Veber's Rules in Terahertz Light

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Research article

Keywords: Pharmacology, Histidine, Veber's rules, Terahertz technique, Attenuated Total Reflection spectroscopy

Posted Date: January 30th, 2020

DOI: https://doi.org/10.21203/rs.2.22281/v1

Abstract

Background: The Attenuated Total Reflection (ATR) technique of spectroscopy in combination using the terahertz technique is being investigated to find rotatable bonds, which are considered as one of the rules of thumb given by Veber et al..

Results: Histidine, as one of the most studied pharmacological preparations, is used in an experiment to test our research. Three rotatable bonds have been found and thus the results published in pharmaceutical data guides have been confirmed.

Conclusion: The results are obtained in a terahertz spectrometer equipped with a Dove prism, where an ATR technique is applied. The samples in two forms, polycrystalline and as a solution in alcohol, are used to check the methodology. Histidine molecules, dissolved in alcohol and thus freed from intermolecular bonds, exposed the rotations around the three bonds. Thus, the validity of the ATR-THz research methodology used in the search for rotating bonds in drug candidates molecules is confirmed. The goal of the method presented in the paper is to support pharmacological research, and pharmaceutical industry, as well.

Background

A number of practical principles have been formulated to support drug search, the so-called rules of thumb. The rules of thumb for the selection of ‘drug-like’ compounds consist of a wide range of properties, such as [1]:

- the number of rotatable bonds (ROTB),
- the number of aromatic rings (AROM),
- the number of hydrogen bond donors and acceptors (HBD and HBA),
- the polar surface area (PSA),
- the acid dissociation coefficient (pKa),
- lipophilicity (logP and logD),
- molecular weight (MW),
- the fraction of sp3 carbons (FSP3).

The best known rule is Lipinski's rule of five, also known as Pfizer's rule of five or simply the rule of five (RO5) [2]. In the RO5, the property criteria were proposed to be:

- lipophilicity (logP) < 5,
- molecular weight (MW) < 500,
- the number of hydrogen bond donors (HBD) < 5,
- the number of hydrogen bond acceptors (HBA) < 10.

In pharmacology, bioavailability (BA or F) is one of the principal pharmacokinetic properties of drugs.

Egan et al. developed a model for predicting bioavailability based on logP and PSA numerical values [3]. Good bioavailability is attributed to a molecule when:

- lipophilicity (logP) ≤ 5,88,
- polar surface area (PSA) ≤ 131,6.

In turn, the Muegge method in the assessment of bioavailability analyzes a much larger number of parameters [4]. According to the author's assumptions, a drug with good bioavailability satisfies the following requirements for its chemical structure:

- 200 ≤ molecular weight (MW) ≤ 600
- -2 ≤ lipophilicity (logP) ≤ 5,
- polar surface area (PSA) ≤ 150,
- number of rings ≤ 7,
- number of carbons > 4,
- number of heteroatoms > 1,
- number of rotatable bonds (ROTB) ≤ 15,
- number of hydrogen bond acceptors (HBA) ≤ 10,
- number of hydrogen bond donors (HBD) ≤ 5.

For a more efficient achievement of the goal, Miles Congreve et al. restricted the RO5 to the rule of three (RO3) [5]:

- number of rotatable bonds (ROTB) ≤ 3
- molecular weight <300,
- number of hydrogen bond donors ≤3,
- number of hydrogen bond acceptors ≤3
- logP ≤3,
- polar surface area (PSA) ≤ 60.

Ghoes et al. provided an analysis of some computable physicochemical properties and chemical constitutions of known drug molecules available in the database of the Comprehensive Medicinal Chemistry (CMC) and seven known drug classes [6]. The study showed that the qualifying range of

- the calculated log P for drug-like molecules is -0.4 to 5.6. The mean value is 2.3, and the preferred range (most populated for an interval having 50% of the drugs) is 1.3 to 4.1.
- For molar refractivity, the qualifying range is 40 to 130. The mean is 97, and the preferred range is 70 to 110.

- For molecular weight, the qualifying range is 160 to 480. The mean molecular weight is 360, and the preferred range is 230 to 390.
- For the total number of atoms, the mean value is 48, and the qualifying range is 20 to 70. The preferred range for the total number of atoms is 30 to 55.

