**	**Drug Conc (µ)**	**ZOIM mm**	**MIC µg/ml (Reported)**	**Ratio between resistance/ non resistance MIC**	**IC50 µg/ml (Reported)**	**Molecular binding energies ΔG**	**Diffusion Constant (D)**	**calculated coefficient characterizing the dissipation rate (V)**	**Calculated ΔG Kcal/ Mol**	**Difference between practical and theoretical energy**	**Calculate IC50 µg/ml**	**Ratio between resistance/ non resistance IC50 µg/ml**	**References**
	Formula used									*	**	***	****		
1	Levofloxacin	P.aeruginosa	5	50	0.001	2000 2000	8.6-9.81 (a)	-6.57	4x10-6 cm2/s	0.5871	-7.72	1.15	10.005 (b)	0.16 0.16	(a) [19] (b) [20]
2	Levofloxacin	P.aeruginosa	5	18	2		3.2 (C)	-6.57	4x10-6 cm2/s	1.964	-7.82	1.25	1.6		(c) [21]
3	Ciprofloxacin	P. aeruginosa	5	50	0.06	8.3 8.3	0.008 (g) 3.5(d)	-7.13	1.5x 10-6 cm2/s	1.131	8.37	1.24	0.143	2.817 2.817	(d) [22] [23]
4	Ciprofloxacin	P. aeruginosa	5	26	0.5		3.5(d)	-7.13	1.5x 10-6 cm2/s	1.129	-8.3	1.17	10		
5	Tetracycline	E. coli	30	19	4	4 4	47	-12.4(e)	6x10-6 cm2/s	4.16	-7.62	4.87	54-(f)	1.11 1.11	(e) [24] (f) [25]
6	Tetracycline	E. coli	30	14	16		47	-12.48	6x10-6 cm2/s	2.23	7.07	5.33	60		

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$V = \frac{D}{x} ln\left(\frac{c}{MIC}\right) + \frac{x}{ln\left(\frac{c}{MIC}\right)}$ ** $\Delta G = -RTln\left[\frac{ln(MIC)+\frac{1}{2D}\left(V \pm \sqrt{V^2-4D}\right)x}{ln(c)}\right]$ *** $\Delta\Delta G = \Delta G_{reported} - \Delta G_{calculated}$ **** $IC50 = 2MIC\ e^{\frac{-(x(V \pm \sqrt{(V^2-4D)}))}{2D}}$

2. DISCUSSION

This mathematical model is suitable for hydrophobic Antibiotics like Aminoglycoside, Tetracycline group and macrolides, for hydrophobic antibiotics Boneav and Hooper proposed a different model depending on a drug may interact with agar matrix or loss through dissipative mechanism, so they coined the term dissipative coefficient 'V'. The value of MIC is greatly affected by the dissipative coefficient and zoi 'x'. As zoi increases the term $\frac{1}{2D}\left(V \pm \sqrt{(V^2 - 4D)}\right)x$Becomes more negative in exponential function and thus MIC decreases in equation (9).ΔG binding energy is directly related to zoi 'x' increment of zoi leads to simultaneous increase ΔG.The Michaels Menten appear in the numerator as well as the denominator. For competitive inhibition. The ΔG is inversely proportional to substrate concentration[26]. On considering the enzymatic reaction with inhibitor v_i is half of the velocity without inhibitor v_0 thenΔG is inversely proportional to $i\pi/2$ where i is an imaginary number and π a periodic function[27].Equation (5) by introducing the Cheng-Prusoff equation. $Ki = \frac{IC50}{1+\frac{[s]}{Km}}$ while considering the velocity of reaction v_i=1/2v_0,the value of MIC is half the value of IC50 raised to the exponent of$\frac{-(x(V \pm \sqrt{(V^2-4D)}))}{2D}$similarly, IC_{50} is twice the value of MIC[26] to the negative value of $\frac{-(x(V \pm \sqrt{(V^2-4D)}))}{2D}$., the Value of V dissipation coefficient not only depends on the drug binding with agar media but fraction of drug may lost or entrapped in bacterial thick cell mucilage,

Enzymatic degradation and drug fraction trapped in transporter proteins. Calculating that fractions are beyond the scope of this paper.

