Original Research Article

Mathematical Modelling of Hydrophilic Antibiotic Concentration, Binding Dynamics, and Drug Resistance under Competitive Inhibition in Disc Diffusion Assays

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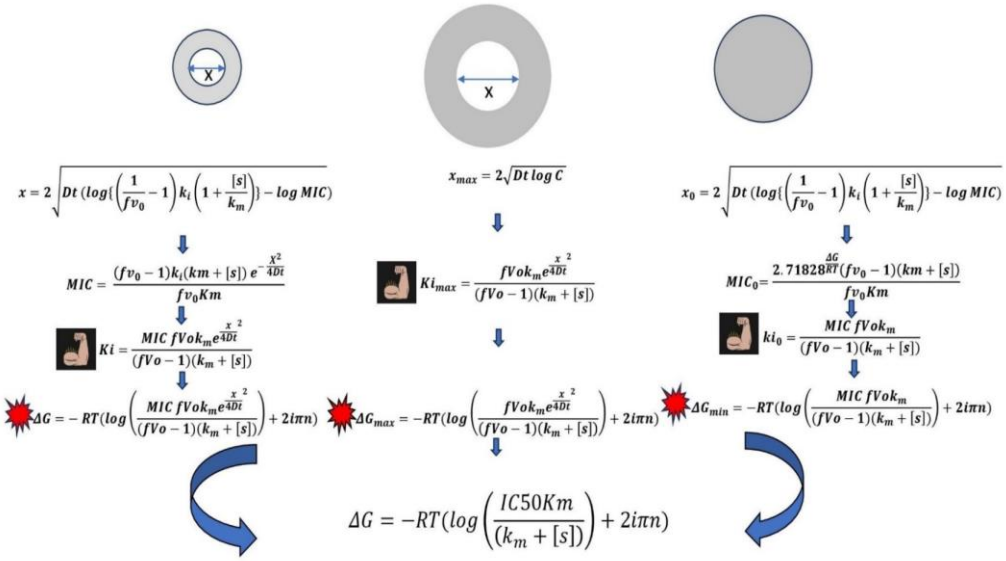
Graphical Abstract



KEYWORDS

Minimum inhibitory concentration, Zone of Inhibition, Gibbs Free energy, inhibition constant. Cheng–Prusoff, Binding affinity and Antibiotic Resistance

ABSTRACT:

Understanding the intricate relationship between minimum inhibitory concentration binding Affinity and drug resistance is paramount for developing effective antimicrobial strategies, in this paper, we explore a mathematical relationship between these factors, under the condition of the disc diffusion method of antimicrobial susceptibility testing of antibiotics.

In our study we considered the response as (ZOI) Zone of Inhibition denoted by "X" in mm, the response X is directly proportional to the concentration of antibiotics, Zone of Inhibition X is directly proportional to binding affinity and ΔG binding free energy of receptor. X is directly proportional to the -log of minimum inhibitory concentration (MIC), which contributes to resistance upon certain values by our mathematical derivation, we tried to give the direct relationship among binding affinity, minimum inhibitory concentration (MIC), zone of inhibition (ZOI) i.e. X and antibiotic concentration 'c' on the hypothesis of enzymatic kinetics the dimensions of Cheng–Prusoff, binding affinity, IC50, were calculated. For hydrophilic antibiotics following competitive inhibition kinetics.

1. Introduction

A traditional technique for figuring out the minimum inhibitory concentration (MIC) in semisolid media is agar plate diffusion [1]. The antibiotic is diffused into the bacterially loaded agarose medium using the disc diffusion method, which inhibits bacterial growth around the source and creates a clear zone devoid of bacterial lawns [2]. The diameter of these zones is directly correlated with the antibiotic concentration. A graph between inhibition zone radii x mm squared values x^2 are plotted against the logarithm of antibiotic concentration. The Intercept of linear regression gives the value of MIC at the squared size of these zoi[3].proposed by Boyan and James (2008)

2. Derivation

$$ln\ ln\ MIC\ = ln\ ln\ C\ - \frac{x^2}{4Dt} \quad \ldots\ldots\ldots\ldots\ldots eq(1)$$

Here "D" is the diffusion coefficient and it is not dependent on the *"C"* concentration of antibiotic and "t" the time of diffusion
Rearranging the equation (1) in terms of zoi = X

$$x = 2\sqrt{DtC\ - log\ log\ MIC}\ ) \ldots\ldots\ldots\ldots eq(2)$$

In Case of competitive Inhibitor, [I]

$$Enzym + I \xleftrightarrow{Kd} Enzy/I \xrightarrow{k_2} Enzy\text{-}I \xrightarrow[H_2O]{k_3} Enzy + I^+$$

