Importance of Thermodynamics in Drug Designing

Project report submitted in partial

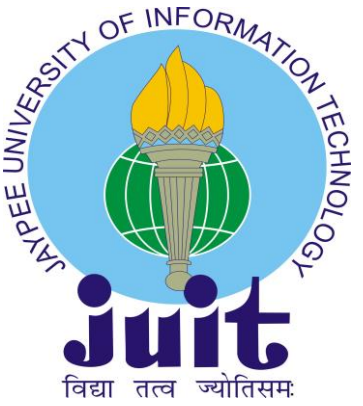
Fulfilment of the requirement for the degree in

BACHELOR OF TECHNOLOG IN BIOTECHNOLOGY

By

Divyanshu Bhuraita

171840



Deptt-BT/BI

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY WAKHNAGHAT, SOLAN-173234, HIMACHAL PRADESH

CERTIFICATE

This is to certify that the work contained in this project entitled "Importance Of Thermodynamics In Drug Designing" submitted by "Diyvanshu Bhuraita" in the partial fulfillment for the award of degree of B.tech (Biotech) at Jaypee university of information technology, waknaghat has been carried out under my supervision. This work has not been submitted partially or wholly to any other university or institute for the award of this or any other degree or diploma**.**

Dr. Poonam Sharma

Asso. Professor

Deptt- BT/BI

Email :

Student's declaration

I hereby declare that the work present in the entire project report entitled "Thermodynamic parameters used for the designing of new drugs" submitted for the partial fulfilment of the requirements for the degree of **Bachelor of Technology** in **Biotechnology** at Jaypee University of Information Technology; Waknaghat in an authentic record of my work that has carried out under the supervision of Dr. Poonam Sharma, Associated Professor.

This work has not been submitted elsewhere for the reward of any other degree or diploma. I am fully responsible for the content of this particular project report.

Acknowledgment

I extend my deep sense of gratitude towards my project guide Dr. Poonam Sharma, Associate Professor, Department of Biotechnology and Bioinformatic Jaypee University of Information Technology for his invaluable guidance and motivation in the time of Covid-19 pandemic helped me very much for the completion of my project. He steered me in the right direction whenever I came across any difficulty throughout my work.

I thank Department of Biotechnology and Bioinformatics at Jaypee University of Information Technology, Waknaghat for providing all the facilities for doing this project.

I also thank all those who were involved indirectly and directly in the completion of my project work.

Date-

Name-Divyanshu Bhuraita

TABLE OF CONTENT

List of Figures

List of Tables

Abstract

The working of any biological process requires molecular recognition, either it is intermolecular, like when binding of a ligand to a macromolecule or could be intramolecular like protein folding. So, our understanding of relationships between the structure of proteins and their energy level changes and their binding and binding affinity to other (bio)molecules is interesting and essential for chemical knowledge and biotech applications. There are many parameters used to characterize the stability of any biological system, some of them being equilibrium constant (K) or the free energy (ΔG°), which calculated by sum of enthalpic (ΔH°) and entropic (ΔS°) terms.

In this thesis, all these thermodynamic parameters like ΔS, ΔH, ΔG measured in the presence of different surfactants(SDS). Clobetasol propionate (antifungal drug) for the studies at different temperatures(25, 30, 35) were measured by using conductance studies. Further Evaluated for the feasibility and spontaneity of process which might be helpful to increase the efficiency and bioavailability of drugs.

Keywords : enthalpic, entropic, stability, biomolecules, thermodynamic parameters, ΔS, ΔH, ΔG.

CHAPTER-1

INTRODUCTION

For new drugs to be produced the identifications of ligands is an essential factor, so as for it to be biologically effective on the target of a particular disease. For a proper biological elicit or reaction necessary for the treatment of disease, the affinity of the ligand which we wish to use should be in consistence with the target of disease and have all the physiochemical requirements.

Optimizing the binding of the chemical to the target is highly important in drug design in order to employ the compound that is the major lead of the medicine planned to use. ΔG (Gibbs free energy), which is further determined by enthalpic (ΔH) and entropic changes (ΔS), can be used to determine K_a, which is the binding affinity towards the target[1,2].

Many different combinations of ΔS and ΔH might result in the same ΔG values, and thus the same binding affinity. However, enthalpically dominated ligands behave differently than entropically dominated ligands[3].

Currently used prototypes in drug development produce conformationally restricted ligands that are very hydrophobic in nature. These ligands are characterized by an entropically .

Because conformationally constrained ligands are unable to respond to changes in binding site geometry, they are particularly vulnerable to drug resistance mutations and genetic polymorphisms that can occur in nature.

The introduction of certain flexible components or the relaxation of conformational limitations are required for creating ligands that can adapt to changing targets. Because these compounds incur a significant conformational entropy cost while binding to the target molecule, the availability of some favourable enthalpy for binding is critical for optimising their binding affinities[4].

Though there are many methods for determining the values required for different affinities, including precise values of thermodynamic parameters, using conductance of different solutes in different solutions have been determined. Other methods are ITC based, DSC based mehods and other calorimeter methods. In this project , conductance measurements have been used to measure various thermodynamics parameters like ΔS, ΔH, ΔG of surfactant SDS in presence of drug clobetasol propionate at different temperature (25,30,35)[5].

Using van't Hoff plot method for determination of such affinities can also be used, but this type of pathway is less reliable than any calorimetric methods. This method involves accurate and precise values of K_d, which is used for the van't Hoff graph to plot for a range of temperature[6]

CHAPTER-2

Review of literature

[2.1]Thermodynamic applications in drug designing

A vital piece of medication plan and improvement is the advancement of sub-atomic connections between a designed medication applicant and its limiting objective.

Thermodynamic portrayal gives data about the equilibrium of vivacious powers driving restricting connections and is fundamental for comprehension and streamlining sub-atomic cooperations.

Well-qualified assessment: The best medication plan and advancement stage comes from a coordinated interaction using all accessible data from primary, thermodynamic and organic examinations. Proceeding with development in our comprehension of the enthusiastic premise of atomic communications and advances in thermodynamic techniques for inescapable application are fundamental to understand the objective of thermodynamically determined medication plan.

Thorough thermodynamic assessment is indispensable from the get-go in the medication advancement cycle to speed drug improvement toward an ideal fiery connection profile while holding great pharmacological properties. Reasonable thermodynamic methodologies, for example, enthalpic improvement, thermodynamic enhancement plots and the enthalpic productivity

list, have now developed to give demonstrated utility in the plan interaction. Improved throughput in calorimetric techniques stays fundamental for much more noteworthy combination of thermodynamics into drug plan[7].

As well as being an advantageous label-free test for considering associations, the warmth change is identified with the limiting enthalpy (ΔH) of the connection and, taken together with the liking KD, can be utilized to ascertain the adjustment of entropy of the cycle[8]. This thermodynamic information gives understanding into the non-covalent powers answerable for driving restricting and acknowledgment.