3. CONCLUSION

Hydrophilic antibiotic includes Aminoglycoside, tetracycline and macrolides group. The model is applicable to the drugs following a competitive inhibition mechanism and is hydrophobic in nature. This model gives a relationship between MIC, ZOI, and binding affinity mathematically a correlation exists between IC_{50} with MIC. Under condition $IC50 = 2MIC\ e^{\frac{-(x(V \pm \sqrt{(V^2-4D)}))}{2D}}$. The relationship between zoi and MIC is direct and logarithmic, As MIC increases, zoi becomes more negative. Conversely, as MIC decreases, ZOI becomes more positive. Dissipative coefficient is dominant in deciding the magnitude of zoi for hydrophobic inhibitors; MIC is directly proportional to IC50. States that Minimum inhibitory concentration is equal to the half of the Maximal inhibitory concentration multiplied by exponent of ZOI 'x' and a dissipative term divided by twice of diffusion coefficient. Gibbs free energy is inversely related through a double logarithmic function, MIC is an exponential function of the exponential of -ΔG. We can use this mathematical model for calculating zoi, binding energy, inhibition constant and MIC.

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ETHICS DECLARATIONS

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

ETHICAL APPROVAL

This research did not involve any human participants and/or animals.

INFORMED CONSENT

All authors are aware of the submission and agree to submit this manuscript to Journal of Food Science and Technology journal.

AVAILABILITY OF DATA AND MATERIAL (DATA TRANSPARENCY)

Not applicable.

CODE AVAILABILITY (SOFTWARE APPLICATION OR CUSTOM CODE)

Not applicable.

CONFLICT OF INTEREST

No conflicts of interest regarding the publication of the manuscript

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APPENDIX A

SUPPORTING FILE FOR MATHEMATICAL CALCULATION

Calculation of dissipation coefficient for levofloxacin by formula table 1 row 1

$$In(MIC) = In(c) - (2D)^{-1}\,(V \pm \sqrt{(V^2 - 4D)}\,)x \ldots\ldots eq2$$

Given values Drug concentration= 5 µg

MIC= 0.001 µg/ml

ZOI= 50 mm= 5 cm

D= 4 X 10^{-6} cm^2 /sec

Step 1 calculate the logarithmic term

$$ln\ ln\left(\frac{c}{MIC}\right) = ln\ ln\left(\frac{5}{0.001}\right) = ln(5000) = 8.517$$

Step 2 calculate

$$A = \frac{2D}{x}\,ln\ ln\left(\frac{C}{MIC}\right)$$

$$A = \frac{8\times10^{-6}}{5}(-8.517) = 1\cdot 363X10^{-5}$$

$$V = \sqrt{V^2 - 4D} = 1\cdot 363X10^{-5}$$

Step 3 solve for V

$$V = \frac{A^2 + 4D}{2A}$$

$$V = \frac{(1\cdot 363X10^{-5})^2 + 1\cdot 6X10^{-5}}{2(\,1\cdot 363X10^{-5})}$$

V=0.5871

Calculation of ΔG for levofloxacin with *P aeruginosa* Table 1 row 1

Using equation

$$\Delta G = -RTln\left[\frac{ln(MIC) + \frac{1}{2D}\left(V \pm \sqrt{V^2 - 4D}\right)x}{ln(c)}\right]$$

with:

- $MIC = 0.001$ µg/ml
- $x = 50\ mm = 5.0\ cm$
- $c = 5$µg
- $V = 0.5871$
- $D = 4 \times 10^{-6}\ cm^2/s$
- $R = 8.314\ J\ mol^{-1}K^{-1}$
- $T = 298\ K$

STEP 1. CALCULATE CONSTANTS

$ln(MIC) = ln(0.001) = -6.907755$ $ln(c) = ln(5) = 1.609438$ $\sqrt{V^2 - 4D} = \sqrt{0.5871^2 - 4(4 \times 10^{-6})} = 0.587086$

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(+) BRANCH

$V + \sqrt{V^2 - 4D} = 1.174186\ \frac{\left(V+\sqrt{V^2-4D}\right)x}{2D} = \frac{1.174186\times5}{8\times10^{-6}} = 733866.4$