Initial rate of reaction V_0 is given by [4]

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

Where Km is Michaelis-Menten constant [s] substrate concentration [I][5] is the concentration of inhibitor, and V_{max} represents the maximum velocity achieved by the system, at maximum (saturating) substrate concentrations.
Rearranging the above equation for inhibitor concentration [I]

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

$K_{i=}$ dissociation constant for the inhibitor [6] k_{-3}/k_3
To compute the concentration of competitive inhibitor [7] [I] that yields the fraction fv_o of velocity v_o where $0 < fv_o < 1$

$$[I] = \left(\frac{1}{fv_0}-1\right)k_i\left(1+\frac{[s]}{k_m}\right) \dots\dots\dots\dots eq(5)$$

Ki value can be interpreted as dissociation constant K_D of competitively binding inhibitor [8]

Substituting the value of equation (5) in equation (2)

$$x = 2\sqrt{\left(\frac{1}{fv_0}-1\right)k_i\left(1+\frac{[s]}{k_m}\right)\} - log\ log\ MIC\ )} \dots\dots\dots\dots eq(6a)$$

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

"C" concentration of antibiotic that can be termed as *an inhibitor* of the enzyme

Zone of inhibition "zoi" is the determining and critical factor in deciding the antimicrobial susceptibility [9] of antibiotic zoi x (directly proportional) $\alpha \frac{1}{fv_0}$ where fv_0 is the ratio of $\frac{v_0}{v_i}$ where v_0 is the velocity without inhibitor and v_i is the velocity with inhibitor. The value of fv_0 decreases in two cases a) when v_0 rate of reaction between enzyme and substrate decreases[10] b) when v_i rate of reaction between inhibitor and enzyme gets increased, it will increase the value of zoi 'x' and decrease the value of MIC minimum inhibitory concentration, which means less inhibitor concentration is required to achieve the desired ZOI similarly, zoi may decrease if the value of fv_0 is increased in the denominator as a function of binding affinity of the enzyme with its natural substrate is more favouring for product formation and v_i velocity with inhibitor is relatively lower, it will increase MIC value, more inhibitor concentration will be required to inhibit the product formation. Inhibition constant *Ki* is inversely proportional to binding affinity[11], which means the higher the value of ki, the lower the value of binding affinity that means the inhibitor is not tightly bound with the enzyme less the inhibitor will compete with the active site and that lowers the response of zoi and *vice-versa*[12].
As the *km* value increases to maintain maximum velocity V_{max} in case of competitive inhibition.

Substituting the value of fv_0 with $\frac{v_0}{v_i}$ equation (6a) becomes $(6b)$

$$x = 2\sqrt{\left(\frac{v_i - v_0}{v_0}\right) k_i \left(1 + \frac{[s]}{k_m}\right)\} - log\ log\ MIC)} \ldots\ldots\ldots\ldots\ldots eq(6b)$$

Rearranging the equation (6a) for minimum inhibitory concentration MIC

$$MIC = \frac{(fv_0 - 1)k_i(km + [s])\ e^{-\frac{X^2}{4Dt}}}{fv_0 Km} \ldots\ldots\ldots\ldots eq(7)$$

As postulated from the equation MIC is directly proportional to the exponent $e^{-\frac{X^2}{4}}$ This is the form of Gaussian function[13]. $e^{-\frac{X^2}{4}}$ that is the negative square of the zone of inhibition(zoi) by 4 as the zoi increases the minimum inhibitory concentration decreases exponentially (MIC= - x^2 /4). The value of inhibition constant Ki influences the MIC directly[14], smaller ki represents greater binding affinity which means smaller amount of ligand (inhibitor) is needed to inhibit the activity of the enzyme, which lowers the value of minimum inhibitory concentration. A high value of *Ki* leads to less binding affinity hence more the value of MIC. In competitive Inhibition Inhibitors and substrate compete for the same active site.
In competitive inhibition, V_{max} remains unchanged and *Km* increases and shifts towards the right, as the *Km* gets increased, affinity of the enzyme with its substrate decreases and the affinity of the enzyme with the inhibitor increases so MIC decreases and *vice-versa*

$$MIC = \frac{e^{\frac{\Delta G}{RT}} e^{-\frac{X^2}{4Dt}}(fv_0 - 1)(km + [s])}{fv_0 Km} Since\ k_i = Exp\left(\frac{\Delta G}{RT}\right) \ldots\ldots\ldots\ldots eq(8a)$$

$$MIC = e^{\frac{\Delta G}{RT} - \frac{x^2}{4Dt}}\left(\frac{1}{fv_0} - 1\right)\left(1 + \frac{[s]}{k_m}\right) \ldots\ldots\ldots\ldots eq(8b)$$