There are other complementary rules.

The rules of Veber et al. are [7]:

- 10 or fewer rotatable bonds (optimally 7),
- polar surface area less than or equal to 140 Ǻ (optimally between 110 Ǻ and 140 Ǻ).

Additionally, we usually add expectations of the beneficial properties of drugs to these rules [1], such as:

- high activity and selectivity,
- synthetic availability,
- no chemically reactive groups,
- the possibility of oral administration,
- pharmacokinetic properties,
- metabolism,
- routes of removal from the body,
- no side effects and toxicity.

Even a cursory analysis of the abovementioned points and research in the direction of the above guidelines have proven that finding simple principles for preserving a drug molecule in a living organism is impossible.

Matthew Segall attempted to find a simple rule based on simple properties that could be used to identify the right components with a significantly greater chance of creating an effective drug [1]. Finally, Segal concluded that no single rule can fulfill this role. This frustrating conclusion is prompting scientists to search for new solutions, methods and theories for the design and technology of effective medicine production.

Before finally finding an effective single rule that allows one to design the perfect medicine, let us address one of Veber's rules: the number of rotatable bonds should not exceed 10. This rule is based on Veber's observations that excessive amounts of rotatable bonds may impede anchoring of the drug to a diseased molecule. We show in this paper that the methodology of terahertz time spectroscopy (THz-TDS) can be an effective way to detect such bonds. In our opinion, such experimental studies are necessary to confirm the theoretical data that are usually obtained with computational chemistry.

Results

One of the amino acids – histidine - is selected for the experiment. Amino acids are the building blocks of proteins in our bodies. Histidine is used in rheumatoid arthritis, allergic diseases, ulcers, and anemia caused by kidney failure or kidney dialysis.

The samples were prepared in two forms: polycrystalline and saturated solutions in alcohol.

Fig. 2 shows a hypothetical crystallographic grid in which histidine molecules are trapped in the nodes. (only the histidine crystalline lattice belongs to the space group [8]). The possible intermolecular vibrations and torsional movements are indicated. For the polycrystalline form of the sample, both translation and rotational vibrations can be observed in a terahertz spectrum. Even from such a simplified drawing, it is visible that the released molecules from the crystallographic lattice should show only torsional/rotational movements. Such a conclusion leads directly to the methodology of designing an appropriate experimental system. Here, the following question should be answered: what test method should be used to “see” the rotating bonds that we are looking for?

- First, from an optical point of view, rotations in complex molecules are relatively slow and can therefore be observed in the far-infrared region, i.e., in the area of terahertz spectroscopy.
- Second, the sample should be prepared in a dissolved form to release molecules from the crystallographic lattice. Very often, the solvent shows very strong absorption for terahertz waves. Fortunately, for the experimenter, with the terahertz spectroscopy technique, it is possible to apply the attenuated total reflection methodology, which allows an investigation of the samples even in an aqueous environment.

The results of the THz spectroscopy measurements are shown in the diagram in Fig. 5. Four measurement results are presented in the same figure for a comparison: (1) the absorbance of pure ethyl alcohol, (2) histidine dissolved in alcohol, (3) the difference between pure alcohol and the saturated solution of histidine, and (4) the spectrum of the polycrystalline form of the histidine sample. The diagram is prepared for two systems of units: frequency in terahertz (THz) and inversed centimeters (). The spectra are calculated from the absorbance of the samples:

[See supplementary files for formula.]

(Absorbance is the common logarithm of the ratio of incident to transmitted radiant power through a material).

In the polycrystalline sample, we can observe all possible vibrations of the molecule - diagram (4). The results are shown in Table 1.

As can be seen, the dissolved sample exhibits a strong absorption peak at 24.9 (0.74 THz) and two weaker peaks at 57.5 cm^{-1} (1.73 THz) and 70.8 cm^{-1} (2.12 THz), indicated with the arrows RB.

Discussion

As expected, diagram (4) in Fig. 3 shows all possible spectral details responsible for both the intermolecular vibrations and torsional/rotational internal movements of the histidine molecule. The true picture of the molecule is revealed when it is free of intermolecular vibrations. Then, we have the opportunity to observe only internal movements belonging only to the molecule—see diagrams (2) and (3). In this case, we observe only rotations [12]. Other sources also indicate 3 rotatable bonds [13].