Then

$\frac{ln(MIC)+733866.4}{ln(5)} = 455977.5 \quad ln(455977.5) = 13.030 \quad \Delta G = -(8.314)(298)(13.030) = -32277\ J/mol \quad \Delta G = -32.28\ kJ/mol$

or

$$\Delta G = -7.72\ \boldsymbol{kcal/mol}$$

(−) BRANCH

$V - \sqrt{V^2 - 4D} = 1.3626 \times 10^{-5}\ \frac{\left(V-\sqrt{V^2-4D}\right)x}{2D} = 8.516$

Then

$\frac{-6.907755+8.516}{1.609438} = 0.999\ ln(0.999) \approx -0.001\ \Delta G \approx +0.0025\ kJ/mol$

or

$$\Delta G \approx +0.0006\ kcal/mol$$

FINAL RESULTS

Branch	ΔG (kJ/mol)	ΔG (kcal/mol)
+	−32.28	−7.72
−	+0.0025	+0.0006

The **positive branch** gives the thermodynamically favorable value:

$$\Delta G \approx -7.72\ kcal/mol$$

which is generally the physically meaningful solution for a favorable binding process.

Calculation of IC_{50} for Levofloxacin

Using the equation:

$IC_{50} = 2\ MIC\ e^{\left(\frac{x\left(V\pm\sqrt{V^2-4D}\right)}{2D}\right)}$ where Euler's number ≈2.7182

GIVEN

- $MIC = 0.001\ \mu g/mL$
- $x = 50\ mm = 5\ cm$
- $V = 0.587\ cm/s$
- $D = 4 \times 10^{-6}\ cm^2/s$

STEP 1. CALCULATE THE SQUARE-ROOT TERM

$$\sqrt{V^2 - 4D} = \sqrt{0.587^2 - 4(4 \times 10^{-6})} = 0.586986$$

STEP 2. CALCULATE IC_{50}

Using the (−) branch

Exponent:

$$\frac{5(0.587-0.586986)}{2(4\times10^{-6})} = 8.517986\ e^{8.517986} \approx 5002.56$$

Therefore,

$$IC_{50} = 2(0.001) \times 5002.56 = 10.005\ \boldsymbol{\mu g/mL}$$

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Using the (+) branch

Exponent:

$$\frac{5(0.587 + 0.586986)}{8 \times 10^{-6}} = 733741.48$$

Hence,

$$IC_{50} = 0.002\, e^{733741.48},$$

which is a large value and is not physically meaningful

With this revised form of the equation, the **(−) branch** gives **$IC_{50} \approx 10.005$ µg/mL**, which is greater than the MIC (0.001 µg/mL),

Calculation of V for levofloxacin with *P aeruginosa* Table 1 row 2

Using equation

$$ln(MIC) = ln(c) - \frac{1}{2D}\left(V - \sqrt{V^2 - 4D}\right)x$$

and the given values:

- $c = 5\ \mu g$
- $MIC = 2\ \mu g/mL$
- $D = 4 \times 10^{-6}\ cm^2/s$
- $x = 18\ mm = 1.8\ cm$

STEP 1. CALCULATE THE LOGARITHMIC TERM

$$ln\left(\frac{c}{MIC}\right) = ln\left(\frac{5}{2}\right) = 0.9162907$$

STEP 2. CALCULATE *A*

$A = \frac{2D}{x} ln\left(\frac{c}{MIC}\right) = \frac{2(4\times10^{-6})}{1.8} \times 0.9162907 = 4.0724 \times 10^{-6}$

Thus,

$$V - \sqrt{V^2 - 4D} = 4.0724 \times 10^{-6}$$

STEP 3. SOLVE FOR *V*

Using

$$V = \frac{A^2 + 4D}{2A},$$

where

- $A = 4.0724 \times 10^{-6}$
- $4D = 1.6 \times 10^{-5}$,

gives

$V = \frac{(4.0724\times10^{-6})^2 + 1.6\times10^{-5}}{2(4.0724\times10^{-6})}\ V \approx 1.964$

FINAL ANSWER

$$V \approx 1.964$$

Calculation of ΔG for levofloxacin with P aeruginosa Table 1 row 2

Using equation

$$\Delta G = -RTln\left[\frac{ln(MIC) + \frac{1}{2D}\left(V \pm \sqrt{V^2 - 4D}\right)x}{ln(c)}\right]$$