Since value of e=2.71828

$$MIC = \frac{2.71828^{\frac{\Delta G}{RT} - \frac{0.25x^2}{Dt}}(fv_0 - 1)(km + [s])}{fv_0 Km} \ldots\ldots\ldots\ldots\ldots eq(8c$$

In the above equation Michaelis-Menten Km unit is mole, R gas constant in kcal/molK and T is Temperature in Kelvin/
Rearranging the equation (6a) concerning the *Ki* inhibition constant

$$Ki = \frac{MIC\ fVok_m e^{\frac{x^2}{4Dt}}}{(fVo - 1)(k_m + [s])} \ldots\ldots\ldots\ldots eq(9)$$

$$k_i = Exp\left(\frac{\Delta G}{RT}\right) \ldots\ldots\ldots\ldots eq(10)$$

Where k_i= inhibition constant [15]
ΔG = Binding Energy kcal/mol
R= Gas Constant 1.985 × 10−3 kcal/mol K
T= Temperature 298.15K

Here the inhibition constant *ki* was obtained from the binding energy by using the formula as mentioned in equation (10)
Substituting the value of *Ki* of equation (10) to equation (6) the value of zoi =x is given by

$$x = 2\sqrt{\left(\frac{1}{fv_0} - 1\right) Exp\left(\frac{\Delta G}{RT}\right)\left(1 + \frac{[s]}{k_m}\right)\} - log\ log\ MIC)} \ \ldots\ldots\ldots eq(11)$$

Substituting the value of *Ki* of equation (10) in equation (9) we get

$$\Delta G = -RT(log\left(\frac{MIC\ fVok_m e^{\frac{x}{4Dt}^2}}{(fVo-1)(k_m+[s])}\right) + 2i\pi n) \ldots\ldots\ldots eq(12)$$

Since $\Delta G = RT(log\ log\ (k_i) + 2i\pi n)$

The value ΔG is the binding energy, R is gas constant and $\left(\frac{MIC\ fVok_m e^{\frac{x}{4Dt}^2}}{(fVo-1)(k_m+[s])}\right)$ is inhibition constant k_i,T is the temperature in kelvin.The function $2i\pi n$ is the periodic function[16],accounts for the multiple branches of the logarithm function in the complex plane, which states that the ΔG may have finite values depending on the integer "n" by setting n=0 the periodic influence of the term $2i\pi n$ can be eliminated, hence a constant value of ΔG can be achieved at a particular T temperature. The equation $\Delta G(directly\ proportional)$ α –log MIC indicates a mathematical relationship where change in the variable ΔG (binding energy) is inversely related to the log MIC, this implies that as the value of MIC increases due to resistance, the term log MIC grow positively leading to the decrease in ΔG
The function shows the logarithmic decline in the value of binding energy in response to increase in minimum inhibitory concentration MIC.
In condition the value of ΔG binding energy increases the value of MIC. the left-hand side of the equation becomes more positive. Since ΔG is defined as −log MIC, an increase in ΔG implies that –log MIC becomes increasingly positive, which in turn means that log MIC must become increasingly negative. This indicates that MIC must decrease because as the logarithm of a positive number becomes more negative.

Rearranging the equation (12) in terms of ΔG is the binding energy

$$\Delta G = -RT\left(log\ log\left(\frac{MIC\ fVok_m e^{2Dt\sqrt{x}}}{(fVo-1)(k_m+[s])}\right) + 2i\pi n\right) \ldots\ldots eq(13)$$

on simplifying the equation (11)

$$x = 2\sqrt{Dt\ log\left[\frac{\left(\frac{1}{fv_0} - 1\right)\ (k_m + [s])}{k_m}\right] - log\ log\ MIC} \ \ldots\ldots\ldots..eq(14)$$

When the IC50 value is known, the Cheng–Prusoff [17] equation is frequently applied to determine the equilibrium dissociation constant (Kb) of a competitive antagonist.

