Biomedical & Pharmacology Journal, December 2021. Vol. **14**(4), p. 1823-1829

Solubility of Nifedipine by Shake Flask UV-Spectrometry; Review of Safety Concerns in Pregnancy

Soham Samajpaty

Department of General Medicine, International Faculty, Russian National Research Medical University named after NI Pirogov, Moscow, Russian Federation.
*Corresponding Author E-mail: sohamsamajpaty@gmail.com

https://dx.doi.org/10.13005/bpj/2281

(Received: 16 August 2021; accepted: 11 October 2021)

Nifedipine is chemically dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate, a dihydropyridine derivative used frequently as anti-hypertensive. It is a L- type calcium channel blocker (CCB). Few analogical discrepancies were found between Nifedipine's clinical output report and chemical analysis of solubility. The ambition of this research is to conduct a re-check and proper quantification of partition co-efficient (logP) of Nifedipine and clarify the discrepancy and rectify if any mistake has been done in recent past. The method used is the "gold standard" shake-flask method followed by analysis through UV-scpectrophotmetry.

Keywords: logP of Nifedipine; Medicine; Nifedipine; Pharmacology; Solubility Of Nifedipine.

Nifedipine is chemically dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate, a dihydropyridine derivative used frequently as anti-hypertensive. During depolarization phase of smooth muscles, calcium influx is noted inside the cell through calcium channels from the extracellular space. These calcium ions form a complex with calmodulin and initiates muscular contraction. It is L-type calcium channel blocker which is widely used to manage emergency hypertension, angina, second grade hypertension, Raynaud's phenomenon and pre-term labour. Blocking of these L-type calcium channels restrict entry of calcium ions (Ca^{2+}) into the intracellular space from extracellular space, hence stopping Ca-CM complex or calcium-calmodulin complex formation and functionally inhibiting vascular smooth muscle and cardimyocyte contractions. The drug has high bioavailability with 84%-86% immediate release and peak concentration in plasma is reached at about 2.5-5 hours. This is metabolized in the liver by cytochrome P450 3A4. The drug has also few reported side effects of concern like reflex tachycardia and a high risk of gingival hyperplasia.

Aims and scope

Aim of this research is to find out lipophilicity of Nifedipine in logP scale. It is essential to know such parameter of a drug so frequently being used to review the pharmacodynamics of it. Literary review side by side has also been done to assess true utility and safety concerns of this drug in pregnancy, taking lipophilicity into concern. Some peer reviewed research articles have claimed Nifedipine to be hydrophilic already but without any calorimetric data to support the calims (Zwieten & Pfaffendorf,

1993 Dec, S3-8). This research however, takes a more scientific approach to definitely ascertain calorimetric values and make few assumptions based on the data obtained.

MATERIALS AND METHODS

The research was conducted in Qualissure laboratory services; 361 Prantik Pally, Kasba, Kolkata – 700107, West Bengal, India; NABL certificate no. – TC-6271

1. Market grade Nifedipine tablet of 20mg were bought in enough quantity from local retailer
2. Separating funnel, measuring cylinder, funnel clamp, weighing machine in gram scale, volumetric flasks and petridish were provided by Qualissure laboratory services.
3. 1-octanol (500ml) was provided by Merk Life Science Private Limited,Mumbai – 400079 which was available is Qualissure laboratory services.
4. Distilled water was provided by Qualissure laboratory services
5. UV-3000nm spectrophotometer was provided by Lab India Analytical, made available in same laboratory as above.

LogP value of Nifedipine was indexed by dissolving it in distilled water and 1-octanol, kept in different random volumetric ratios and absorbance and thus concentration was measured in UV-spectrophotometer.