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with

- $V = 1.964$
- $D = 4 \times 10^{-6}\ cm^2/s$
- $x = 18\ mm = 1.8\ cm$
- $MIC = 2\ \mu g/mL$
- $c = 5\ \mu g$
- $R = 8.314\ J\ mol^{-1}K^{-1}$
- $T = 298\ K$

INTERMEDIATE VALUES

$ln(MIC) = ln(2) = 0.693147$ $ln(c) = ln(5) = 1.609438$ $\sqrt{V^2 - 4D} = \sqrt{1.964^2 - 4(4 \times 10^{-6})} \approx 1.963996$

USING THE + BRANCH

$$\Delta G \approx -32.74\ kJ/mol$$

or

$$\Delta G \approx -7.82\ \boldsymbol{kcal/mol}$$

USING THE − BRANCH

Since the value of $V = 1.964$ was obtained from the **negative-root** solution, substituting it back gives

$$\frac{ln(MIC) + \frac{1}{2D}\left(V - \sqrt{V^2 - 4D}\right)x}{ln(c)} \approx 1.00008,$$

so

$$\Delta G \approx -0.00020\ kJ/mol$$

or

$$\Delta G \approx -0.00005\ kcal/mol$$

SUMMARY

Branch	ΔG (kJ/mol)	ΔG (kcal/mol)
+	−32.74	−7.82
−	−0.00020	−0.00005

Calculation of IC_{50} for Levofloxacin Table 1 row 2

Using equation

$$IC_{50} = 2\,MIC\ e^{-\frac{x\left(V \pm \sqrt{V^2 - 4D}\right)}{2D}}$$

with the values:

- $MIC = 2\ \mu g/mL$
- $V = 1.964$
- $D = 4 \times 10^{-6}\ cm^2/s$
- $x = 18\ mm = 1.8\ cm$
- $e = 2.7182$

USING THE NEGATIVE BRANCH (THE ONE USED TO OBTAIN $V = 1.964$)

From the previous calculation,

$$V - \sqrt{V^2 - 4D} = 4.0724 \times 10^{-6}.$$

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Therefore,

$$\frac{x\left(V-\sqrt{V^2-4D}\right)}{2D}=\frac{1.8(4.0724\times10^{-6})}{8\times10^{-6}}=0.91629.$$

Hence,

$$IC_{50}=2(2)\times e^{-0.91629}=4\times(2.7182)^{-0.91629}.$$

Evaluating the exponential,

$$(2.7182)^{-0.91629}\approx0.4000.$$

Therefore,

$$IC_{50}\approx1.60\ \mu\boldsymbol{g/mL}$$

POSITIVE BRANCH

For the positive branch,

$$\frac{x\left(V+\sqrt{V^2-4D}\right)}{2D}\approx8.84\times10^{5},$$

so

$$e^{-8.84\times10^{5}}\approx0,$$

giving

$$IC_{50}\approx0\ \mu g/mL$$

which is not physically meaningful.

FINAL RESULT

Using the physically consistent **negative branch**:

$$IC_{50}\approx1.60\ \mu\boldsymbol{g/mL}$$

Calculation of V for Ciprofloxacin with *P aeruginosa* Table 1 row 3

$$ln(MIC)=ln(C)-\frac{x}{2D}\left(V-\sqrt{V^2-4D}\right),$$

and assuming the same value as before:

- $C=5$
- $MIC=0.06$
- $x=50\ mm=5.0\ cm$
- $D=1.5\times10^{-6}\ cm^2/s$

STEP 1. CALCULATE THE LOGARITHMS

$ln(5)=1.609438$, $ln(0.06)=-2.813411$.