In summary, considering Veber's rules of thumb, we have:

- 10 or fewer rotatable bonds—here, only 3 rotatable bonds [this paper and [13]],
- polar surface area less than or equal to 140 Å—here, 92 Å according to [13]—and we can find that Veber's rules are fulfilled for histidine.

Conclusion

The THz technique appears to be a sensitive and selective technique that allows the detection and identification of details of a molecular structure. In this work, the THz-ATR methodology was used to identify rotatable bonds (ROTB) in a histidine molecule. It is known that rotatable bonds are taken into account as one of the rules of thumb given by Veber et al. As we have shown, the methodology of terahertz time spectroscopy including an ATR system can be an effective way to detect rotatable bonds. Usually, the published data are obtained using computational chemistry [13]. We show a method to experimentally confirm some of the data. Thus, the research methodology presented in the paper should produce measurable benefits for pharmacological research and thus may also support the pharmaceutical industry.

Methods

Fig. 4 shows the scheme of the terahertz time-domain spectrometer (THz-TDS) [9]. The setup is driven by a pulsed femtosecond laser (83 fs) with a repetition frequency of 80 MHz and a wavelength of 780 nm. The terahertz radiation is produced by a photoconductive antenna, Tx, made of LT-GaAs (low-temperature galium arsenide). The same kind of photoantenna is used as a detector, Rx. The terahertz beam (2) is formed and focused with parabolic off-axis mirrors, PM. The samples are placed on the surface of a Dove prism (made of high-resistivity silicon with a resistivity of approximately 1 kΩm). The Dove prism is an optical element prepared in such a way that the direction of the THz wave does not change. Arms (1) and (2) form the sampling path, and arm (3) forms the detection path. Attenuated total reflection (ATR) spectroscopy is used in the experiment [10]. The ATR technique employs the total internal reflection phenomenon, where an evanescent wave (EV) develops along the boundary surface of the prism (see Fig. 5).

The EV penetration depth into the sample is relatively small – usually between 0.5 and 2 μm. The exact value depends on the wavelength of the probing beam, the angle of incidence and the refractive indices. The evanescent effect only works if the crystal is made of an optical material with a higher refractive

index than the sample being studied (here, 3.4). The experiment is prepared with two arrangements: samples in polycrystalline form and dissolved samples, shown in Figs. 4a) and 4b), respectively. In the case of the solid samples, polycrystalline powder is firmly clamped to ensure good contact and to remove trapped air that would reduce the signal intensity. An important advantage of the ATR method is the possibility of measuring strongly absorbing media with an infrared (IR) or terahertz wave. In terms of measuring the spectrum of the molecules released from intermolecular bonds, the ATR method is a convenient solution. Another advantage appears even for polycrystalline sample measurements. A disadvantage of scientists being involved in the synthesis of molecules (drugs) is the small amount of material obtained. The ATR method provides the opportunity to overcome this inconvenience. Comparing Fig. 5a) and Fig. 5b), 1 mg of the synthetized molecules is sufficient to perform the measurements successfully. The samples, as mentioned, are prepared as a powder (polycrystalline form) and saturated solutions in alcohol.

Declaration

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Freely available to any scientist wishing to use them for noncommercial purposes.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by Wroclaw University of Science and Technology and Wroclaw Medical University.

Authors’ contributions

EFP—conception. SP—interpretation of the data. Both authors have equally contributed to the writing of the manuscript and read and approved the final paper.

Acknowledgements.

The authors wish to express their deep gratitude to Mr. Przemyslaw Jarzab for assistance with the measurement.

References

[1]. Segall M., Drug Discovery, „Rules for drug discovery: can simple property criteria help you to find a drug?”, *15(2),* 19–24 (2014).

[2]. Lipinski C. A, Lombardo F., Dominy B. W., Feeney P. J., (March 2001). “Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings”, Adv. Drug Deliv. Rev. *46 (1–3),* 3–26 (2001).

[3]. Egan W. J., Merz K. M., Baldwin J. J., “Prediction of Drug Absorption Using Multivariate Statistics”, J. Med. Chem., *43,* 3867–3877 (2000).