The Experiment with UV- spectrophotometry

In experimental pharmacology many methods have been developed for assessment of solubility of drugs and likewise logP and log D determinations of these. But till date, *shake flask* method is the most reliable method for intentions of such calculations. Theoretically, in this method an aqueous solution with or without buffer solution is prepared, preferably distilled water and another lipid solvent preferably n-octanol is taken and the solute in question is dissolved in them. At first measured volumes of aqueous and lipid phases are taken and poured in a separating funnel and then solute is added in it. The separating funnel is then rigorously shaken in order to ensure dissolution of solute in both the phases and then both the phases are separately collected in test-tubes or volumetric flasks, this is followed by spectrometric analysis (Veseli, Zakelj & Kristl, 2019Nov, 1717-1724).

The experiment was performed in laboratory facility provided by company as has been mentioned before. The setup was in room temperature. At first 9 strips of market grade Nifedipine with each strip having 9 tablets were purchased from local retailer, each tablet being of 20mg. The tablets were at first mechanically grinded with mortar and pestle to obtain dusted form of the drug. It was visible that the dusted powder of market grade Nifedipine contained binding material as impurity which was orange in colour, whereas pure Nifedpine is bright yellow in colour. Hence, the impure Nifedipine was passed through filter fabric net with rigorous shaking and the obtained powder now was bright yellow and pure Nifedipine, orange coloured impurity was separated out.

The next target was separation of specifically weighed Nifedipine powder separately. Hence, on paper pieces of negligible weight, 0.00 g in calibrated weighing machine 10mg, 16mg, 20mg and 30mg Nifedipine were taken respectively. The 10mg placed in a controlled manner in sample number CS-1, 16mg is sample number CS-2, 20mg in sample number CS-3 and 30mg in sample number CS-4, respectively. Now, separate volumes of 1-octanol and distilled water were taken in random and different volumetric ratios suing measurement cylinder under sample groups as mentioned above. After measurement the solvents i.e. 1-octanol and distilled water were poured into separating funnel of 100ml capacity fixed to a clamp and specific weights of Nifedipine powder was mixed in definite groups as determined. This was done to enable the findings to be more random.

Volumes of solvents taken, weight of solute mixed and ratio of their volumes in specific sample groups has been mentioned below in tabular form;-

In such setup, each sample group was taken in separating funnel and was rigorously shaken to ensure proper mixing in both the phases for 5 minutes each. After shaking, the separating funnel was fixed on clamp and was allowed time to settle for 5 minutes. A clear division of lipid and aqueous phases was visible. Distilled water was found at the bottom of the flask and 1-octanol being of lesser specific gravity (0.822 – 0.830) was found over the water (FAO, 1997,vol 4). After this, distilled water – Nifedipine solution and 1-octanol – Nifedipine solution were taken out

from separating funnel into separate volumetric flasks. Such process was repeated two more times to get three separate batches of prepared samples.

The eight volumetric flasks grouped in three sample groups labelled as CS-1, CS-2, CS-3 and CS-4, respectively were taken for spectrophotometry. The analysis was done in UV-300 nm spectrophotometer of India Lab Analytical owned by Qualissure Laboratory Services, Kolkata. At, first a sample of CS-1 from 1-octanol-Nifedipine solution was taken and was scanned to find out the peak wavelength. The peak was observed at 555nm. This is also validated by similar findings where Nifedipine was reduced with Zn/HCl reduction system, followed by diazo coupling reaction where the wavelength peak was determined at 470nm (Aderibigbe, S.A., Adegoke, O.A. & Idowu, 2012, art no. 5). Same was done with Nifedipine in distilled water solution taken from CS-3 and the peak was again observed at 555nm.

Fig. 1. Pure Nifedipine dust

Hence, peak wavelength i.e. λ_{max} = 555 nm

For a systemic understanding, now the solutions are regrouped into two major groups; Nifedipine dissolved in 1-octanol labelled as "CSO" and that dissolved in distilled water labelled as "CSW".