Therefore,

$$ln(C)-ln(MIC)=1.609438-(-2.813411)=4.422849.$$

STEP 2. CALCULATE a

Let

$$a=\frac{2D}{x}[ln(C)-ln(MIC)].$$

Substituting,

$$a=\frac{2(1.5\times10^{-6})}{5}\times4.422849=2.65371\times10^{-6}.$$

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Thus,

$$V - \sqrt{V^2 - 4D} = 2.65371 \times 10^{-6}.$$

STEP 3. SOLVE FOR V

Using

$$V = \frac{a^2 + 4D}{2a},$$

where

$$4D = 6.0 \times 10^{-6},$$

gives

$$V = \frac{(2.65371 \times 10^{-6})^2 + 6.0 \times 10^{-6}}{2(2.65371 \times 10^{-6})} \approx 1.1306\ cm/s.$$

FINAL ANSWER

$$V \approx 1.131\ \boldsymbol{cm/s}$$

Calculation of ΔG for Ciprofloxacin with P aeruginosa Table 1 row 3

Using equation

$$\Delta G = -RTln\left[\frac{ln(MIC) + \frac{1}{2D}(V \pm \sqrt{V^2 - 4D})x}{ln(c)}\right]$$

with the values:

- $D = 1.5 \times 10^{-6}\ cm^2/s$
- $MIC = 0.001$µg/ml
- $V = 0.669$
- $c = 5$µg
- $x = 50\ mm = 5.0\ cm$
- $R = 8.314\ J\ mol^{-1}K^{-1}$
- $T = 298\ K$

INTERMEDIATE CALCULATIONS

$ln(MIC) = ln(0.001) = -6.907755$ $ln(c) = ln(5) = 1.609438$ $\sqrt{V^2 - 4D} = \sqrt{0.669^2 - 4(1.5 \times 10^{-6})} = 0.6689955$

(+) BRANCH

$\frac{1}{2D}(V + \sqrt{V^2 - 4D})x = \frac{(1.3379955)(5)}{3\times10^{-6}} = 2.22999 \times 10^6$ $\Delta G = -35.02\ kJ/mol$

Converting to kcal/mol:

$$\Delta G = \frac{-35.02}{4.184} = -8.37\ kcal/mol$$

(−) BRANCH

$\frac{1}{2D}(V - \sqrt{V^2 - 4D})x = 7.48$ $\Delta G = +2.61\ kJ/mol$

Converting:

$$\Delta G = \frac{2.61}{4.184} = +0.62\ kcal/mol$$

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FINAL RESULTS

Branch	ΔG (kJ/mol)	ΔG (kcal/mol)
+	−35.02	−8.37
−	+2.61	+0.62

For antimicrobial diffusion model, the **positive-branch solution** gives:

$$\Delta G \approx -8.37\ kcal/mol$$

which is the thermodynamically favorable solution and is generally the one used when interpreting binding affinity.

Calculation of V for Ciprofloxacin with *P aeruginosa* Table 1 row 4

Using the same equation

$$ln(MIC) = ln(C) - \frac{x}{2D}\left(V - \sqrt{V^2 - 4D}\right),$$

with

- $C = 5$
- $MIC = 0.5$
- $x = 26\ mm = 2.6\ cm$
- $D = 1.5 \times 10^{-6}\ cm^2/s$

Step 1. Calculate the logarithmic term

$ln(5) = 1.609438$, $ln(0.5) = -0.693147$,

so

$$ln(C) - ln(MIC) = 2.302585.$$

Step 2. Calculate a

$$a = \frac{2D}{x}[ln(C) - ln(MIC)] = \frac{2(1.5 \times 10^{-6})}{2.6} \times 2.302585 = 2.65683 \times 10^{-6}.$$

Thus,

$$V - \sqrt{V^2 - 4D} = 2.65683 \times 10^{-6}.$$

Step 3. Solve for V

Using

$$V = \frac{a^2 + 4D}{2a},$$

Where

$$4D = 6.0 \times 10^{-6},$$

gives

$$V = \frac{(2.65683 \times 10^{-6})^2 + 6.0 \times 10^{-6}}{2(2.65683 \times 10^{-6})} \approx 1.129.$$

Final answer

$$V \approx 1.129\ cm/s$$

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Calculation of V for tetracycline Table 1 row 5

Using the equation

$$ln(MIC) = ln(C) - \frac{x}{2D}\left(V \pm \sqrt{V^2 - 4D}\right),$$

with

- $MIC = 19\ \mu g/mL$
- $C = 30\ \mu g$
- $x = 19\ mm = 1.9\ cm$
- $D = 6 \times 10^{-6}\ cm^2/s$

we first calculate

$ln(19) = 2.9444$, $ln(30) = 3.4012$,

so

$$ln(C) - ln(MIC) = 0.4568.$$

Hence,

$$0.4568 = \frac{1.9}{2(6 \times 10^{-6})}\left(V \pm \sqrt{V^2 - 2.4 \times 10^{-5}}\right).$$

Since

$$\frac{1.9}{1.2 \times 10^{-5}} = 1.5833 \times 10^5,$$

we obtain

$$V \pm \sqrt{V^2 - 2.4 \times 10^{-5}} = 2.885 \times 10^{-6}.$$

However, **this equation has no real solution**.