The linkage among selectivity and restricting thermodynamic profiles has been another significant inquiry in judicious medication plan. Such a linkage has likewise featured significance of enthalpic or entropic commitments in hitting the ideal objective. Statins, which are advertised cholesterol bringing down drugs, act by restricting to 3-hydroxy-3-methylglutaryl coenzyme A. An examination of the limiting information on various statins over a time of around 12 years obviously showed meaning of enthalpic commitment to the limiting free energy and subsequently rise of more up to date class of more powerful statins and different medications[9].

There have been reports of calorimetric based profiling of blood plasma from colorectal malignant growth patients . The approval of calorimetry as an indicative device is asserted since this procedure

could screen changes in colorectal malignancy at various phases of tumor improvement. The development of proteomics in unwinding biochemical changes which lead to malignant growth has assumed an extremely critical part[10] . Clearly the part of thermodynamics in proteomics which could be applied in the advancement of prognostic biomarkers and procedures for viable malignancy treatment can't be disregarded at any stage. The meaning of thermodynamics in malignant growth fixed states has been featured, and entropy age in disease advancement has been associated with new conceivable anticancer treatments.

The role of entropy in sticking to the Second Law of Thermodynamics in the living frameworks in creation and upkeep of underlying request is all around perceived, and its association with the heart has additionally been depicted. As indicated by this depiction, the heart can counterbalance the body's expanding entropic trouble by sending out entropy to the environmental factors by utilizing its energy[11]. Subsequently thermodynamic parts of cardiovascular physiology and heart infections additionally should be tended to. Further, the significance of thermodynamics in displaying mind action has additionally been perceived . Clear cut laws of thermodynamics giving connection among data and energy can absolutely be extremely helpful in neuroscience. The utilization of entropy in setting of conditions of awareness and related neurodynamics with explicit spotlight on hallucinogenic state has been considered. Direct relationship of the First Law of Psychology and Second Law of Thermodynamics has been called attention to . There are numerous organic cycles which have not been tended to

dependent on thermodynamic contemplations. One such model is flexibility of blood bonding and its association with thermodynamics of the cycle.

[2.2]Thermodynamics of ligand binding

The medication plan and advancement have profited certainly from thermodynamic investigations.

It has had a major effect in both the scholastic and modern areas. Unique accentuation on underlying complementarity and streamlining of relationship between the medication and target restricting locales are significant segments of techniques to produce lead compounds. Solvency, selectivity, adsorption, dissemination, digestion, discharge and toxicology are different worries inside limitations of Lipinski Rule of Five[12].

The rule of five (Ro5) is a set of in silico guidelines used in drug development to prioritise molecules having a high chance of oral absorption.

Thermodynamically determined medication configuration will keep on being significant and effective on the grounds that it tends to sub-atomic cooperations based on energetics and licenses spanning association among underlying and thermodynamic data.

[2.3]How thermodynamic parameters can carve the path for a variety of new medications

With the completion of Human genome Project, it has created a new scientific realm of its own. About 35,000 genes encoding for the proteins required are known, even the genomes of several pathogens are also complete, and many more are being recognized and will be available in future.

Many properties like the structure, function or the types of interactions they make with other molecules need to be identified. Because many of the proteins recognized from the genomic data will further become the source of studies for new drug development against many of the diseases present. But surely, these futuristic developments require the need for designs that precisely focus on the binding affinity, specificity, biological acceptance and toxicity[13].

The methods which are being used for the lead identification and improvement are of generally two types which include:-

Computational methods and

Experimental methods

Even though the two methods are often used in conjunction with each other, can be used in parallel or even in a defined sequence, a real theory-of-knowledge link is missing between the two methods and continuously a meticulous sync is absent.

And it is seeming that thermodynamics or specifically thermodynamic parameters involving the binding of ligand and other such quantities, namely Gibbs free energy, enthalphy, entropy, heat capacity changes, which can be measured by experiment or can be determined by structure will be able to provide this link that has been missing.

But to further make things difficult, in most of the cases involved with such type of needs the link is very confined to comparison of binding affinities that are calculated and experimental.

[2.4]Basic thermodynamic qualities of test compounds

A basic knowledge of binding is necessary for understanding the processes which are being carried out at the target site. Intracellular receptors are known to bind chemical messengers, such as hormones, enzymes bind to substrate, G-proteins bind agonists and antagonists, and some ions channels are also being regulated by ligand bindings.

During the process of transport of drug, the compounds which are being identified or being designed are progressed because they are do the job of modulating the disease processes which is associated with the aberrant activities at target site. Therefore, binding process with a suitable affinity along with the effect we want on the biological level activity of the target protein is the main purpose or goal the drug[14].

Although it is necessary to note that the energetic contribution may be different, even if interaction of different test compounds with test protein have similar structures and affinities.

In order for the characterization of binding interactions, both of the enthalpic and entropic contributors need to be determined and related to the structures of both of these partners, the complex or any other changes in the physical or chemical properties of the binding process, maybe such as change in hydration properties, protonation effects, etc[15,16].

[2.5]Parameters contributing to affinity and the type of interactions at molecular level

The thermodynamic parameters, ΔH, ΔS and ΔC_p are all being affected by the interactions of different types, including anionic and cationic interactions, vander waal forces, covalent bondings, H_2 bonding, and more.

Keeping in mind the main desired character of the drug, the optimization of the ligand protein binding can be done to various contributors of affinity.

Even so, many factors are difficult to resolve while understanding he working of how these interacting may occur or effect the process, a basic frame work has been constructed many few years ago[17].

However, the results acquired from the general trends of these different types of interactions can surely be a starting point to understand in depth of thermodynamic relations to the effectiveness of the drug. It is safe to say that there might surely be a point in between these interactions a particular state of enthalpic and entropic values may show dominance for any specific interaction[18].

The types of bonds which might be considerable are

[2.5.1]Hydrogen bonds, cationic and anionic pairs

Hydrogen bonds, individually have a very little impact on the affinity. The contribution results from redistribution of the hydrogen bonding network on both sides which results in H_2 bond exchange.

So, it is obvious that the magnitude of contribution to enthalpic values is dependent on the bond length and the angel of the bond. A net gain or net loss of favored H_2 bonding interactions determines the sign of contribution.

[2.5.2]Hydrophobic molecules

The water molecules behave differently when around hydrophobic molecules. They tend to form strong H_2 bond between themselves than bulk molecules. Therefore, non-polar solvents have do not have the ability to satisfy the potential required for H_2 bonds.

On interacting with hydrophobic solvents these water molecules get released back to solvent in bulk and usually has a little enthalpy and favored change of entropy.

A negative effect on ΔC_p is also observed.

