An Analysis of Tetrahydrocannabinol (THC) and Its Analogs Using Surface Enhanced Raman Scattering (SERS)

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Abstract

We present an analysis of the adsorption geometry of THC and its analogs (cannabinol, cannabidiol and HU-210) on silver using Surface Enhanced Raman Scattering (SERS) technique along with DFT calculations. Our study shows that these cannabinoids are oriented parallel to the silver surfaces and form bonds using primarily the benzene rings and oxygen atoms. In addition, we examine the effect of four different salts: $MgCl_2$; $MgSO_4$; KNO_3; and Na_2SO_4 on SERS enhancements of these drugs. We observe that $MgCl_2$ causes the greatest enhancement for most of these drugs. We attribute this enhancement to the increase of the electric field induced by Mg^{2+} salts and the formation of SERS active sites. This work also provides evidence that each salt most likely affects the orientation of all four cannabinoids in a similar way on a silver surface, resulting in a production of similar SERS spectra.

Key Words: Cannabinoids, THC, SERS

Introduction

In recent years, the abuse of psychoactive drugs has been attributed to the rise of criminal activity and an increase in serious health issues[1,2]. Cannabis, also known as marijuana, is one of the most commonly used psychoactive drugs containing more than 60 psychoactive cannabinoids[3]. The abuse of these psychoactive cannabinoids has been associated with higher crime rates in major cities across United States[4]. As a response to this crisis, developing a sensitive, fast, efficient and reliable technique for the detection of these forensic substances has been of utmost importance for the forensic community[5]. In response to this challenge, Surface Enhanced Raman Scattering (SERS) has recently gained considerable attention as a sensitive, rapid and non-destructive technique for forensic analysis[6,7,8,9]. The advantage of SERS relies on the fact that this technique can greatly amplify Raman signals from molecules adsorbed on the surface of silver particles. Factors that typically effect this enhancement include the formation of "hot spots" and the adsorption of the molecules on the silver substrates. Hot spots are gaps between adjacent silver particles where the strength of the electric field is highly increased[10]. As a part of the SERS technique, salts are commonly added to silver nanoparticles in order to generate these hot spots. On the other hand, the adsorption of molecules on the silver substrates results in the enhancement of the Raman signal due to a charge transfer between the molecules and the substrates[11,12]. Charge transfer transitions have been attributed not only in the enhancements of weak normal Raman vibrational modes but also in the enhancements of Raman forbidden modes appearing in the SERS spectra. Therefore, an understanding of the bonding geometry of molecules on silver has been an essential part of the SERS technique due its effect on Raman signal enhancements.

During the past several years, SERS has been used for the detection and analysis of many synthetic opioids and cannabinoids including fentanyl, carfentanyl, 5F-PB-22, synthetic variations of K2 spice, and both the psychoactive substances flakka and AMB-FUBINACA[9,13,14,15,16]. In addition to the detection of these drugs, the development of a reliable and effective SERS technique has been a current focus of research. For this purpose, the examination of bonding of molecules and their orientation on SERS substrates has been gaining considerable momentum over the years[14,17,18,19]. Using DFT calculations and SERS, Alkaseem *et. al.* has studied the adsorption geometry of the synthetic cannabinoid F5-PB-22 on silver and its correlation to the SERS spectrum[13]. In our recent study of trace analysis of the synthetic cannabinoid AMB-FUBINACA, we utilized DFT calculations to explain the possible binding geometry of this drug on a silver surface[14]. In this study, we demonstrated the relationship between the binding geometry and the observed enhancements of the weak Raman vibrational modes in the SERS spectrum of this drug.

In addition to investigating the molecular interaction with the surface, the role of salts in the enhancements of Raman signals has also been an important area of research[20,21,22.] A recent

publication by Davis *et. al.,* reported the effect of cations on the electric field on the surface of TiO_2 substrates[23]. The author observed an increase in the electrical field on the TiO_2 based on the magnitude of the charge on the cations. Furthermore, it has also been reported that the presence of Cl^- ions increases SERS active sites therefore increases the enhancements of the Raman signals[24]. In addition, the influence of Cl^- ions on the orientation of F5-PB-22 has also been demonstrated on silver particles contributing to the SERS enhancements of this drug[13].

In our present study, we have undertaken the SERS study of the most controversial psychoactive component of cannabis, tetrahydrocannabinol (THC) and three of its analogs: cannabinol, cannabidiol and HU-210. We examine the adsorption geometry of these drugs on silver particles and their corresponding SERS enhancements using DFT calculations. By analyzing the surface orientations of these drugs, we provide possible correlations between the surface geometry and their observed SERS enhancements. The SERS results obtained from this work show a strong signal to noise ratio for all of these drugs. A recent study has reported the trace detection of THC in body fluid while another study has focused on the development of SERS substrates for the detection of THC[3,25]. However, neither of these studies focused on the adsorption geometry of THC with the SERS substrates nor examined the effect of salts on SERS enhancements of this drug. Understanding the mechanisms of interaction between a cannabinoid and a SERS substrate is not only crucial in understanding the SERS enhancements but also necessary in the development of a fast, effective and reliable SERS technique in the detection of these drugs.