The wavelength now being determined, samples were ready to be tested. In spectrophotometer absorbance of each sample from both "CSO" and "CSW" groups were measured. The results are being mentioned below in tabular form;-

Concentration Calculation

The absorbance thus was recorded. Now, it is required to find out the concentration

Fig. 2. Nifedipine dissolved in upper layer of 1–octanol and lower layer of distilled water

Table 1. Sample grouping in random volumes of solvents and weight of solute

Sample . group No	Volumetric ratio	Volume of 1-octanol (ml)	Volume of distilled water (ml)	Weight of Nifedipine powder (mg)
CS-1	1:1	25	25	10
CS-2	3:4	15	20	16
CS-3	7:5	35	25	20
CS-4	9:10	45	50	30

of Nifedipine in each group of solutions. For the purpose, Beer – Lambert Law is used. The Beer-Lambert law is the most essential and significant law in the field of optical spectroscopy and analytical chemistry. The principle of this law is very simple where solute particle with different vibrational or electrical state is determined and detected by the help of different scattering of light compared to the solvent or medium of solution. Principle is somewhat based on Maxwell's theory interpreting light as electromagnetic wave (Maxwell, 1865, p.459-512). The law states the following;-

$$A = \varepsilon l = \log (I_0/I)$$

A = absorbance; ε = molar absorption co-efficient; l = light path length I_0 = incident light; I = optical path length and c = concentration (Mayerhofer, Pahlow & Popp,14 July 2020,vol 21).

In the experiment, the value of molar absorption co-efficient i.e. was determined to be 14.9112 L mol^{-1} cm^{-1} and optical path length i.e. was 1cm.

Hence, analogically from the Beer Lambert law we can write;-

$$C = A / \varepsilon l$$

Thus, concentration of solute from absorbance thus is found out. The concentration of solute calculated for each group along with their absorbance is written down in tabular form;-

Thus we have complete data of concentration of Nifedipine in each group of solution.

The final aim is to find whether the drug Nifedipine is hydrophilic or lipophilic i.e. it's solubility in aqueous and lipid phases. In discovery or pharmacodynamic of any drug this parameter is of utmost importance, although neglected in case of Nifedipine. The parameter is useful in determining absorption of the drug is specific anatomical regions inside the human body. This may be derived experimentally or using computation technology (K Jacek, P Hanna, M Anna, D Beata, B Marek K, 2012, p. 81-88). This parameter is calculated by measuring the partition co-efficient. The partition co-efficient is by definition concentration of a particular compound in two immiscible solvents