[2.5.3]Conformational changes

The ligands accommodated are reflected by the conformational changes that are seem generally during binding. The reason for these subtle conformational changes is the flexibility and dynamic nature of proteins and ligands in solution. Therefore, they are regarded with low unfavorable values for the entropy and enthalpy with –ve values for the $\Delta C_{p.}$

[2.5.4]The water molecules

The molecules of water are seen to react to free proteins, ligands and protein-ligand complexes as well. The molecules which are close to binding vicinity are known to be more structured than those which are present in bulk solvent. Both these have very different behaviors.

The structured molecules can either be released back or retained when binding interaction occurs. On release of water molecules is seen to have a favored change of ΔS because these are being displaced in the bulk. Although this has an unfavorable effect on the ΔH because of now being weaker bonds in the bulk than stronger bonds which were present with the macro mol. or other water mol.

Obvious result would be encountered from the retention of H_2O because of more H_2 bonding and an unfavorable change in entropic value.

[2.5.5]Thermodynamic relations and changes in structures

The thermodynamic parameters, also the changes in polar and apolar surface areas came into light a few years back, by studies on protein folding and unfolding.

Due to very low changes in the surface areas and a lot of varieties of different ligands produced by medicinal chemistry, these studies can be a little difficult to accurately predict the thermodynamic parameters.

[2.6] The methods used for measuring

Till now we have discussed that during protein-ligand binding the involves recognition and binding which results in the desired complexes and during all this the thermodynamic parameters are changed. Now to measure these parameters there are many ways, 2 types of measurements are the Direct measurement methods and the Indirect measurement methods[19].

The direct methods are often used and also more precise and accurate, although indirect methods are used when there is practical reasons.

[2.6.1]Direct measurements

ITC provides for highly sensitive measurements of the enthalpy and affinity of binding reactions at a fixed experimental temperature. The amount of ligand bound during each injection determines the heat change of the interaction in the ITC experiment. The equilibrium dissociation constant can be calculated as the free ligand concentration when the free target protein concentration equals the bound target protein concentration because the total test compound and total target protein concentrations are known and the bound concentration can be determined. When [P] = [P'.L'], the Ka value is [L]free if the binding interaction is characterised by the reaction above[20].

$$\Delta G=\Delta H-T\Delta S=RTIn K_p$$

This equation can be used for complete thermodynamic characterization.

[2.6.2]Indirect measurements

Non-calorimetric approaches can be used to determine ligand binding affinities, which mainly involve the measurement of a signal proportional to the bound concentration of protein[21].

Below are the observations and the values of CMC and X_{cmc} , measured by the conductivity meter.

In this thesis, we have used conductivity meter for the determination of values of various parameters of thermodynamic.

[2.6.3]Measuring from the change in the stability of protein

There is a release of free energy on complexation because the binding of a test chemical to a target protein is a spontaneous process linked with a negative Gibbs enthalpy free energy shift[22]. As a result, the complex formed by the target following protein and the test drug is more stable than the free partners, with the degree of stabilization being determined by the binding energy magnitude. Thus, the difference between the free energy of unfolding of the unliganded study protein and that of the complex can be used to estimate the free energy of binding.

CHAPTER-3

Materials used for experimentation

[3.1] Materials used for the experimental deduction of thermodynamic parameters

[3.1.1] SDS (Sodium docecyl sulphate)

SDS is also sometimes known as to be sodium laurilsulfate. The formula of SDS is $CH_3(CH_2)_{11}OSO_3Na$, and it is an organic compound. It is a white to pale yellow looking paste like or mild solid phase, it is miscible in water. It is an anionic surfactant, which is composed of sodium alkyl sulfates, lower surface tension of aqueous sol., and is used as an important piece of research tool in protein biochemistry.

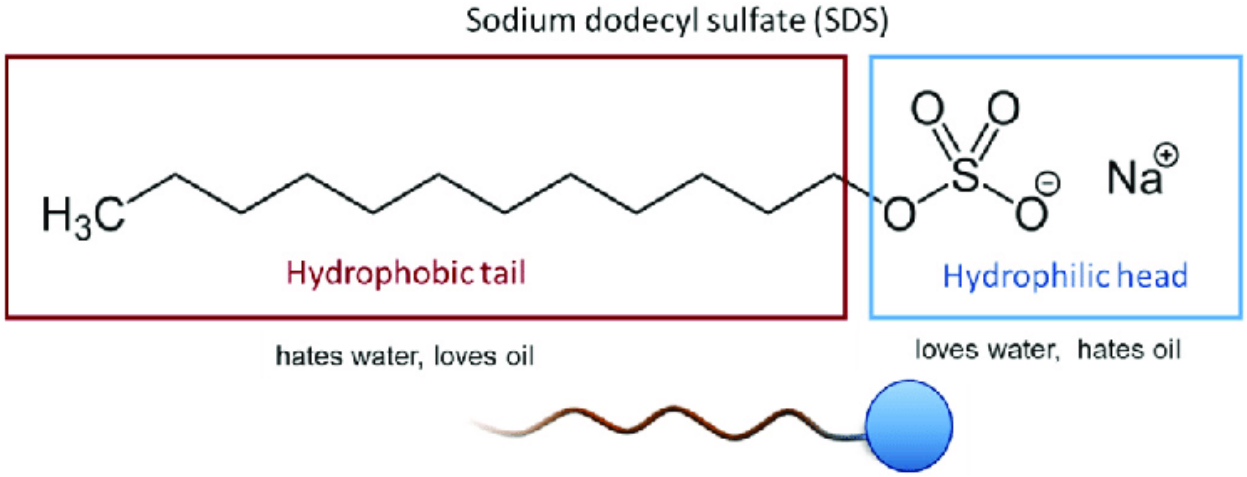


Figure 1: Surfactant SDS

[3.1.2]C-TAB (Cetyltrimethyl ammonium bromide)

CTAB extraction buffer has the components 0.5 M EDTA (pH 8.0) 1 M Tris-Cl (pH 8.0). It is usually prepared immediately before use; buffer is only good when freshly prepared. A Store 10% CTAB stock solution can be stored at a constant temperature of 37. It may be stored at 37°C which helps in avoiding precipitation. And it can be stored at that temperature for further future uses for several years.

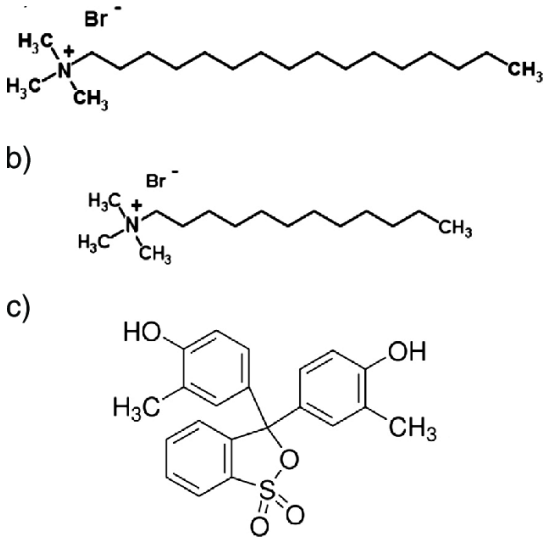


Figure 2: Ceyltrimethyl ammonium bromide

[3.1.3]Clobetasol propionate(anti-fungal drug)

It is a corticosteroid which has anti fungal purposes. This is generakly used to treat many skin conditions or skin related problems such as eczema, psoriasis, contact dermatitis.