Furthermore, we carried out our present work using four different monovalent and divalent salts: $MgCl_2$; $MgSO_4$; Na_2SO_4; and KNO_3 to investigate their effect on the adsorption geometry of the four cannabinoids and the corresponding SERS enhancements on silver particles. We show here that $MgCl_2$ causes the strongest Raman signal enhancements among the four salts. Results obtained from this study exhibit remarkably similar SERS spectra for the four cannabinoids when the SERS measurements are conducted using the same salt. This observation provides evidence that each salt most likely affects the orientation of the four drugs in a similar way on the silver particles generating almost identical SERS spectra. However, details of the spectral fingerprints allow for identification.

Material and Methods

Chemicals

(−)-*trans*-Δ^9-tetrahydrocannabinol (THC) (≥ 98% purity), cannabinol (≥ 98% purity), cannabidiol (≥ 98% purity) and (–)-1,1-dimethylheptyl analog of 11-hydroxy- Δ^8- tetrahydrocannabinol (HU-210) (≥ 98% purity) in methanol at 1mg/100µL concentration were purchased from Cayman Chemical. Methanol (≥ 99% purity) and magnesium chloride, $MgCl_2$ (≥ 99% purity), potassium

nitrate, KNO_3 (≥ 99% purity), magnesium sulfate, $MgSO_4$ (≥ 99% purity) and sodium sulfate, Na_2SO_4 (≥ 99% purity) were purchased from Sigma- Aldrich.

Experimental

Solutions of 0.1 mM of THC, cannabinol, cannabidiol and HU-210 were prepared by diluting the stock solutions of these cannabinoids in pure methanol. 0.5 M solutions of magnesium chloride ($MgCl_2$), potassium nitrate, KNO_3, magnesium sulfate, $MgSO_4$ and sodium sulfate, Na_2SO_4 were prepared by dissolving these salts in deionized water (18.2 MΩ·cm at 25 ^{0}C). Silver nanoparticles were prepared using a microwave synthesis method, as previously reported by Leona *et. al.* [26].

Raman spectra of these cannabinoids were investigated using a Renishaw Confocal Raman microscope (with spectral resolution 0.02 – 0.5 cm^{-1} and using 1200 lines/mm grating) equipped with a 633 nm diode laser. The laser spot size was 1 µm, which was focused on the sample using a 50X objective lens attached to a confocal microscope. The laser power on stage was 10 mW.

The Raman measurements of these samples were collected with three accumulations over 10 s of acquisition and an average of these spectra were recorded by the spectrometer software "in Via" for our results. To ensure that the obtained spectra were comparable, the settings, including laser power and exposure time, were all kept constant. An average of 10 different spots were probed to confirm the reproducibility of the measurements for the detection of the drugs.

The normal Raman spectra of the four cannabinoids were acquired from the solid crystals of these drugs. These solid crystals were formed by depositing a few drops of these drugs on a glass surface and by evaporating the methanol from the sample. The SERS measurements were obtained from liquid samples which was prepared by first mixing 4µL of silver nanoparticles with 2µL of each of the different salts in an Eppendorf tube. 2µL of 0.1 mM of THC was then added to each of the Eppendorf tubes and kept in the tubes for a few seconds before depositing the sample mixture on a glass slide for SERS measurements. Similar procedures were followed for the remaining three drugs for SERS measurements. The plotting and analysis of the graphs were performed using graphing software Origin 9.1.

DFT calculations

All calculations for the cannabinoids studied were made with the b3lyp density functional with the 6-311+G(d,p) basis set using the Gaussian 16 program[27]. The Gaussian default convergence criteria for maximum and RMS force and maximum and RMS displacement were used. The frequency calculations showed no imaginary frequencies for all compounds indicating a global minimum for the optimizations. Both IR and normal Raman spectra were calculated. GaussView 6.0.16 was used to view data, output images, and analyze results.

Results/Discussion

Molecular structure of THC and its analogs

In Figure 1, we show the structures of tetrahydrocannabinol (THC) and three of its analogs: cannabinol, cannabidiol and HU-210. Among these four cannabinoids, THC and cannabinol have the most similar chemical structures as they are distinguished by two benzene rings with different number of hydrogens (H). HU-210 and cannabidiol also have chemical structures which are very similar to THC. Both of these drugs have an additional hydroxyl group (OH) compared to that of THC. Besides this OH group, HU-210 has two additional methyl groups (CH_3) and an alkyl chain which is two carbon longer than that of the THC whereas cannabidiol has an additional methylidene ($=CH_2$) group compared to that of the THC.