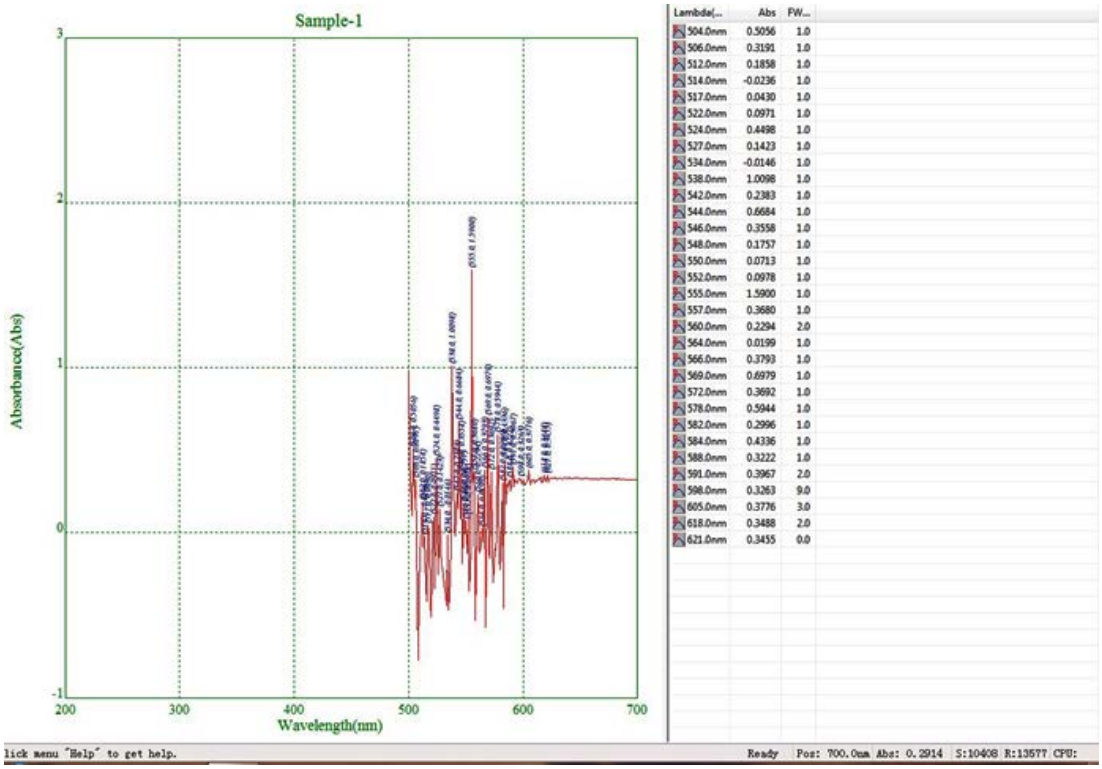

Fig. 3. Wavelength determined by taking Nifedipine in 1-octanol solution in UV – 3000nm spectrophotometer

at chemical and physical equilibrium and is symbolized by "P". The formula is expressed as following;-

$$\log P = \log ([\text{solute}]_{\text{octanol}} / [\text{solute}]_{\text{water}})$$

The logP value accounts for how soluble a compound or solute is in lipid and in aqueous phases. On scale of solubility, "0" value is considered to be a neutral equilibrium point where the solute is neither soluble in lipid nor in aqueous phase. More negative the value, more hydrophilic is the solute and more positive the value, more lipophilic is the compound or the solute dissolved (G. Johanson, 2018, p. 165-187).

Using the above mathematical relation, the value of partition co-efficient has been calculated and logP chart has been made. The logP values for all groups as has been described at first i.e. "CS-1, CS-2 and CS-3" of test samples has been described below in a tabular form;-

Contradictions with previous data

It is to be noted that till date, many methods of measuring physical and thermodynamic parameter of compounds have emerged with time like HPLC, pH metric determination, electrochemical and others. But the *shake flask method* still remains "gold standard" for such analysis in optical and analytical chemistry. In 1995 Masumoto K et Al measured the logP of Nifedipine using high performance liquid chromatography (HPLC) and quantified its value to be 2.20 (Masumoto K, Takeyasu A, Oizumi K & Kobayashi T, 1995, p. 213-220). Such values states that Nifedipine is highly lipophilic, but bioavailability of pure Nifedipine i.e. when binding material and other sugar coatings are excluded in approximated at 44.5 + 7.5% for swallowed Nifedipine and 48.7 + 8.3% when administered sublingually. A highly lipophilic drug as reported by Masumoto K should have greater bioavailability (Aguirre, G Alcazar, JM Rodriguez, F Urrea & MA Cabrera, 1989, p. 129-135). The HPLC system is

Table 2. Nifedipine in 1-octanol solution at 555nm wavelength

Group No.	Absorbance (Au)
CSO-1	0.0559
CSO-2	0.0906
CSO-3	0.1617
CSO-4	0.1911

Table 3. Nifedipine in distilled water solution at 555nm wavelength

Group No.	Absorbance (Au)
CSW-1	0.0517
CSW-2	0.1411
CSW-3	0.0436
CSW-4	0.0981

Table 4. Concentration of Nifedipine in 1-octanol

Group No.	Absorbance (Au)	Concentration (M)
CSO-1	0.0559	0.00374
CSO-2	0.0906	0.00607
CSO-3	0.1617	0.01084
CSO-4	0.1911	0.01281

Table 5. Concentration of Nifedipine in distilled water

Group No.	Absorbance (Au)	Concentration (M)
CSW-1	0.0517	0.00346
CSW-2	0.1411	0.00946
CSW-3	0.0436	0.00292
CSW-4	0.0981	0.00658

Table 6. logP chart of each group of trial

Group no.	logP chart
CS-1	0.033
CS-2	- 0.192
CS-3	0.569
CS-4	0.513

Hence, from logP chart we may conclude that Nifedipine is sparingly soluble in lipid and is mildly lipophilic in nature.