It is generally used as a product that is applied to the skin as a cream, an ointment or any other form like shampoo. Although its use is recommended only if any other form of corticosteroids are ineffective.

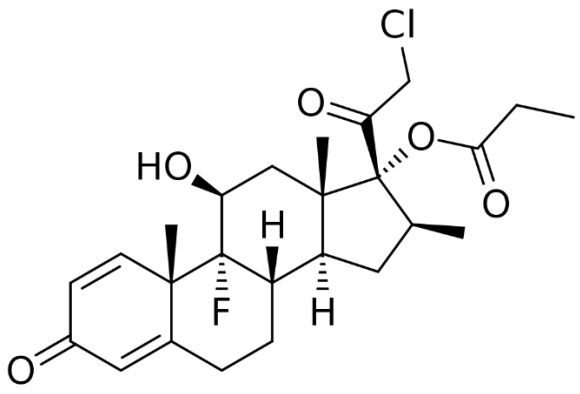


Figure 3: Clobetasol propionate (anti-fungal drug)

[3.1.4] Water

Water being one of the major solvent in the study which is also employed in calibration of instruments or apparatus was obtained by double distillation process. By volume, 1000ml of pure water was collected from double distillation unit which was further subjected to distillation on acidified $KMnO_4$ over a 750mm long fractionating column.

[3.2] Methodology

[3.2.1] Measuring Conductance

A calibrated digital conductivity meter was used for the determination of conductance measurements (Cyber Scan CON 510). At different temperatures, 25°C, 30°C, 35°C and 40°C, specific conductance (**κ)** was measured.

[3.2.2] CMC (critical micelle concentration)

The concentrations of surfactants above which the micelles form and all other surfactants which are present in the observed in the system will form micelles, in surface chemistry.

CMC is related to the surface tension, which is being observed to change strongly before the CMC values are reached, but once the CMC values are reached they remain constant, or change with a very slow or lower slope.

The values of CMC depend on various conditions, in the following experiments, we change he temperature to get the desired results or varied CMC.

[3.2.3] Thermodynamic Parameters

Enthalpy is the amount of heat energy transferred (heat absorbed or emitted) in a chemical process under constant pressure. It is expressed as a change in enthalpy (ΔH) because the total enthalpy (H) of a system cannot be measured directly.

Entropy measures the amount of heat dispersed or transferred during a chemical process. Entropy can be thought of as the degree to which energy is dispersed throughout a system. For example, water has a greater entropy than ice because energy is more spread out in water than in ice.

Gibbs Energy is also known as energy available to initiate a chemical process and is determined under constant pressure and temperature. Some reactions are spontaneous (eg. rusting). A spontaneous process happens by itself without any energy added to the system (apart from the activation energy). A non-spontaneous process will not take place unless it is driven by an external source of energy.

Chapter-4

Results and Discussion

Conductance measurements

By experimentally measuring the conductance of Sodium Carbonate in H_2O, we calculate the CMC, X_{CMC} , and therefore the required thermodynamic parameters.