Normal Raman and SERS spectra

Figure 2 shows the comparison between the normal Raman (NR) and the SERS spectra of THC and its analogs. In figure 2a, the SERS spectrum of THC shows an appearance of new peaks at 708 cm^{-1}, 1090 cm^{-1} and 1200 cm^{-1} compared to the normal Raman spectrum. The figure also exhibits strong enhancements of peaks at 415 cm^{-1}, 1004 cm^{-1} and 1060 cm^{-1} corresponding to those of the normal Raman peaks at 412 cm^{-1}, 1002 cm^{-1} and 1068 cm^{-1}. These three peaks show a shift between 3 to 8 cm^{-1} compared to those observed in the normal Raman spectrum. SERS enhancements of the similar peaks for THC have also been previously observed[3,25]. In addition, while the relative intensity of the peak at 1608 cm^{-1} remains almost the same both in the normal and the SERS spectra, the peaks at 1108 cm^{-1}, 1155 cm^{-1}, 1304 cm^{-1}, 1444 cm^{-1} and 1501 cm^{-1} in the normal Raman spectrum of THC disappear in the SERS spectrum of this drug.

In Figure 2b, we present the normal Raman and the SERS spectra of cannabinol. A close examination of the SERS spectrum of the cannabinol shows the appearance of strongly enhanced peaks at 420 cm^{-1}, 706 cm^{-1}, 1004 cm^{-1}, 1062 cm^{-1}, 1094 cm^{-1} and 1202 cm^{-1} compared to those in the normal Raman spectrum. The enhancements of these peaks are very similar to those of the THC. These peaks show a shift between 2 to 10 cm^{-1} compared to those observed in the normal Raman spectrum of this drug. In addition, while the relative intensity of the strong peak at 1606 cm^{-1} remains almost the same, the peaks at 1156 cm^{-1}, 1302 cm^{-1} and 1507 cm^{-1} in the normal Raman spectrum of cannabinol disappear in the SERS spectrum of this drug. Nonetheless, unlike THC, cannabinol has an additional strong peak at 1623 cm^{-1} in the normal Raman spectrum which is absent in the SERS spectrum of this drug.

In figure 2c and figure 2d, we provide the Raman enhancement of cannabidiol and HU-210, respectively. Similar to the SERS spectra of THC and cannabinol, the SERS spectra of cannabidiol and HU-210 show strong enhancements of the four peaks around 1000 cm^{-1}, 1060 cm^{-1}, 1090 cm^{-1} and 1200 cm^{-1} compared to the normal Raman spectra of these drugs. The SERS spectrum of cannabidiol also shows Raman enhancements of peaks at 807 cm^{-1}, 1580 cm^{-1} and 1609 cm^{-1} whereas the SERS spectrum of HU-210 shows strong enhancements of peaks at 457 cm^{-1}, 616 cm^{-1}, 638 cm^{-1}, 666 cm^{-1}, 1220 cm^{-1} and 1576 cm^{-1}. Similar to the SERS spectra of THC and cannabinol, these peaks also show a shift between 2 to 10 cm^{-1} compared to those in the normal Raman spectra. Furthermore, some of the peaks in the normal Raman spectrum of cannabidiol at 602 cm^{-1}, 852 cm^{-1}, 1108 cm^{-1}, 1301 cm^{-1} and 1630 cm^{-1} have disappeared in the SERS spectrum of this drug. For HU-210, some of the prominent peaks in the normal Raman spectrum at 517 cm^{-1}, 563 cm^{-1}, 717 cm^{-1}, 1304 cm^{-1}, 1332 cm^{-1}, 1438 cm^{-1}, 1458 cm^{-1}, 1622 cm^{-1} and 1675 cm^{-1} whose Raman intensity either diminished or disappeared in the SERS spectrum of this drug. In table 1, we listed these peaks in order to further clarify the SERS enhancements of the normal Raman spectra of these drugs.

It is well known that SERS spectra often appear to display quite different relative intensities from the normal Raman spectra and the relative wavenumbers sometimes vary between 10 - 15 cm^{-1} depending of the strength of bonding between the molecules and the substrate[9,11]. Strong peaks in normal Raman can be considerably diminished while weak peaks can be strongly enhanced. This variation in intensity between the SERS and the normal spectra is due to the nature of orientational bonding between a compound and the substrate[11]. When a compound forms bonds to a substrate, it lowers the symmetry of the molecule and allows the appearance of some Raman peaks in the SERS spectrum which are previously forbidden in the normal Raman while it diminishes the intensity of some of the strong peaks present in the normal Raman[28,29]. The adsorption of a molecule on the surface of a substrate with a favorable orientation can cause some vibrational lines to be enhanced strongly while diminishing others[17,18]. This same effect is most likely responsible for the differences in the intensities for these cannabinoids in the SERS spectra relative to the normal spectra as it appeared in our study.