Thus; on an average the mean value of logP = [(0.033 -0.192 + 0.569 + 0.513) / 4] = 0.231

a newer, faster and cheaper method to determine parameters of compounds but HPLC is not flawless. In many experimental results, discrepancy between HPLC computed value and shake-flask method value has been found. Especially, if intermolecular interactions are not enlisted in the database then there is a big chance of getting flawed result and it should be remembered that Masumoto K conducted his experiment during the days of development of CS-905 Nifedipine drug (C Vraka, L Nics, KH Wagner, M Hacker, W Wadsak & M Mitterhauser, 2017, p. 1-10).

Supporting evidences from clinical practice of gynaecology

Nifedipine is frequently used in pregnancy as an anti-hypertensive. Being a dehydropyridine vasodilator it has been frequently used in pre-eclampsia with good clinical results (KD Tripathi, 2019, p. 618). In controlled clinical experimental setup on pregnant women between 26-36 weeks with severe grade pre-eclampsia 31% reduction in hospitalization was noted, concluding it to be safe and convenient in clinical practice of gynaecology (K Fenakel, G Fenakel, Z Appleman, S Lurie, Z Katz & Z Shoham, 1991, p. 331-337). In a larger controlled clinical trial study between 1st April, 2015 and 21st August, 2017, total 298 women with severe hypertension were administered Nifedipine regimen among whom only 3 women i.e. 1% did not show improvement (T Easterling et al., 2019, p. 1011-1021). Any fetotoxicity or embryotoxicity has also been not reported in human fetus.

These are supportive evidences from clinical practice that Nifedipine is mildy lipophilic but its lipophilicty is not as high as has been claimed previously. The human placenta is a discoid shaped structure with 15-20 cm diameter and central foci thickness of approximately 3cm. It is anatomically divided into dull reddish coloured maternal and smooth and glistening amnion visible foetal parts with amnion attached to it (DC Dutta, 2019, p. 26). Experimentally it has been confirmed that both parts are almost inseparable. When desiccated material from placenta was treated with petroleum-ether solvent and dried, both maternal and foetal parts showed rich lipid component (F Fenger, 1917, p. 19-23). Thus a highly lipophilic drug would easily cross placenta and act on the foetus. In case of Nifedipine truly being that much lipophilic (logP = 2.20) it would have blocked calcium channels in the foetus and resulted into immense vasodilatation and cases of foetus wasting or IUGR or abortion might have been clinically visible.

DISCUSSION

The findings determining partition co-efficient (logP) of Nifedipine at 0.231 has clarified the discrepancy between analogy from the view point of pharmacology and analytical chemistry and real clinical practice. It has also made a significant step in understanding pharmacodynamics of Nifedipine, a CCB which was being used from early 1970s which is a big leap in perspective of chemistry. It has also pointed out fallacies which might been obtained in results using HPLC method for determining calorimetric parameter and thermodynamic parameters of different compounds and the paper expects that in future researchers will be more cautious in using HPLC.

CONCLUSION

Nifedipine still remains an useful calcium channel blocker (CCB) in reducing hypertension and cardiovascular deaths and reducing associated psychiatric co-morbidity in the dependants of those succumbing to the cardiovascular diseases. Further it has been found safe in pregnancy in clinical practice and now is being proven the chemical basis of such clinical output. Thus this research has efficiently eradicated the discrepancy between clinical findings and data from the perspective of analytical chemistry.

ACKNOWLEDGEMENT

The author recognizes the aid provided by Qualissure Laboratory Services, Kasba, Kolkata, West Bengal, India for providing the amenities, laboratory facility along with instruments and reagents in order to make this project a success.

REFERENCES

1. van Zwieten, P. A., & Pfaffendorf, M. Pharmacology of the dihydropyridine calcium antagonists: relationship between lipophilicity and pharmacodynamic responses. *Journal of*

hypertension. Supplement : official journal of the International Society of Hypertension, **11**(6): S3–S8 (1993).