Thermodynamic parameters experimentally found for Clobetasol propionate

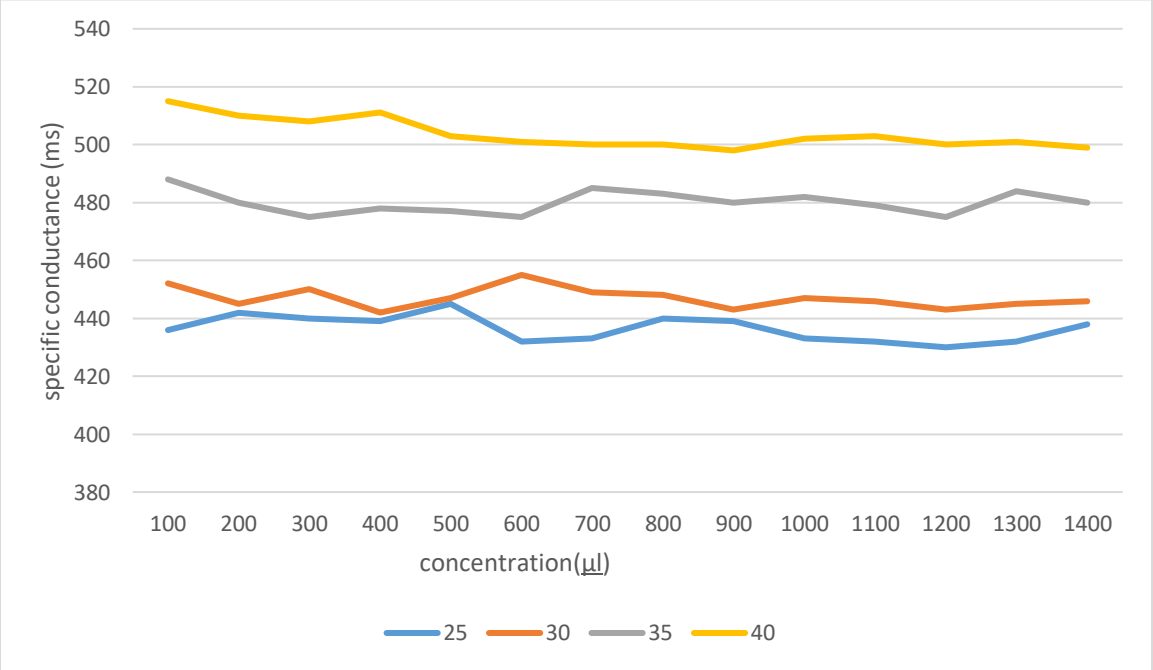


Figure 4:CMC values of clobetasol propionate over varying temperature

For 25°C

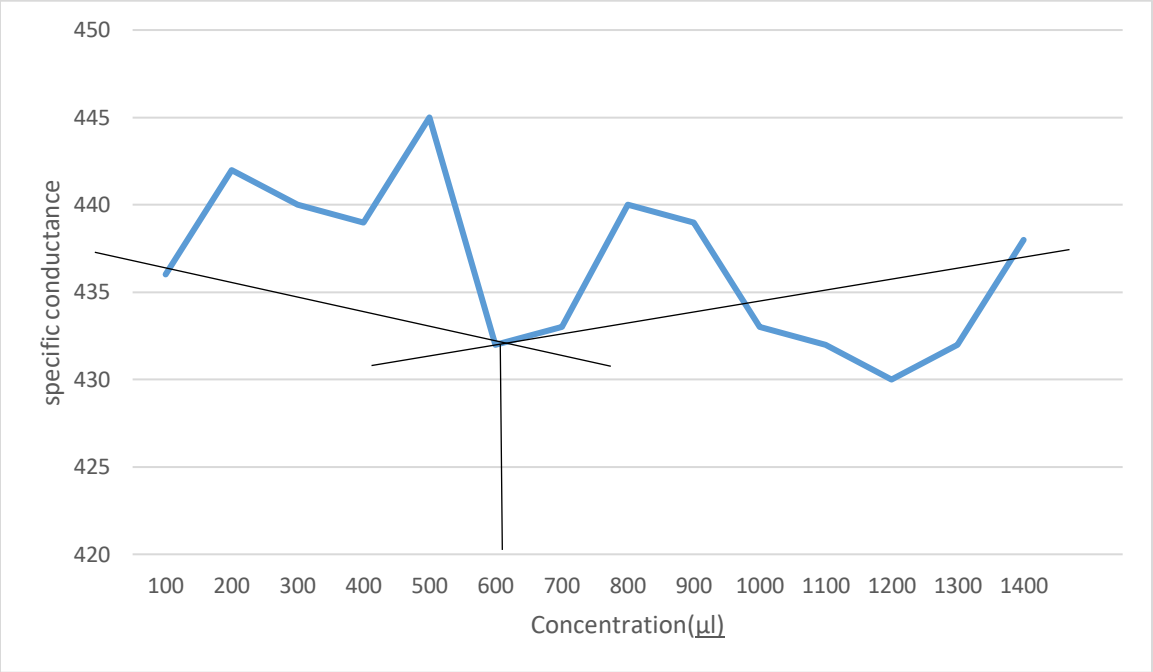
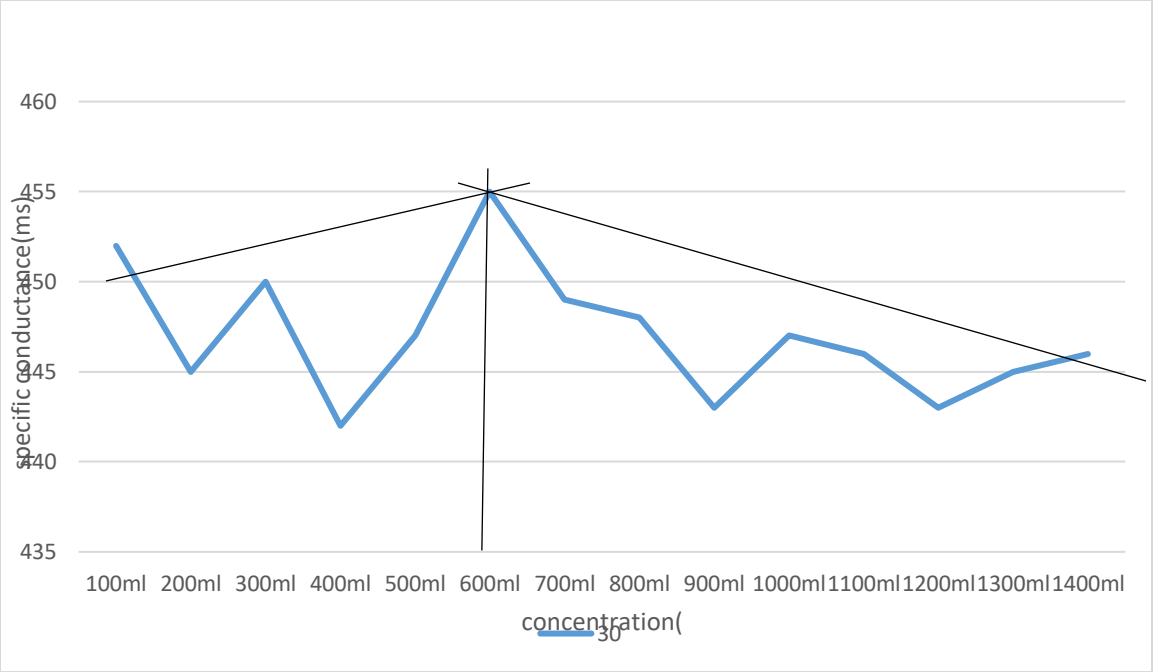


Figure 5:CMC values at temperature 25°C

CMC is 0.0072

For 30°C



F

Figure 6:CMC values at temperature 30°C

Cmc is 0.006

For 35°C

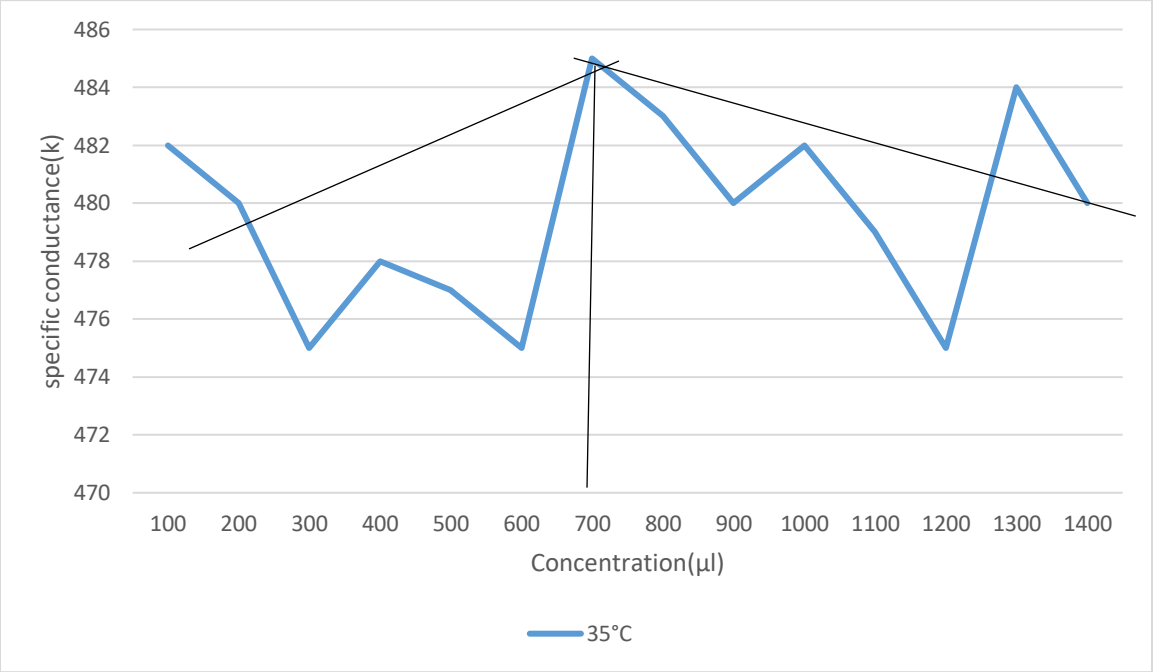


Figure 7: CMC values at temperature 35°C

Cmc is 0.007120

Temp	CMC	X_{CMC}	T^2	$L_N X_{CMC}$
25	0.0072	0.0001295262219	625	-4.933674253
30	0.006	0.0001079408484	900	-5.11599581
35	0.00712	0.0001280872 26	1225	-4.944847554

Table 1:CMC values measured by conductivity meter for clobetasol propionate

Temperature	DhoM	Dgo	Dso
25	-0.1143175	-1.025464193	0.03644586774
30	-0.1646172	-1.276031675	0.03704714916
35	-0.2240623	-1.43890119	0.03470968256

Table 2: Thermodynamic parameters calculated by using CMC for antifungal drug

ΔH, ΔG and ΔS is calculated by the cmc values at different temperatures.