In Figure 3, we present the SERS spectra for all four of these cannabinoids in order to compare the Raman enhancements observed for all of these drugs. A close examination shows that all these cannabinoids have four peaks in common at 1004 cm^{-1}, 1060 cm^{-1}, 1090 cm^{-1} and 1200 cm^{-1} which are enhanced in the SERS spectra. The figure also shows that the SERS spectra of THC and cannabinol exhibit almost identical Raman enhancements. The only noticeable differences are the two peaks at 804 cm^{-1} for cannabinol and at 1409 cm^{-1} for THC. Compared to THC, cannabidiol has two strong SERS peaks at 805 cm^{-1} and at 1448 cm^{-1} and HU-210 has four peaks at 457 cm^{-1}, 616 cm^{-1}, 638 cm^{-1} and 666 cm^{-1} which can be used to distinguish these analogs from THC. Because of the fact that all these cannabinoids have very similar chemical structures, which could make it difficult to differentiate these drugs in a real-world sample, especially if they are found in a mixture. The enhanced Raman peaks observed in our study show a strong signal to noise ratio which can be utilized as “finger print” peaks for the identification of these

cannabinoids. At the same time, the SERS technique can also be applied to distinguish THC from its analogs, based on their differences in the SERS spectra.

Vibrational assignments

In addition to identifying the cannabinoids, our study focuses on the interaction of these drugs on the surface of silver substrates contributing to the Raman enhancements. In our previous study, we attributed the bonding of FUBINACA on the surface of silver substrates to the electronegative oxygen and nitrogen atoms found in its ester and amide functional groups[14]. We determined the possible binding geometry of FUBINACA on the surface of silver by examining the change in intensity of the Raman peaks which appeared in the normal Raman and in the SERS spectrum of this drug.

In our present study, we have undertaken DFT calculations in order to understand the adsorption geometry of these cannabinoids on the surface of silver particles contributing to the corresponding SERS enhancements. In Table 2, we present spectral assignments of some of the strong peaks of THC, cannabinol and cannabidiol observed in the normal Raman and the SERS spectra of these drugs. The SERS spectra of THC show a disappearance of peaks in the normal spectrum at 1108 cm^{-1}, 1155 cm^{-1}, 1304 cm^{-1}, 1444 cm^{-1} and 1501 cm^{-1}. These peaks primarily correspond to the vibrations of the benzene and the hexene rings, hydroxyl (OH) group and the C-O vibrations of the tetrahydropyran ring. On the other hand, the vibrations of most enhanced peaks at 1004 cm^{-1} and 1090 cm^{-1} and 1200 cm^{-1} in the SERS spectrum of THC belong to the benzene ring and the alkyl chain of this drug. Changes of these vibrations indicate that THC is most likely attached to the silver particles using primarily the benzene and the hexene rings in addition to the oxygen atoms (O) of the hydroxyl (OH) group and the tetrahydropyran ring. The benzene and the hexene rings in THC are nearly parallel to each other. Since THC is attached to silver using the benzene and the hexene rings, it is most likely that this drug is oriented parallel to or lying flat on the silver surface.

The SERS spectrum of cannabinol shows the disappearance of three peaks at 1156 cm^{-1}, 1302 cm^{-1} and 1507 cm^{-1} which are similar to those of THC. In addition, the strong peak at 1623 cm^{-1} in the normal Raman spectrum also disappeared in the SERS spectrum of this drug. The vibrations of these peaks are assigned to the vibrations of the two benzene rings as well as the oxygen atoms (O) of the hydroxyl (OH) and the pyran ring. Moreover, similar to THC, the strongly enhanced peaks at 1004 cm^{-1}, 1094 cm^{-1} and 1202 cm^{-1} correspond to the vibrations of the benzene ring and the alkyl chain. Based on the similarities that these two drugs share, it is reasonable to assume that cannabinol has a parallel orientation on the surface of the silver, attached using the benzene rings and the oxygen atoms (O) found in the hydroxyl (OH) group and the pyran ring.