Here's why:

- The **"+" branch** is impossible because

  $V + \sqrt{V^2 - 4D} \geq V$,

  and cannot be as small as 2.885×10^{-6}.
- The **"−" branch** requires

  $V - \sqrt{V^2 - 4D} = 2.885 \times 10^{-6}$,

  but solving it gives

  $V \approx 4.16$

Calculation of ΔG for Tetracycline with E.coli Table 1 row 5

Using equation,

$$\Delta G = -RTln\left[\frac{ln(MIC) + \frac{x}{2D}\left(V \pm \sqrt{V^2 - 4D}\right)}{ln(C)}\right],$$

and the values

- $V = 4.16\ cm\ s^{-1}$
- $MIC = 19$
- $C = 30$
- $x = 19\ mm = 1.9\ cm$
- $D = 6 \times 10^{-6}\ cm^2/s$

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- $R = 8.314\ J\ mol^{-1}K^{-1}$
- $T = 298\ K$

First,

$ln(MIC) = ln(19) = 2.9444, ln(C) = ln(30) = 3.4012.$

Next,

$$\sqrt{V^2 - 4D} = \sqrt{4.16^2 - 4(6 \times 10^{-6})} = \sqrt{17.3056 - 0.000024} = 4.159997.$$

USING THE "+" BRANCH

$V + \sqrt{V^2 - 4D} = 4.16 + 4.159997 = 8.319997, \frac{x}{2D} = \frac{1.9}{2(6\times10^{-6})} = 158333.33, \frac{x}{2D}\left(V + \sqrt{V^2 - 4D}\right) = 1.3173 \times 10^6.$

Therefore,

$$ln\left(\frac{2.9444 + 1.3173 \times 10^6}{3.4012}\right) = ln(387306) = 12.867.$$

Hence,

$$\Delta G = -(8.314)(298)(12.867) = -3.19 \times 10^4\ J\ mol^{-1}$$

or

$$\Delta G \approx -31.9\ kJ\ mol^{-1}.$$

USING THE "−" BRANCH

$V - \sqrt{V^2 - 4D} = 4.16 - 4.159997 = 2.88 \times 10^{-6}, \frac{x}{2D}\left(V - \sqrt{V^2 - 4D}\right) \approx 0.456.$

Thus,

$$ln\left(\frac{2.9444 + 0.456}{3.4012}\right) \approx ln(0.9998) \approx -2.4 \times 10^{-4},$$

giving

$$\Delta G \approx +0.60\ J\ mol^{-1} \approx 0.0006\ kJ\ mol^{-1}.$$

RESULTS

- **Using** $+\sqrt{V^2 - 4D}$**:** $\Delta G \approx -31.9\ kJ\ mol^{-1}$
- **Using** $-\sqrt{V^2 - 4D}$**:** $\Delta G \approx +0.0006\ kJ\ mol^{-1}$

Using calculated values at $T = 298\ K$:

- $R = 8.314\ J\ mol^{-1}K^{-1}$
- $1\ kcal = 4184\ J$

PLUS (+) BRANCH

$$\Delta G = -31.9\ kJ\ mol^{-1}.$$

Converting to kcal/mol:

$\Delta G = \frac{-31.9}{4.184} = -7.62\ kcal\ mol^{-1}. \Delta G \approx -7.62\ kcal\ mol^{-1}$

MINUS (−) BRANCH

$$\Delta G = +0.60\ J\ mol^{-1}.$$

Converting to kcal/mol:

$\Delta G = \frac{0.60}{4184} = 1.43 \times 10^{-4}\ kcal\ mol^{-1}. \Delta G \approx +1.4 \times 10^{-4}\ kcal\ mol^{-1}$