[4]. Muegge I., “Pharmacophore features of potential drugs”, Chem. Eur. J., *8,* 1976–1981 (2002) No. 9.

[5]. Congreve M., Carr R., Murray C., Jhoti H., “A ‘rule of three’ for fragment-based lead discovery?”, Drug Discov. Today. *8 (19),* 876–877 (2003).

[6]. Ghose A. K., Viswanadhan V. N., Wendoloski J. J. “A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases”. J. Comb. Chem., *1 (1),* 55–68 (1999).

[7]. Veber D. F., Johnson S. R., Cheng H. Y., Smith B. R., Ward K. W., Kopple K. D., “Molecular properties that influence the oral bioavailability of drug candidates”. J. Med. Chem. *45 (12),* 2615–2623 (June 2002).

[8]. Suresh C. G., Vijayan M., “Occurrence and geometrical features of head-to-tail sequences involving amino acids in crystal structures”, Int. J. Peptide Protein, *22,* 129–143 (1983).

[9]. Karolewicz B., Plinski E. F., Fuglewicz B., Nowak K., Jarzab P. P., Plinska S., Walczakowski M. J., Augustyn Ł., Mikulic M., Pałka N., Szustakowski M.: “Selected aspects of terahertz spectroscopy in pharmaceutical sciences”, Acta Pol. Pharm.—Drug Research, *72,* 851–856 (2015) No. 5.

[10]. Minnes R., Nissinmann M., Maizels Y., Gerlitz G., Katzir A. & Raichlin Y., “Using Attenuated Total Reflection–Fourier Transform Infra-Red (ATR-FTIR) spectroscopy to distinguish between melanoma cells with a different metastatic potential”, Scientific Reports, 7, 4381–7 (2017).

[11]. True A. B., Schroeck K., French T. A., Schmuttenmaer Ch. A., “Terahertz spectroscopy of histidine enantiomers and polymorphs”, J. Infrared Milli. Terahz. Waves (2011) 32:691–698.

[12]. Matei A., Drichko N., Gompf B., Dressel M., “Far-infrared spectra of amino acids”, Chem. Phys., *316,* 61–71 (2005).

[13]. https://www.guidetopharmacology.org.

Table

Table 1. The results of the histidine absorbance terahertz spectrum measurements. Spectral details are marked in order of appearance a, b, c, d, e and f. (Values in parenthesis according to [13]). Rotatable bonds are indicated with RBs.

a	b	c	d	e	f	
0.74	1.44	1.73	2.12	2.53	2.88	THz
(0.76)	(1.41)	(1.73)	(2.09)			
24.9	47.9	57.5	70.8	84.3	95.9	cm^{-1}
(25.4)	(46.9)	(57.6)	(69.7)			
RB		RB	RB			

Figures

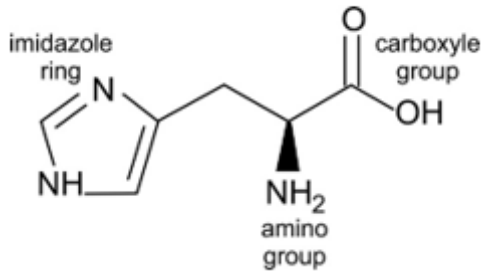

Figure 1

Molecular structure of histidine. Three components are indicated: the imidazole ring, amine group, and carboxyl group.

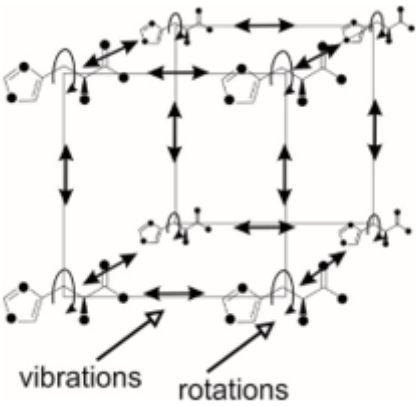

Figure 2

The figure roughly presents histidine molecules trapped in the centers of the P-type crystallographic lattice.

