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RAMAN ANALYSIS OF DIFFERENT METFORMIN TABLETS

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Abstract

One of the oldest and most commonly used blood sugar lowering drugs is metformin (N, N-dimethylbiguanide). Having limited side effects, it has been used since the late 1950s as the first line of treatment for patients suffering from diabetes mellitus. It has also been reported to have pleiotropic actions as anticancer agents. In this study, different tablets of metformin were analyzed by Raman spectroscopy. These tablets, coming from the United States and the Philippines, contain different amount of metformin which were compared. The effect of glipizide on the Raman spectra was also determined in one of the tablets. Results showed common peaks found in all samples with increased signals observed in tablets containing a higher amount of metformin. These peaks matched with that obtained using density functional theory (DFT) calculations. Lastly, reduction of the Raman signal was observed in tablets containing glipizide.

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Introduction

Diabetes mellitus is one of the most common metabolic diseases, and the rate of prevalence in the population is reaching alarming levels[1]. In 2017, it was reported that 425 million adults are living with diabetes and this number is expected to rise to 629 million by 2045[2]. This metabolic disorder is recognized as the world fastest growing chronic condition and the number of people with Type 2 diabetes is growing around the world. Causes of the disease involve a combination of genetic and environmental factors. Obesity, unhealthy lifestyle, alcohol, smoking, and other risky health behaviors increase the chances of developing diabetes. Diabetes mellitus is a progressive disease, characterized by low glucose responsiveness, hypoglycemia due to impaired insulin secretion or action defects, as well as insulin resistance. Insulin is required for carbohydrate, fat and protein metabolism.[3] If the body is resistant to insulin, its action in liver and muscles is impaired.[4]

The end goal of Type 2 diabetes treatments is to increase the quality of life and lifespan through prevention, or progression of vascular complications. Some of the drugs used to treat diabetes include α-glucosidase inhibitor, thiazolidine, and metformin. These drugs are used in combination with proactive lifestyle changes and improvements for individuals with diabetes. This kind of treatment approach is cost effective as well.[4] Metformin, or its IUPAC name of 3-(diaminomethylidene)-1,1-dimethylguanidine (Figure 1) has been used as a prescription drugs to treat Type 2 diabetes since the 1950s. It counteracts insulin resista nce by increasing insulin sensitivity.[5]

Metformin tablets are a member of a class of drugs called biguanides that help lower blood glucose concentration levels by improving the way the body handles insulin. It contains two methyl groups at one end of the molecule and was originally discovered in the *Galega officinalis* plant and proved to be one of the most effective antidiabetic drugs[5]. Metformin hydrochloride is one of the two peroral antidiabetic drugs included in the World Health Organization (WHO) Model List of Essential Medicines[6]. Metformin was observed carefully during the initial years of its availability and was even banned at first in many countries including the USA. It is now one of the most prescribed oral drugs in the world.

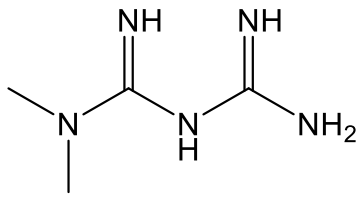

Figure 1. Chemical structure of metformin.

Metformin is more potent when used in combination therapy with insulin, sulfonylureas and zolidinediones, as opposed to a stand-alone treatment. The effects are the same in every individual regardless of their age, body weight, ethnicity, etc. It decreases body weight because of its appetite suppressive effects. It is usually taken at dosages of 500mg to 1500 mg with a large meal. The most common side effects are gastrointestinal or digestive, and include nausea, vomiting, stomach upset, diarrhea, weakness, and a metallic taste in the mouth. However, most of the symptoms disappear within two weeks of treatment and can be avoided through slowly increasing dosage and adaptation to the drug.[7]

The main objective of this study is to use Raman spectroscopy to determine any differences between five different metformin tablets that are commercially marketed. Although, the main active ingredient is the metformin, the presence of other excipients such as glipizide can possibly have an effect on the Raman spectra of a given tablet. The Raman spectra obtained experimentally in metformin tablets were also compared with calculated Raman spectra to aid in assigning the functional group responsible for the observed peaks.

Experimental Methods

Five types of metformin tablets were used in the study. Three of the metformin were manufactured in the United States and supplied by Maxwell Pharmacy while the remaining two were from the Philippines. Table 1 shows the information about the 5 tablets used in the study. Companies that manufactured the tablets in the US are Teva, Zydu, and Epic Pharmacy. Companies manufactur-

ing metformin in the Philippines are Watsons and Unilab. Teva contains 500 mg metformin + 2.5 mg glipizide. Zydu and Watsons contain 500 mg metforming HCl. Epic Pharmacy and Unilab Glumet contain 1000mg metformin HCl.

All Raman spectra were obtained on a Jasco NRS-3100 confocal dispersive Raman spectrometer equipped with a macro-Raman measurement accessory (Easton, MD). Raman scattering was induced by a 12mW 488 nm laser and collected on a thermoelectrically cooled CCD detector. The macro-Raman assembly permitted direct measurements of the solid sample in quartz slide. The Raman spectra of the metformin tablets were taken in their pure form without any sample preparation.