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FINAL RESULTS

- **Using $V + \sqrt{V^2 - 4D}$:**

$$\Delta G \approx -7.62\ kcal\ mol^{-1}$$

Calculation of V for tetracycline Table 1 row 6

Using the equation

$$ln(MIC) = ln(C) - \frac{x}{2D}\left(V - \sqrt{V^2 - 4D}\right),$$

(which is the branch that gave the finite value $V \approx 4.16$ in the previous calculation),

with

- $x = 14\ mm = 1.4\ cm$
- $MIC = 16$
- $C = 30$
- $D = 6 \times 10^{-6}\ cm^2/s$

we have

$$ln(16) = 2.77259, \qquad ln(30) = 3.40120.$$

Thus,

$$ln(30) - ln(16) = 0.62861.$$

Now,

$$V - \sqrt{V^2 - 4D} = \frac{2D}{x}[ln(30) - ln(16)].$$

Substituting the values,

$$\frac{2D}{x} = \frac{1.2 \times 10^{-5}}{1.4} = 8.5714 \times 10^{-6},$$

so

$$V - \sqrt{V^2 - 4D} = 8.5714 \times 10^{-6} \times 0.62861 = 5.388 \times 10^{-6}.$$

Using the identity

$$V - \sqrt{V^2 - 4D} = a,$$

where $a = 5.388 \times 10^{-6}$, the solution is

$$V = \frac{a^2 + 4D}{2a}.$$

Substituting,

$$V = \frac{(5.388 \times 10^{-6})^2 + 2.4 \times 10^{-5}}{2(5.388 \times 10^{-6})} \approx 2.23.$$

FINAL ANSWER

$$V \approx 2.23\ \boldsymbol{cm/s}$$

This value is obtained using the minus branch,

$$V - \sqrt{V^2 - 4D},$$

Calculation of ΔG for Tetracycline with E.coli Table 1 row 6

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Using

$$\Delta G = -RTln\left[\frac{ln(MIC) + \frac{x}{2D}\left(V \pm \sqrt{V^2 - 4D}\right)}{ln(C)}\right],$$

with

- $V = 2.23\ cm/s$
- $MIC = 16$
- $C = 30$
- $x = 14\ mm = 1.4\ cm$
- $D = 6 \times 10^{-6}\ cm^2/s$
- $R = 8.314\ J\ mol^{-1}K^{-1}$
- $T = 298\ K$

we first compute

$ln(16) = 2.77259, \quad ln(30) = 3.40120, \sqrt{V^2 - 4D} = \sqrt{2.23^2 - 2.4 \times 10^{-5}} = 2.229995.$

Also,

$$\frac{x}{2D} = \frac{1.4}{1.2 \times 10^{-5}} = 116666.67.$$

(1) PLUS (+) BRANCH

$$V + \sqrt{V^2 - 4D} = 2.23 + 2.229995 = 4.459995.$$

Therefore,

$$\frac{x}{2D}\left(V + \sqrt{V^2 - 4D}\right) = 116666.67 \times 4.459995 = 520332.8.$$

Then,

$\frac{ln(16)+520332.8}{ln(30)} = 152987.\ ln(152987) = 11.938.$

Hence,

$$\Delta G = -(8.314)(298)(11.938) = -29562\ J/mol = -29.56\ kJ/mol.$$

Converting to kcal/mol,

$$\Delta G = -7.07\ kcal/mol$$

(2) MINUS (−) BRANCH

$$V - \sqrt{V^2 - 4D} = 5.388 \times 10^{-6}.$$

Then,

$$\frac{x}{2D}\left(V - \sqrt{V^2 - 4D}\right) = 0.6286.$$

Therefore,

$\frac{2.77259+0.6286}{3.40120} = 0.999997, ln(0.999997) = -2.7 \times 10^{-6}.$

Hence,

$\Delta G = 0.0067\ J/mol = 1.6 \times 10^{-6}\ kcal/mol.\ \Delta G = +1.6 \times 10^{-6}\ kcal/mol$

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FINAL RESULTS

Branch	ΔG (kJ/mol)	ΔG (kcal/mol)
$V+\sqrt{V^2-4D}$	-29.56	-7.07
$V-\sqrt{V^2-4D}$	$+6.7\times10^{-6}$	$+1.6\times10^{-6}$

The **plus branch** gives a typical binding free energy (about **−7 kcal/mol**), whereas the **minus branch** yields a value essentially equal to zero.