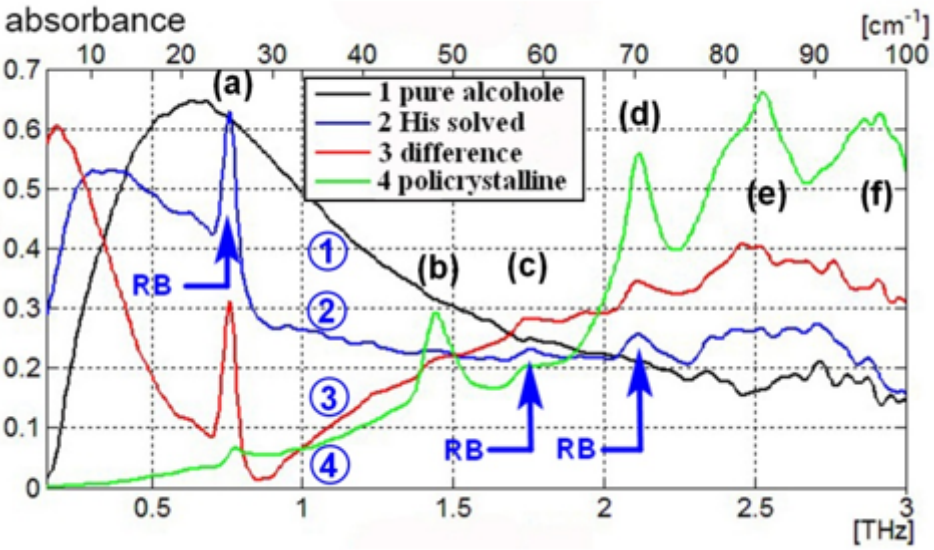

Figure 3

Histidine absorbance in the THz band: 1) pure alcohol, 2) dissolved histidine (His), 3) difference of the alcohol-dissolved sample, and 4) polycrystalline form of the sample (powder). The six spectral details marked as a, b, c, d, e, and f along the curve (4) are shown in Table 1. The rotatable bonds, RBs, are indicated with arrows.

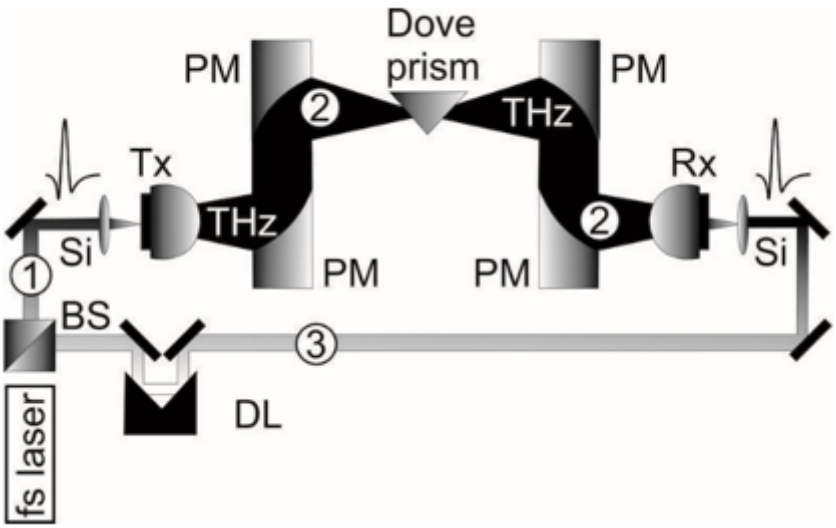

Figure 4

Schematic illustration of the THz-TDS spectrometer. 1+2 – probing arm of the spectrometer, 3 – detecting arm, Tx – THz transmitter, Rx – THz receiver, BS – cubic beam splitter, DL – optical delay line, PM – parabolic off-axis mirror, Si – microscopy lenses.

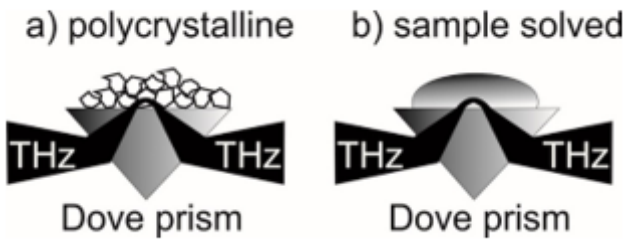

Figure 5

Attenuated total reflection (ATR) spectroscopy: a) Investigations of the polycrystalline forms and b) investigations of the dissolved samples.

Supplementary Files

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