Conductivity measured of C-TAB

In the following experiment, the conductivity of CTAB (Cetyltrimethyl ammonium bromide) at different temperatures.

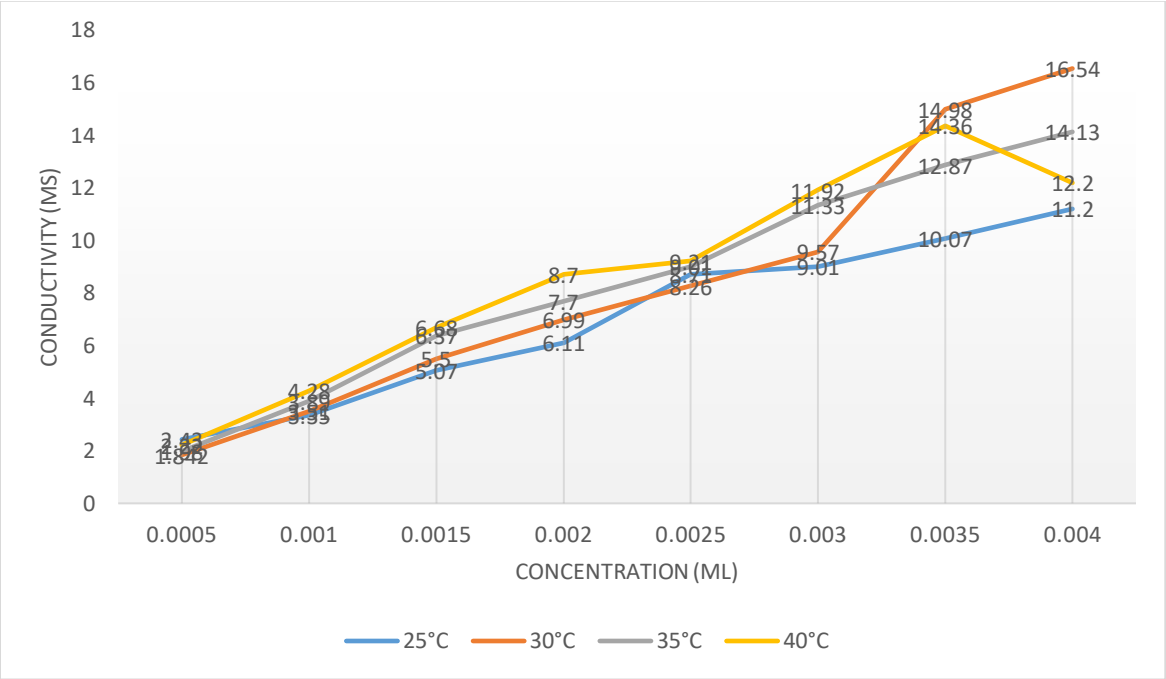


Figure 8: CMC values at various temperature

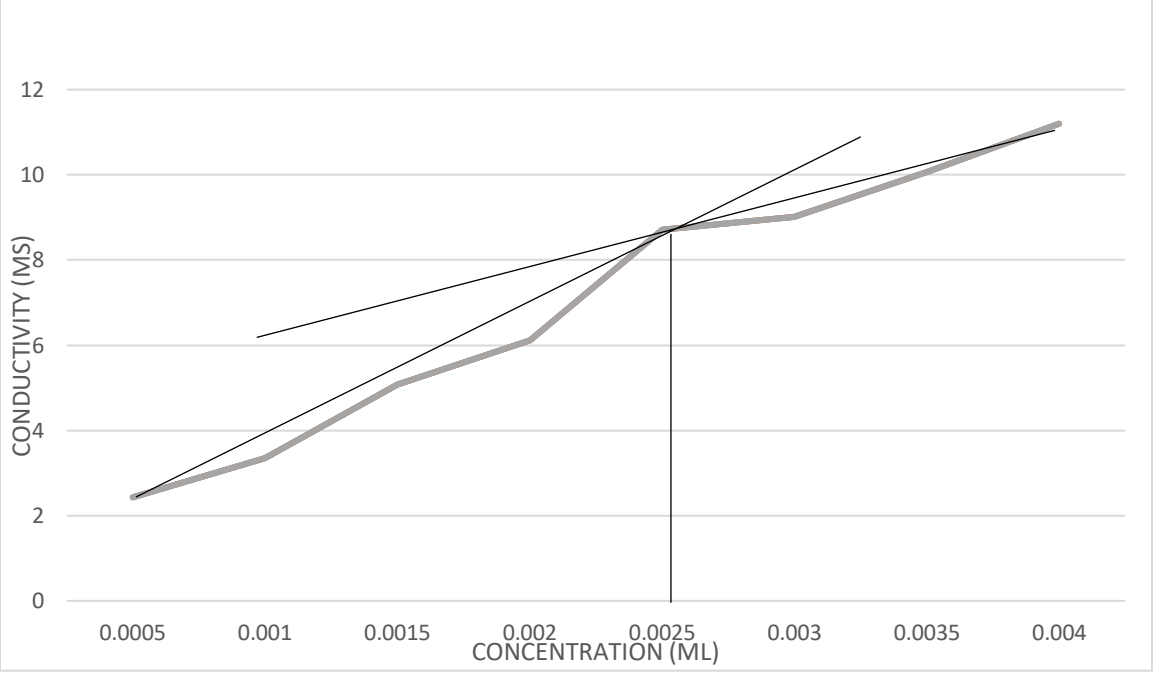


Figure 9: CMC values at temperature 25°C

For 30°C

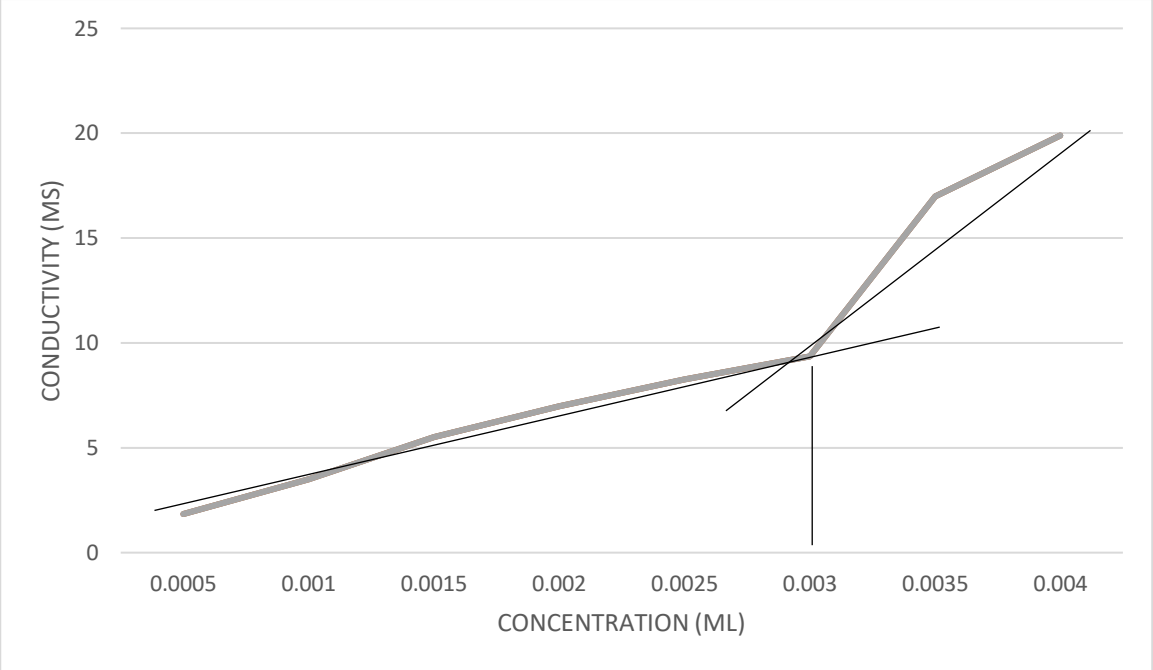


Figure 10: CMC values at temperature 30°C

For 35°C

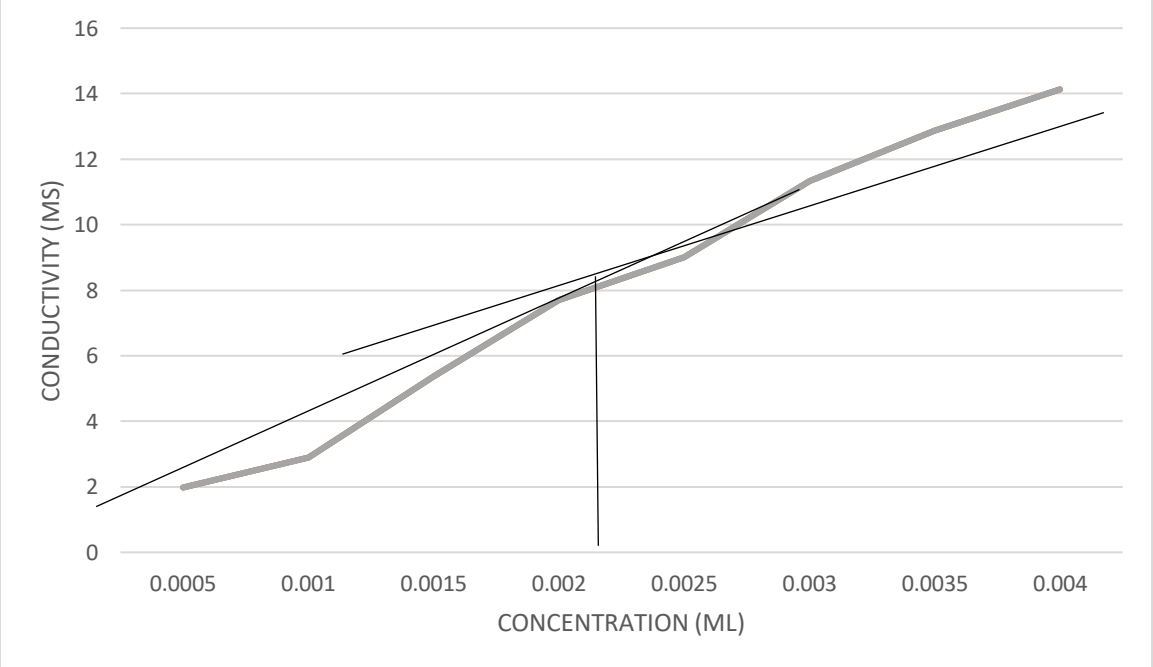


Figure 11: CMC values at temperature 35°C

For 40°C