$$Ki = \frac{IC50}{1 + \frac{[s]}{Km}} \ldots\ldots\ldots..eq(15)$$

Substituting the value of Ki of equation (17) in equation (6)

$$x = 2\sqrt{\left(\frac{1}{fv_0} - 1\right)\frac{IC50}{1 + \frac{[s]}{Km}}\left(1 + \frac{[s]}{k_m}\right)\} - log\ log\ MIC)} \ \ldots\ldots\ldots..eq(16)$$

The term $1 + \frac{[s]}{Km}$ cancelled out from the equation

$$x = 2\sqrt{\left(\frac{1}{fv_0} - 1\right)IC50\ \} - log\ log\ MIC)} \ \ldots\ldots\ldots..eq(17)$$

Rearranging the above equation (19) for IC50

$$IC50 = \frac{fv_0\ MIC e^{\frac{x^2}{4Dt}}}{(fv_0 - 1)} \ldots\ldots\ldots..eq(18)$$

Driving the above equation for MIC by equation (20)

$$MIC = \frac{IC50(fv_0 - 1)e^{-\frac{x^2}{4Dt}}}{fv_0} \ldots\ldots\ldots..eq(19)$$

MIC is the minimum inhibitory [18] concentration used to determine the lowest concentration of the substance that inhibits visible growth of microorganism

The term $e^{-x^2/4}$this term involves the exponential function with negative argument, as ZOI 'x' increases the value of MIC decreases. The factor 4 in the denominator inside the exponent makes this change more sensitive

The diffusion coefficient 'D' represents the rate at which molecules diffuses through the medium. The increased rate of diffusion can potentially lead to more effective inhibition of microbial growth thereby radius of MIC

IC_{50} half maximal concentration is directly related to the MIC as they both measure the potency of antibiotic in inhibiting the growth of microorganism
A lower IC_{50} generally corresponds to a lower MIC, indicating the greater potency and effectiveness in inhibiting microbial growth.

In the condition of resistance where the zoi x_0, x=0 or x ≥ 0 then the exponent $e^{-\frac{x^2}{4Dt}}$ becomes one, the value of inhibition constant Ki_0, minimum inhibition concentration MIC_0, and change in binding energy ΔG_{min} is given by.

$$x_0 = 2\sqrt{\left(\frac{1}{fv_0} - 1\right) k_i \left(1 + \frac{[s]}{k_m}\right)\} - log\ log\ MIC\ )} \ldots . (20)$$ (where x =0 or x ≥ 0)

MIC_0 = C……. . (21)
The minimum inhibitory concentration (MIC) is almost equal Antibiotic concentration "c"

$$MIC_0 = \left(\frac{1}{fv_0} - 1\right) k_i \left(1 + \frac{[s]}{k_m}\right) \ldots\ldots\ldots\ldots eq(22a)$$

$$MIC_0 = \frac{k_i(fv_0 - 1)(km + [s])}{fv_0 Km} \ldots\ldots\ldots eq(22b)$$ where $k_i = e^{\frac{\Delta G}{RT}}$

$$MIC_0 = \frac{2.71828^{\frac{\Delta G}{RT}}(fv_0 - 1)(km + [s])}{fv_0 Km} \ldots\ldots\ldots\ldots eq(23)$$ (Where e=2.7182)

$$ki_0 = \frac{MIC\ fVok_m}{(fVo - 1)(k_m + [s])} \ldots\ldots\ldots eq(24)$$

Here the ki_0 Value is given when x=0 or x ≥ 0 this reveals that the enzyme had loses the binding affinity with the inhibitor it is the critical value with no zoi or negligible zoi showing resistance to Antibiotics

$$\Delta G_{min} = -RT\left(log\left(\frac{MIC\ fVok_m}{(fVo - 1)(k_m + [s])}\right) + 2i\pi n\right) \ldots\ldots\ldots eq(25)$$

The binding energy is at its critical value denoted by ΔG_{min} at it lowest below which no zoi will be detected as no microorganism killed on nutrient agar plate, the exponent $e^{\frac{x^2}{4Dt}}$ must have certain value (x) so that ΔG in negative should have certain value more than ΔG_{min} to show some antimicrobial activity and zoi to be appeared. In equation (25)

$Ki = \left(\frac{MIC\ fVok_m}{(fVo - 1)(k_m + [s])}\right)$ is the Inhibition constant, and R is the gas constant T
is temperature and $2i\pi n$ is the periodic function.
Substituting the value zoi of x=0, or x ≥ 0 in equation (19) the condition of resistance to antibiotic is given by.