To help analyze the Raman data, computational studies were performed to aid in the peak assignments. The geometry of metformin was optimized in the gas phase with the structure generated using the Chem3D sub-structure library and the frequency calculated using Gaussian 09W software. The calculation was carried out using density functional theory (DFT) approximation implementing the Becke's three-parameter exchange functional in combination with the Lee, Yang, and Parr correlation function (or B3LYP).

Results and Discussion

Code	*Description*	*Source*	*Manufacturer*
M1	500 mg metformin + 2.5 mg glipizide	United States	Teva
M2	500 mg metformin HCl	United States	Zydu
M3	1000 mg metformin HCl	United States	Epic Pharmacy
PM1	500 mg metformin HCl	Philippines	Watsons
PM2	1000 mg metformin HCl	Philippines	Unilab Glumet

Table 1. Metformin tablets used in the study.

M1	M2	M3	PM1	PM2	Band Assignment
400	425	428	429	429	C-N-C deformation
518	519	522	523	521	C-N-C deformation
	564	567	569	568	C-N-C deformation
642				634	C-N-C deformation
	726	730	730	730	C-N-C deformation
	740	743	744	744	N-H wagging
	801	801	806	806	N-H wagging
	940	942	944	943	N-H wagging
	1039	1040	1043	1043	C-N stretching
	1084	1086	1987	1086	C-N stretching
	1165	1165	1169	1169	C-N stretching
				1250	C-N stretching
	1280	1280	1281	1283	C-N stretching
	1424	1424	1422	1425	CH_3 sym. deformation
	1454	1454	1454	1456	CH_3 asym. deformation
	1470	1472	1471	1473	CH_3 asym. deformation
	1512	1512	1511	1500	N-H in plane deformation
	1513	1513	1513	1515	N-H in plane deformation
	1567	1568	1566	1569	C=N stretching
	1651	1651	1650	1654	C=N stretching
	2818	2818	2820	2821	CH_3 sym. stretching
	2886	2886	2888	2889	CH_3 sym. stretching
	2942	2942	2944	2945	CH_3 asym. stretching
	2978	2978	2980	2980	CH_3 asym. stretching
		3012	3015.6	3016	N-H sym stretching
	3195	3196	3198	3198	N-H asym stretching

Table 2. Peaks observed in metformin tablets used in the study

The Raman spectra of the different metformin samples are shown in Figures 2-4 with the peaks listed in Table 2 that also includes the functional groups responsible for the peaks as assigned by theoretical calculations. Common peaks can be observed in all tablets with the exception of the M1 tablet. This tablet contain glipizide and it is observed that all M1 tablets have lower signals in all regions in comparison to the other tablets. In addition, the signals are observed to be higher on tablets containing higher amount of metformin (1000 mg vs 500 mg).

Upon closer inspection of the peaks, it has been observed that

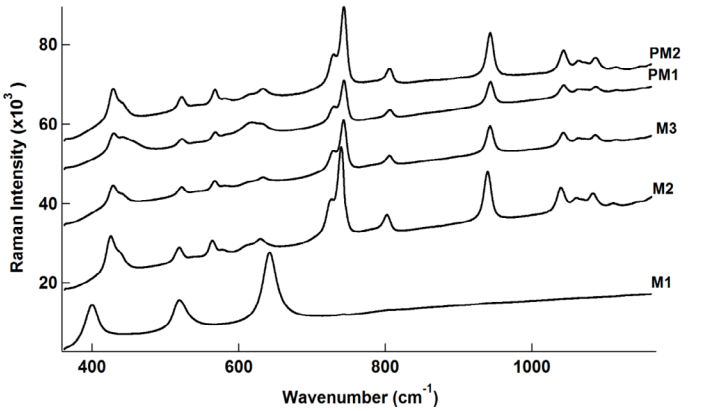

Figure 2. Raman spectra of the metformin tablets in the 400-1050 cm^{-1} region.

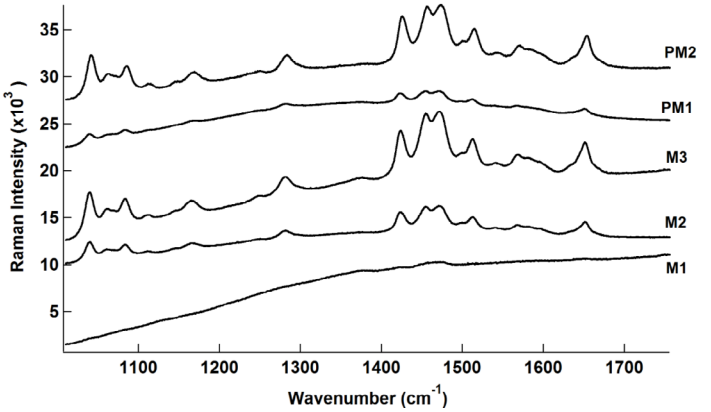

Figure 3. Raman spectra of the metformin tablets in the 1070-1760 cm^{-1} region.