As previously described, cannabidiol has peaks at 602 cm^{-1}, 852 cm^{-1}, 1108 cm^{-1}, 1301 cm^{-1} and 1630 cm^{-1} in the normal Raman spectrum which are absent in the SERS spectrum of this drug. The vibrations of these peaks are predominantly from the benzene ring, the two hydroxyl

(OH) groups and the methylidene (=CH_2) group. In addition, SERS spectrum of cannabidiol shows the enhancements of three peaks at 807cm^{-1}, 1580 cm^{-1} and 1609 cm^{-1} cm along with the two usual peaks at 1005 cm^{-1} and 1095 cm^{-1}. The assignments of these peaks primarily belong to the vibrations of alkyl chain and the benzene ring. It can be seen that most of the vibrations which are affected in cannabidiol are similar to those of the THC and cannabinol. The only other functional group whose vibrations are affected in this drug is methylidene (=CH_2) which is absent in the molecular structure of THC and cannabinol. Therefore, it can be concluded that, similar to the previous drugs, cannabidiol has a parallel surface geometry on silver using the benzene ring, the oxygen atoms (O) of the hydroxyl (OH) groups and the methylidene (=CH_2) group.

Some of the prominent peaks in the normal Raman spectrum of HU-210 which are either absent or diminished in intensity in the SERS spectrum are 517 cm^{-1}, 563 cm^{-1}, 717 cm^{-1}, 1304 cm^{-1}, 1332 cm $^{-1}$ and 1622 cm^{-1}. The vibrations for these peaks are generally assigned to the cyclic functional groups (benzene, hexene and tetrahydropyran rings of this drug) and the two hydroxyl groups attached to the benzene and the cyclohexene rings. The functional groups which are affected due to the change in the vibrations of these peaks are similar to those observed in THC, cannabinol and cannabidiol. In addition, the SERS spectrum of HU-210 also shows the enhancements of the two familiar peaks at 1000 cm^{-1} and 1095 cm^{-1} and the appearance of two new peaks at 616 cm^{-1} and 638 cm^{-1}. The vibrational assignments of these peaks, unlike the rest of the analogs, belong only to the benzene and the hexene rings, and the 2nd hydroxyl (OH) group. The absence of the enhancements of the alkyl chain vibrations indicates that while HU-210 is attached to silver using the functional moieties similar to those of the other three cannabinoids, it may have a different orientation compared to those of the other cannabinoids. This difference in orientation is most likely responsible in producing Raman enhancements which are somewhat different compared to the other cannabinoids.

The effect of salts

In addition to determining the interactions of these cannabinoids on a silver surface, our present study also focused on analyzing the role of salts as aggregating agents and their effect on the SERS enhancements. In figure 4 (a-d), we present the SERS spectra of each of the cannabinoids using all four salts. It can be seen that except for HU-210 (fig. 4d), the rest of the cannabinoids experienced the largest Raman signal enhancements when $MgCl_2$ salt was used as an aggregating agent. For HU-210, the largest Raman enhancements seem to occur using Na_2SO_4 salt. In order to eliminate any possibility of interference from the peaks of the silver and the four salts used in this study, we have provided both the Raman spectra of silver and the four salts (fig. 1 Supplemental Information).

A generation of strong electric fields due to both single and doubly charged cations was previously observed on TiO_2 and silver[23,24,30]. In addition, the formation of SERS active sites on silver particles due to the presence of Cl^- ions has been attributed to an increase in SERS

enhancements[24]. It is possible that the strong Raman signal enhancements of THC, cannabinol and cannabidiol are due to both an increase of the electromagnetic field in the presence of Mg^{2+} cations and the formation of SERS-active sites due to the presence of Cl^- ions. Our previous study on the trace analysis of synthetic drugs AMB-FUBINACA and α-PVP and in a toxicological analysis of synthetic cannabinoids, the application of $MgCl_2$ salts also produced the strongest SERS enhancements[14,16].

In order to further understand the role of these salts on the orientations of these drugs affecting the SERS enhancements, in Figure 5, we display the SERS spectra of all the four cannabinoids using each of the salts. A close examination of the figure shows that the enhancements of the Raman peaks in the SERS spectra of these cannabinoids look very similar using the same salt. Figure 5a shows the SERS spectra of all the cannabinoids obtained using only $MgCl_2$ salts. It can be seen that enhancements of the normal Raman peaks observed in the SERS spectra appear remarkably similar for all four drugs. This similarity among the SERS spectra using the same salt can be also observed in Figure 5 (b – d) where all these four drugs are examined using KNO_3, $MgSO_4$ and Na_2SO_4, respectively. Furthermore, a comparison between the SERS spectra of all four cannabinoids using $MgCl_2$ in Figure 5a and to those using KNO_3 in Figure 5b shows Raman enhancements of several different peaks when different salts are used in the measurements. This phenomenon can also be observed in Figure 5c and Figure 5d, where the SERS experiments are performed using $MgSO_4$ and Na_2SO_4.