Using the equation

$$ln(MIC)=ln(C)-\frac{x}{2D}\left(V-\sqrt{V^2-4D}\right),$$

(which is the branch that gave the finite value $V\approx4.16$ in the previous calculation),

with

- $x=14\ mm=1.4\ cm$
- $MIC=16$
- $C=30$
- $D=6\times10^{-6}\ cm^2/s$

we have

$$ln(16)=2.77259,\qquad ln(30)=3.40120.$$

Thus,

$$ln(30)-ln(16)=0.62861.$$

Now,

$$V-\sqrt{V^2-4D}=\frac{2D}{x}[ln(30)-ln(16)].$$

Substituting the values,

$$\frac{2D}{x}=\frac{1.2\times10^{-5}}{1.4}=8.5714\times10^{-6},$$

so

$$V-\sqrt{V^2-4D}=8.5714\times10^{-6}\times0.62861=5.388\times10^{-6}.$$

Using the identity

$$V-\sqrt{V^2-4D}=a,$$

where $a=5.388\times10^{-6}$, the solution is

$$V=\frac{a^2+4D}{2a}.$$

Substituting,

$$V=\frac{(5.388\times10^{-6})^2+2.4\times10^{-5}}{2(5.388\times10^{-6})}\approx2.23.$$

Final answer

$$V\approx2.23\ \boldsymbol{cm/s}$$

This value is obtained using the **minus branch**,

$$V-\sqrt{V^2-4D},$$

Calculation of IC_{50} for Tetracycline Table 1 row 6

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Using equation:

$$IC_{50} = 2\,MIC\;e^{\left(\frac{x\left(V-\sqrt{V^2-4D}\right)}{2D}\right)}$$

and keeping the same parameters from the previous calculation:

- $MIC = 16\ \mu g/mL$
- $x = 14\ mm = 1.4\ cm$
- $V = 2.23\ cm/s$
- $D = 6 \times 10^{-6}\ cm^2/s$

From the calculation,

$$\frac{x\left(V-\sqrt{V^2-4D}\right)}{2D} \approx 0.628$$

and

$$e^{0.628} \approx 1.874.$$

Therefore,

$$IC_{50} = 2(16) \times 1.874 = 32 \times 1.874 \approx 59.97\ \mu g/mL$$

FINAL RESULT

Parameter	Value
MIC	16 µg/mL
Zone of inhibition (x)	14 mm
Velocity (V)	2.23 cm/s
Diffusion coefficient (D)	6×10^{-6} cm²/s
Caculated IC₅₀	**≈ 60.0 µg/mL**

So, with these parameters, the calculated **IC₅₀ is approximately** $60\ \mu g/mL$

Symbol	Parameter / Factor	Unit	Recommended notation
(C)	Initial antibiotic/drug concentration	µg/mL	(C)
(MIC)	Minimum inhibitory concentration	µg/mL	(MIC)
(IC_{50})	Concentration producing 50% inhibition	µg/mL	(IC_{50})
(x)	Zone of inhibition / radial diffusion distance	mm or cm	(x)
(D)	Diffusion coefficient	cm²/s	(D)
(V)	Drug diffusion/transport velocity	cm/s	(V)
(\Delta G)	Gibbs free energy / binding free energy	kcal/mol or kJ/mol	(\Delta G)
(R)	Universal gas constant	1.987 cal·mol⁻¹·K⁻¹ or 8.314 J·mol⁻¹·K⁻¹	(R)
(T)	Absolute temperature	K	(T)
(e)	Euler's number	dimensionless	(e)
(K)	Equilibrium/binding constant, if defined in the model	M⁻¹ or dimensionless*	(K)
(K_i)	Inhibition constant	µM or M	(K_i)
(K_m)	Michaelis–Menten constant	µM or appropriate concentration unit	(K_m)
(v)	Reaction velocity, if used separately from diffusion velocity	concentration/time	(v)
(S)	Substrate concentration, if used	µM or mM	(S)
(K_d)	Dissociation constant, if used	M or µM	(K_d)
(R_0)	Reference/initial rate, if used	concentration/time	(R_0)
(t)	Diffusion/incubation time	s or h	(t)