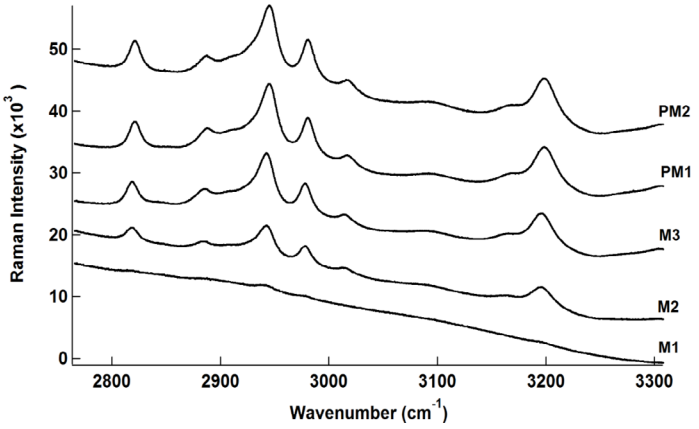

Figure 4. Raman spectra of the metformin tablets in the 2750-3300 cm^{-1} region.

there are slight shifts in some of the peaks in some of the tablets. For instance, a peak at 740 cm^{-1} observed in M2 tablet slightly blue shift to 743 cm^{-1} in M3 tablets and to 744 cm-1 in both tablets from the Philippines (PM1 and PM2). A blue shift of 2-3 cm^{-1} can be observed from M2 to M3. PM1 and PM2. Tablets from the Philippines are consistently blue shifted in comparison to the US based metformin tablets. It can also be observed that a broaden peak at 614 cm^{-1} in PM1 tablet which is not as obvious in the other tablets.

Being a small molecule containing the functional groups such as amine and methyl, peak assignment was much easier with aid from theoretical calculations (Figure 5). The peaks below 700 cm^{-1} can be assigned to C-N-C deformation while peaks in the region of 700-1000 cm^{-1} can be due to N-H wagging. The vibrational mode C-N stretching can be for the 1000-1200 cm^{-1} region while C-H deformation in the methyl group can be assigned between 1200-1500 cm^{-1} region. The N-H in plane deformation can be assigned to peaks observed in the 1500-1600 region which can be overlap with C=N stretching in the 1550-1700 cm^{-1} region. The peaks in the 2800-3000 cm^{-1} region can be due to the C-H modes in the methyl group while peaks above 3000 cm^{-1} can be due to N-H stretching. The peaks observed in all tablets are in agreement with those reported in literature.[8,9]

The presence of glipizide on the metformin tablet has a direct effect on the Raman spectra. Glipizide is a second-generation sulfonylurea that is also widely used to treat Type 2 diabetes. Sulfonylureas help the pancreas to make more insulin and help the cells to respond better to insulin and help lower blood sugar, and keeps it under control.[9] Although the amount of glipizide is only 2.5 mg in comparison to 500 mg of metformin, the analyzed tablet was found to be fluorescent hence affecting the Raman signal. However, there is no reported study on the fluorescent nature of glipizide. It has been reported that it is a fluorescent quencher upon binding with bovine serum albumin.[10] It is possible that the source of fluorescence came from the excipients present in the sample. The identity of the excipients is hard to determine and test since the tablet formulations depend on the manufacturing company and their proprietary.

For the tablets that have slight shifts in peaks, the main reason for this is the environment where the metformin is. It has been observed that there is a distinct shift between the tablets from the United States and the Philippines. The two tablets from the Philippines are blue shifted in comparison to those locally available. This means that the excipients (other components in the tablets) in these tablets are more non-polar in comparison to those manufactured in the United States. This trend has been observed in vibrational modes of cyano- and nitro- groups where samples containing these functional groups blue shifts when place in a more non-polar environment.[11-12]

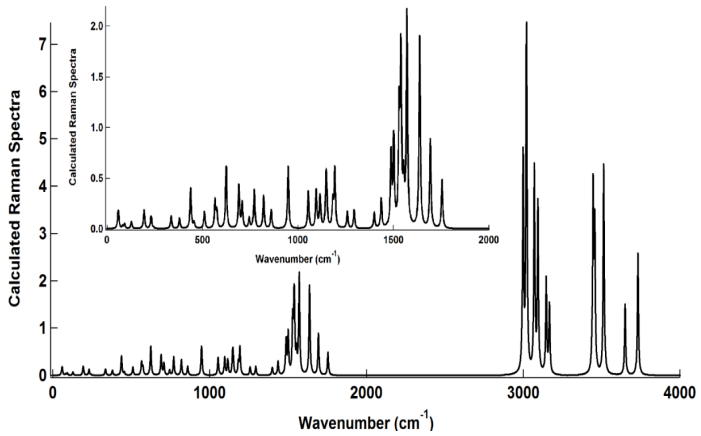

Figure 5. Calculated Raman spectra of metformin.

Conclusion

Metformin is a potent hypoglycemic and glucose-lowering drug. Research has shown that it is powerful when used alone, with stronger Raman signals in higher concentrations. Other excipients present in the metformin drugs have decreased its peak signal strength. All tablets showed same peaks, with slight shift differences between them except M1 tablet which can be due to the presence of glipizide.

Acknowledgements

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