$$x = 2\sqrt{\left(\frac{1}{fv_0} - 1\right) IC50\ \} - log\ log\ \ MIC_0)} \ \ldots\ldots\ldots eq(26)$$

$$\left(\frac{1}{fv_0} - 1\right) IC50 = \ MIC_0 \ \ldots\ldots\ldots eq(27)$$

$$MIC_0 = \frac{IC50(fv_0 - 1)}{fv_0} \ldots\ldots\ldots eq(28)$$

$$IC50_0 = \frac{fv_0\ MIC}{(fv_0 - 1)} \ldots\ldots\ldots eq(29)$$

Relative comparison of MIC with resistant and non-resistant species

When x ≥ 0, then Value of MIC_0 is given by eq (28) the value of minimum inhibitory concentration MIC (without resistance) is always lower than MIC_0 (with resistance) by the factor of $e^{-\frac{X^2}{4Dt}}$ therefore MIC= $(MIC_0)\ e^{-\frac{X^2}{4Dt}}$ can be written as

MIC= $MIC_0\ exp(-\frac{X^2}{4Dt}) \ldots\ldots\ldots eq(30)$

The magnitude of resistance between resistance strain of microorganism and non-resistance strain of microorganism can be explained mathematically by x' it is the difference of zoi in between resistance and non-resistance strain of microorganism subjected to be treated by same antimicrobial agent and same species with different strains.

$$x' = \sqrt{log(\frac{MIC}{MIC_0}) + 2i\pi n\ 2\sqrt{Dt}\ } \ldots\ldots\ldots eq(31)$$

putting n=0 the value of $2i\pi n = 0$

$$x' = \sqrt{log(\frac{MIC}{MIC_0})\ 2\sqrt{Dt}} \ldots\ldots\ldots eq(32)$$

rearranging the $eq(32)\ x' = \sqrt{log(MIC) - log(MIC_0)}2\sqrt{Dt}) \ldots\ldots\ldots$eq (33)

$$x' = 2\sqrt{Dt(MIC)\ -log\ log\ (MIC_0\ )} \ldots\ldots\ldots\ldots eq\ (34)$$

This value of x zoi will give the difference between resistance and non-resistance strains and the where MIC_0>MIC

Ideal Condition

While considering a hypothetical condition where MIC value is negligible then eq (2) becomes

$x_{max} = 2\sqrt{DtC\ -log\ log\ MIC)}$ ∵Log MIC is negligible

$x_{max} = 2\sqrt{Dt\ log\ log\ C}$. …… ……..eq (35)

The eq (35) it is the hypothetical case where the MIC is negligible and all the concentration of drug converts to the response x_{max} the maximum response of zoi at negligible value of log MIC. For maximum response $K_{i\ max}$ is given by

$$Ki_{max} = \frac{fVok_m e^{\frac{x}{4Dt}^2}}{(fVo-1)(k_m+[s])} \ldots\ldots\ldots\ldots..eq(36)$$

Similarly, max energy without MIC is given by

$$\Delta G_{max} = -RT(log\left(\frac{fVok_m e^{\frac{x}{4Dt}^2}}{(fVo-1)(k_m+[s])}\right) + 2i\pi n) \ldots\ldots\ldots(37)$$

Gibbs-Cheng equation for competitive inhibition.

The intensity of an inhibitor in preventing an enzyme's activity is measured by the inhibition constant (Ki). It is a particular type of equilibrium dissociation constant that shows how well an inhibitor binds to its specific enzyme [19]

The relationship between the Gibbs free energy change and the inhibition constant can be expressed mathematically by the equation

$$\Delta G = -R\ T\ ln\ Ki \ldots\ldots\ldots..eq(38)$$

This relationship implies that as the inhibition constant decreases (indicating stronger inhibition), the Gibbs free energy change becomes more negative, reflecting increased stability of the enzyme-inhibitor complex[20]

$$Ki = \frac{IC50}{1+\frac{[s]}{Km}} \ldots\ldots\ldots..eq(39)$$

$$k_i = Exp\left(\frac{\Delta G}{RT}\right) \ldots\ldots\ldots..eq(40)$$

$$Exp\left(\frac{\Delta G}{RT}\right) = \frac{IC50}{1+\frac{[s]}{Km}} \ldots\ldots\ldots..eq(41)$$

The IC50 is the concentration of an inhibitor at which the enzyme's activity is reduced by 50%. A lower IC50 indicates a more potent inhibitor. Km represents the substrate concentration at which the reaction rate is half of its maximum value. It reflects how tightly the enzyme binds to the substrate**.** [S] is the concentration of substrate available for the enzyme.

$$\Delta G = -RT(log\left(\frac{IC50Km}{(k_m+[s])}\right) + 2i\pi n) \ldots\ldots\ldots..eq(42)$$

ΔG is the change in free energy, which tells us if the reaction or process is favourable (negative ΔG = spontaneous) or unfavourable (positive ΔG = non-spontaneous).