By using Raman experiments and DFT calculations, the effect of Cl^- ions on the orientations of synthetic cannabinoid (F5-PB-22) on a silver surface was previously demonstrated[13]. In our present work, the appearance of almost identical SERS spectra using a single salt must be due to the similar effect of the salts on the orientation of these molecules on silver particles. It is possible that each salt has its own unique way of influencing the orientation, and thereby the adsorption of these drugs on silver particles generating similar SERS spectra. $MgCl_2$ salts must have similar effects on the interaction of THC and its analogs on a silver surface producing similar SERS spectra. When the same cannabinoids are studied using a different salt, such as KNO_3, SERS spectra with the enhancements of several different peaks are generated. This indicates that KNO_3 must affect the orientation of the cannabinoids differently than that of $MgCl_2$. This might be true for $MgSO_4$ and Na_2SO_4 salts as well. Thus, while the SERS spectra of all the cannabinoids using the same salt appear remarkably similar, the SERS spectra of the same drugs using different salts look different from those of the other salts.

Conclusion

The SERS results obtained from our current studies show possible surface interactions of THC and three of its analogs on silver contributing to strong Raman signal enhancements of these drugs. We conclude that these enhanced Raman peaks can be utilized for the purpose of identification and differentiation of these drugs found in a mixture. Our study also examined the

effect of four salts on the Raman enhancements for these cannabinoids. Based on the similarity observed among the SERS spectra of the four cannabinoids, we reasoned that salts most likely affect the orientation of these drugs on a silver surface. We further conclude that this effect of the salt on the orientation of these drugs is based on the unique chemical nature of the salt. Our current SERS results also demonstrate that $MgCl_2$ produces the largest Raman enhancements due to the increase of an electric field and due to the creation of SERS active sites on silver particles. Note that while the largest Raman enhancements due to $MgCl_2$ are observed for THC, cannabinol and cannabidiol, we are not sure why the same enhancements are not observed for HU-210. Further careful studies need to be conducted to better understand the effect of salts on the adsorption geometry of these drugs and their contribution to the SERS enhancements. This understanding of SERS mechanisms is important as it will further improve the efficiency and reliability of SERS and present this technique as an important analytical tool for trace detection in the field of forensics.

Acknowledgments

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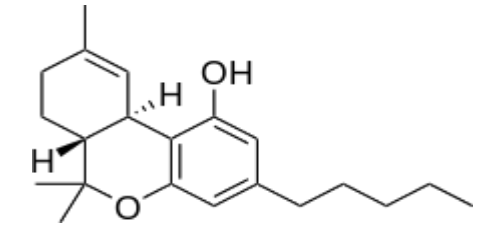
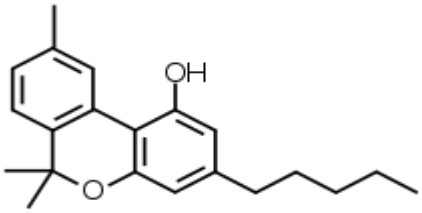
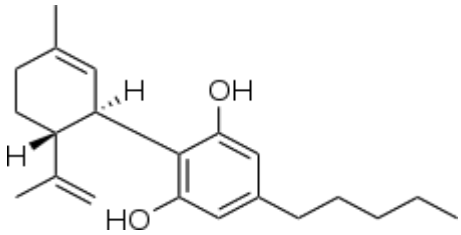
Figures



Tetrahydrocannabinol (THC)