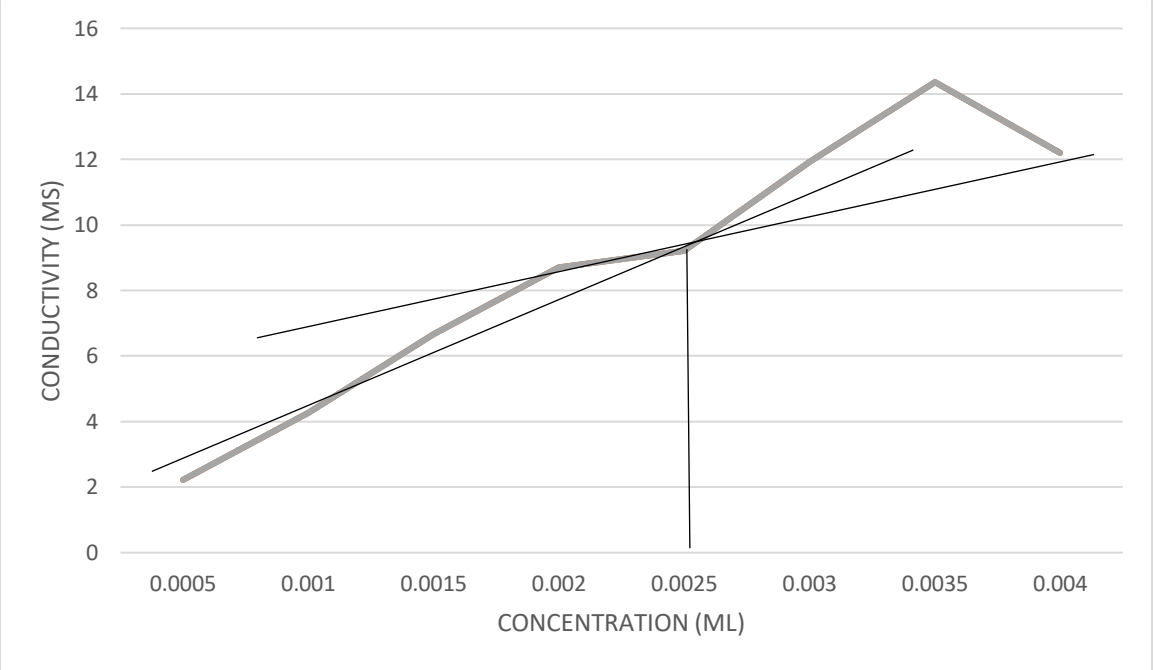


Figure 12: CMC values at temperature 40°C

Temp	CMC	X_{CMC}	T^2	L_NX_{CMC}	dL_NX_{CMC}
25	0.0025	4.5E-05	625	-5.9915	0.01
30	0.003	5.4E-05	900	-5.80901	0.01
35	0.0025	3.6E-05	1225	-6.2146	0.01

Table 3: CMC and X_{CMC} value measured by conductivity meter for C-TAB

Temp	DhoM	Dgo	Dso
25	-0.052	-1.2453	0.04773
30	-0.0748	-1.4489	0.0458
35	-0.1018	-1.8084	0.04876

Table 4: Thermodynamic parameters calculated by using CMC for C-TAB

Cmc is calculated, which is the critical micelle concentration.