The numerator IC50·Km combines the effect of inhibitor potency (IC50) and the enzyme-substrate binding affinity (Km). A lower IC50 (stronger inhibition) increases this value. A lower Km (tighter substrate binding) also increases this value. The denominator km+[S] captures the contribution of the substrate concentration ([S]) and another kinetic factor, km. As [S] increases, the denominator grows, reducing the overall value of the fraction, which could mean a lower ΔG The km term represents the intrinsic catalytic activity or background rate, and can act as a baseline in the absence of a high substrate concentration. ΔG becomes more negative (favorable) as The IC50 decreases (stronger inhibition), The Km decreases (higher substrate affinity), The [S] increases (more substrate present).

3. Discussion

A mathematical model of antibiotics concentration, binding energy, minimum inhibition concentration, Inhibition constant and IC50 is developed. It is a novel approach to incorporate the kinetics in the equation (2) Boyan and James (2008) for competitive Inhibition.
On introducing Inhibitory concentration as a function of fv_0fractional velocity, inhibitory concentration *Ki* and substrate /Michales Menten. ratio.
Further Inhibition constant *Ki* is substituted with the equation $\Delta G = -RT\ log\ Ki$ and Ki was substituted by Cheng–Prusoff, The equation reflecting the two dimensions in term of ΔG binding free energy and IC50 Inhibitory concentration 50%, by eq (6b) Zoi is related to the square root logarithmic term changes in Michales Menten will directly affect the value of "x", in two cases scenario a) Michales Menten will change according to the kind and nature of antibiotics use. b) on fixing the antibiotic, value of Michales Menten is constant for that particular moiety. If the Km value increases the ratio of [S]/km decreases, this in term reduces the value of $1 + \frac{[s]}{k_m}$ so the logarithmic value leading to reduce zoi. The value of zoi is significantly decreases by -log MIC value.
The MIC is (directly proportional) α $(fv_0 - 1)$ where fv_0 is the ratio of $\frac{v_0}{v_i}$ where v_0 is the velocity without inhibitor and v_i is the velocity with inhibitor, as enhancement of v_i leads to decrease the MIC and *vice-versa*, similarly v_0 progression will promote the value of MIC on higher side. The relationship (MIC α $\frac{v_0}{v_i} - 1$) On the condition's if $v_0 > v_i$ MIC will have a positive value, if $v_0 < v_i$, MIC becomes negative because the ratio $\frac{v_0}{v_i}$ is less than 1, so subtracting 1 yield negative result that's a hypothetical condition. If $v_0 = v_i$, then MIC = 0 it is similar to ideal situation as describe in equation (35). Contrary to that fv_0 is in denominator eq (8c),21 and 28. It give an undefined value of MIC. The expression $e^{-\frac{X^2}{4Dt}}$ .This term relates to the exponential function with negative input, as the value of MIC drops as ZOI 'x' increases. This adjustment is extremely sensitive because of the factor 4 in the denominator inside the exponent.

4. Conclusion

The mathematical model can be used to simulate the antimicrobial susceptibility test disk diffusion method in silico and to predict and corelate resistance, intermediate and susceptibility mathematically, we can calculate the binding energy, zoi and minimum inhibitory concentration

of antibiotic. We can also relate the various factors involved in invitro conditions, for drugs Hydrophilic in nature. This model also gives an insight of IC50 and MIC as they both represents concentration of the antibiotic.

IC50 is the concentration needed to inhibit process (e,g, enzyme activity or microbial growth) by 50% while MIC refers to the lowest concentration that inhibits the visible growth. For antimicrobial activity the value of binding energy ΔG should be greater than ΔG_{min} for the particular inhibitor to show antimicrobial activity. The Gibbs-Cheng equation a novel attempt that effectively models how the balance of inhibitor potency, enzyme-substrate affinity, and substrate concentration contribute to the overall free energy of the system. It's a hybrid equation connecting thermodynamic principles (ΔG) with enzyme kinetics (IC50, Km, [S]).

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