Cannabinol



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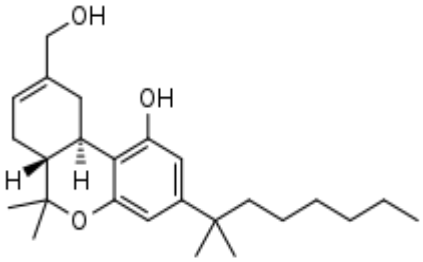
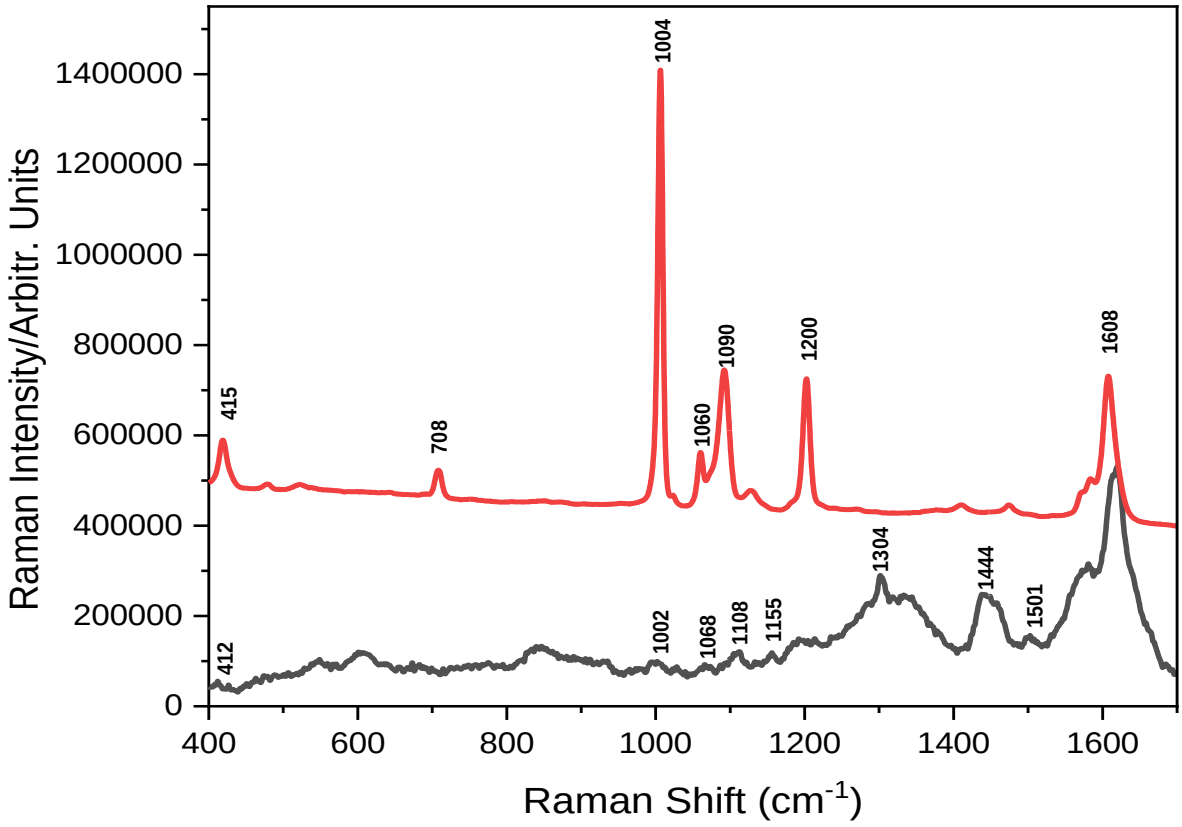