2. Veseli, A., Žakelj, S., & Kristl, A. A review of methods for solubility determination in biopharmaceutical drug characterization. *Drug development and industrial pharmacy*, **45**(11):1717–1724 (2019). https://doi.org/10.1080/03639045.2019.1665062
3. Food and Agricultural Organization of the United Nations. *Specifications of flavouring*s; Analytical methods, **4**; (1997)
4. Aderibigbe, S.A., Adegoke, O.A. & Idowu, O.S. A new colorimetric method for the determination of nifedipine tablets by derivatization using 4-carboxyl-2,6-dinitrobenzene diazonium ion. *Int J Ind Chem*, **3**(5):(2012). https://doi.org/10.1186/2228-5547-3-5
5. Maxwell James Clerk 1865VIII. A dynamical theory of the electromagnetic fieldPhil. *Trans. R. Soc.* 155;459–512
6. Thomas G. Mayerhofer, Susanne Pahlow, & Jurgen Popp. The Bouguer-Beer-Lambert Law: Shining Light on the Obscure. *Chem Phys Chem;* **21**(18): (2020).
7. Kujawski Jacek , Popielarska Hanna, Myka Anna, Drabiñska Beata, Bernard Marek K. The logP Parameter as a Molecular Descriptor in the Computer-aided Drug Design – an Overview. *Computational Methods in Science and Technology*. **18**(2): 81-88 (2012).
8. G. Johanson, 1.08 - Modeling of Disposition, Editor(s): Charlene A. McQueen, Comprehensive Toxicology (Second Edition), Elsevier, 2010, Pages 153-177. https://doi.org/10.1016/B978-0-08-046884-6.00108-1.
9. Masumoto K, Takeyasu A, Oizumi K, Kobayashi T. [Studies of novel 1,4-dihydropyridine Ca antagonist CS-905. I. Measurement of partition coefficient (logP) by high performance liquid chromatography (HPLC)]. Yakugaku Zasshi : *Journal of the Pharmaceutical Society of Japan.* **115**(3):213-220 (1995). DOI: 10.1248/yakushi1947.115.3_213. PMID: 7738779.
10. Palma-Aguirre, J. A., Rosas-Alcázar, G., Rodríguez, J. M., León-Urrea, F., & Montoya-Cabrera, M. A. Bioavailability and pharmacokinetics of nifedipine administered by different routes in healthy volunteers. *Archivos de investigacion medica*, **20**(2): 129–135 (1989).
11. Chrysoula Vraka, Lukas Nics, Karl-Heinz Wagner, Marcus Hacker, Wolfgang Wadsak, Markus Mitterhauser, LogP, a yesterday's value?, *Nuclear Medicine and Biology,* **50**: 1-10 (2017) ISSN 0969-8051, https://doi.org/10.1016/j.nucmedbio.2017.03.003.
12. KD Tripahi; Essentials of Medical Pharmacology. 8th Edition; 2019. Page – 618
13. Fenakel, K., Fenakel, G., Appelman, Z., Lurie, S., Katz, Z., & Shoham, Z. Nifedipine in the treatment of severe preeclampsia. *Obstetrics and gynecology*, **77**(3): 331–337 (1991).
14. Winikoff, Beverly (2019/09/21). Oral antihypertensive regimens (nifedipine retard, labetalol, and methyldopa) for management of severe hypertension in pregnancy: an open-label, randomised controlled trial. *The Lancet*, **394**:1011-1021. doi: 10.1016/S0140-6736(19)31282-6
15. DC dutta. DC Dutta's Textbook of Obstetrics. 9th Edition; 2019. Page – 26
16. Frederic Fenger, THE CHEMICAL COMPOSITION OF THE PLACENTA, *Journal of Biological Chemistry*, **29**(1): 19-23 (1917) ISSN 0021-9258, https://doi.org/10.1016/S0021-9258(18)86803-X.