CMC is then converted to mole fraction of it, and then used to determine different thermodynamic parameters.

$\Delta H_m^\circ = -RT^2 [d(\ln X_{CMC})/dT]$ (1)

$\Delta G_m^\circ = RT(\ln X_{CMC})$ (2)

$\Delta S_m^\circ = (\Delta H_m^\circ - \Delta G_m^\circ)/T$ (3)

CHAPTER-5

Conclusion

Measurements of binding affinity rely heavily on thermodynamics. Understanding the thermodynamic contributions to free energy changes, and thus affinity, is essential for rational drug development. There are several direct and indirect ways for measuring the enthalpic and entropic components that produce a certain binding free energy. Information content dictates the employment of various technologies at specific phases in the pharmaceutical sector.

Using conductivity meter for measurement of various parametres can be useful and when, these measurements are combined with structural and kinetic data, a complete picture of the binding interaction can be drawn, allowing medicinal chemistry efforts to be directed in directions that are most likely to yield successful results in terms of increased potency and acceptable physicochemical properties associated with efficacy.

These binding affinities can be understood and optimized accordingly to suit the best possible characteristic values of thermodynamic parameters for best possible interactions of lead to ligand or protein.

The various parametric values of enthalpy and entropy, and finally the Gibbs free energy to evaluate many characteristic behavior at the target-ligand binding sites are calculated using conductance by conductivity meter.

Medicinal chemists can attempt to leverage enthalpy-entropy compensation by examining the thermodynamics of the binding interaction, since bioisosteric substitutions on a molecule with adequate affinity can be created to increase physical qualities without compromising the desired potency. Increased use of thermodynamic approaches has resulted in a better knowledge of protein-ligand interactions, which will definitely continue to influence modern drug development.

References

1. Chaires JB. Possible origin of differences between van't Hoff and calorimetric enthalpy estimates. Biophys Chem 1997;64(1-3):15-23
2. A. Uehara, M. Kasuno, T. Okugaki, Y. Kitatsuji, O. Shirai, Z. Yoshida, S. Kihara, J. Electroanal. Chem. 604 115–124
3. Chen W., Tian X., He W., Li J., Feng Y., Pan G. Emerging functional materials based on chemically designed molecular recognition. *BMC Mater*. 2020;2:1–22. doi: 10.1186/s42833-019-0007-1
4. Holdgate GA. Thermodynamics of binding interactions in the rational drug design process. *Expert Opin Drug Discov*. 2007;2(8):1103–1114. Very comprehensive and useful discussion of the thermodynamic characterization of binding interactions with a critical discussion of different experimental approaches and the application of thermodynamic measurements to the drug design process
5. Holdgate GA, Ward WHJ. Measurements of binding thermodynamics in drug discovery. *Drug Discov Today.* 2005;10(22):1543–1550.
6. Chaires JB. Possible origin of differences between van't hoff and calorimetric enthalpy estimates. *Biophys Chem.* 1997;64(1–3):15–23
7. Do enthalpy and entropy distinguish first in class from best in class? *Drug Discov Today*. 2008;13(19–20):869–874. Excellent discussion of the use of thermodynamics to guide drug development with particular focus on the enthalpic optimization approach
8. Ruben AJ, Kiso Y, Freire E. Overcoming roadblocks in lead optimization: A thermodynamic perspective. *Chem Biol Drug Des.* 2006;67(1):2–4. Introduction of the enthalpy funnel approach

and its use for faster thermodynamic optimization of drug leads during drug development.

9. Whitesides GM, Krishnamurthy VM. Designing ligands to bind proteins. *Q Rev Biophys.* 2005;38(4):385–395. Interesting perspective on the progress towards the goal of true rational drug design highlighting important technological developments alongside limitations in our current understanding of molecular interactions
10. The use of calorimetry to study ligand-DNA interactions. In. In: Aldrich-Wright J, editor. *Metallointercalators.* Springer-Verlag/Wien; Heidelberg, Germany: 2011. pp. 299–324.
11. Theoretical calculations of the helix-coil transition of DNA in the presence of large, cooperatively binding ligands. *Biopolymers.* 1976;15 (7):1345–1375.
12. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ: Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 1997, 23:3-25
13. Terstappen GC, Schlupen C, Raggiaschi R, Gaviraghi G. Target deconvolution strategies in drug discovery. *Nat Rev Drug Discov.* 2007;6:891–903
14. Perozzo R., Folkers G., Scapozza L. Thermodynamics of protein-ligand interactions: History, presence, and future aspects. *J. Recept. Signal. Transduct. Res.* 2004;24:1–52. doi: 10.1081/RRS-120037896
15. Ferrante A, Gorski J. Enthalpy–Entropy Compensation and Cooperativity as Thermodynamic Epiphenomena of Structural Flexibility in Ligand–Receptor Interactions. *J Mol Biol.* 2012;417:454–467.
16. Olsson TSG, Ladbury JE, Pitt WR, Williams MA. Extent of enthalpy-entropy compensation in protein-ligand interactions. *Protein Science.* 2011;20:1607–1618

17. Abagyan R, Totrov M, Kuznetsov D. ICM: A new method for protein modeling and design: Applications to docking and structure prediction from the distorted native conformation. *J Comput Chem.* 1994;15:488–506

18. Aqvist J, Medina C, Samuelsson JE. A new method for predicting binding affinity in computer-aided drug design. *Protein Eng.* 1994;7:385–391

19. Wiseman T, Williston S, Brandts JF, Lin LN. Rapid measurement of binding constants and heats of binding using a new titration calorimeter. *Anal. Biochem.* 1989;179:131–137

20. Jelesarov I, Bosshard HR. Isothermal titration calorimetry and differential scanning calorimetry as complementary tools to investigate the energetics of biomolecular recognition. *J. Mol. Recognit.* 1999;12:3–18

21. Gilson M.K., Zhou H.X. Calculation of protein-ligand binding affinities. *Annu. Rev. Biophys. Biomol. Struct.* 2007;36:21–42. doi: 10.1146/annurev.biophys.36.040306.132550

22. Gibbs J.W. A method of geometrical representation of the thermodynamic properties of substances by means of surfaces. *Trans. Conn. Acad. Arts Sci.* 1873;2:382–404