HU-210

Figure 1: The chemical structures of THC, cannabinol, cannabidiol and HU-210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Raman Shift (cm-1)
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1000000
800000
600000
400000
200000
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800
1000
1200
1400
1600
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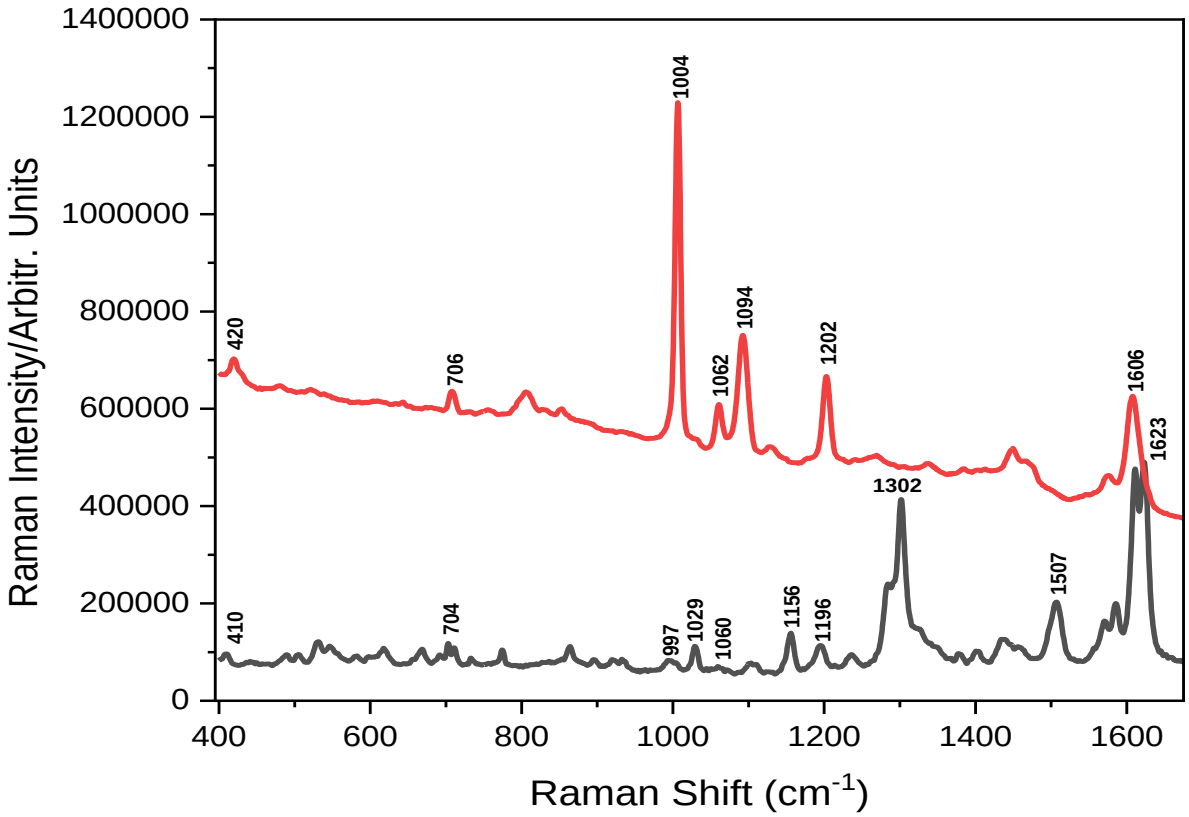
Figure 2a.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Raman Shift (cm-1)
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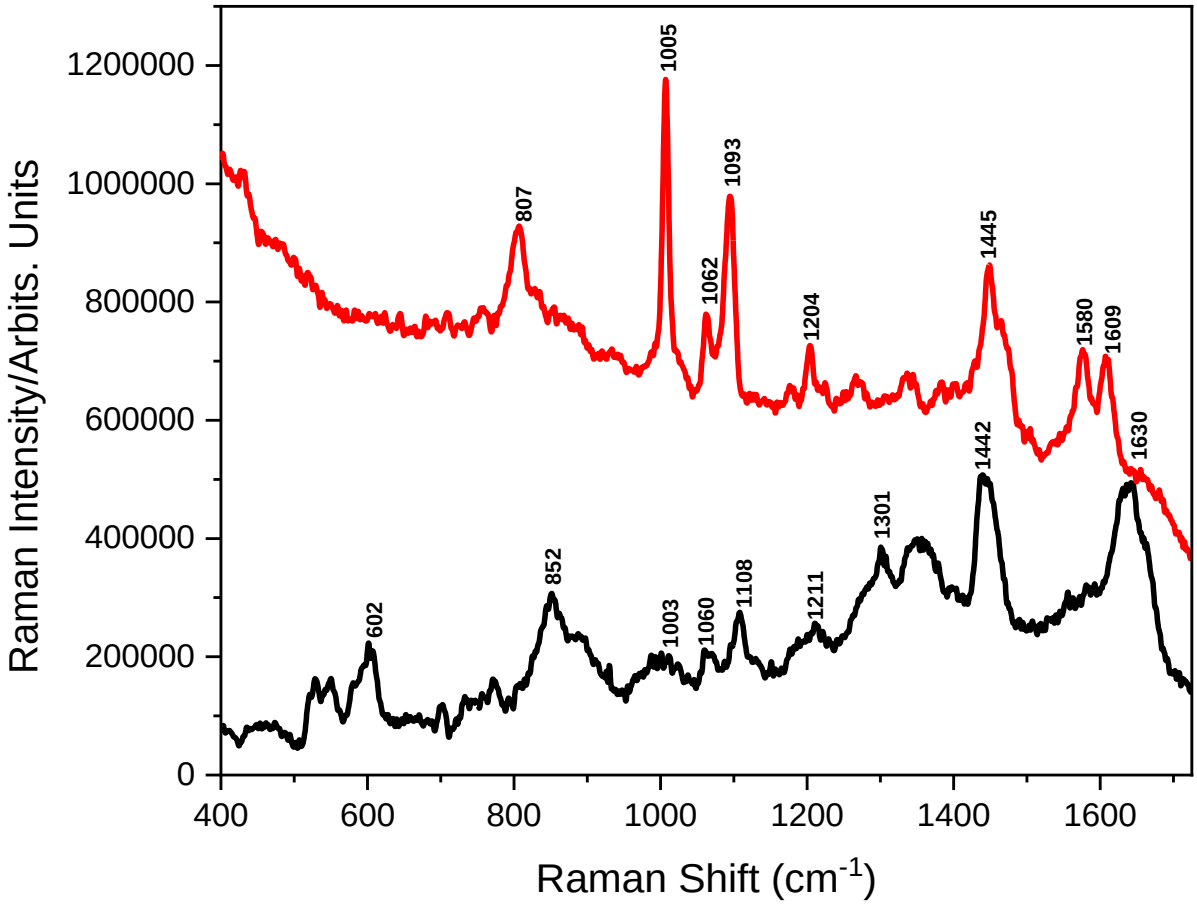
Figure 2b.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Raman Shift (cm-1)
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Figure 2c.

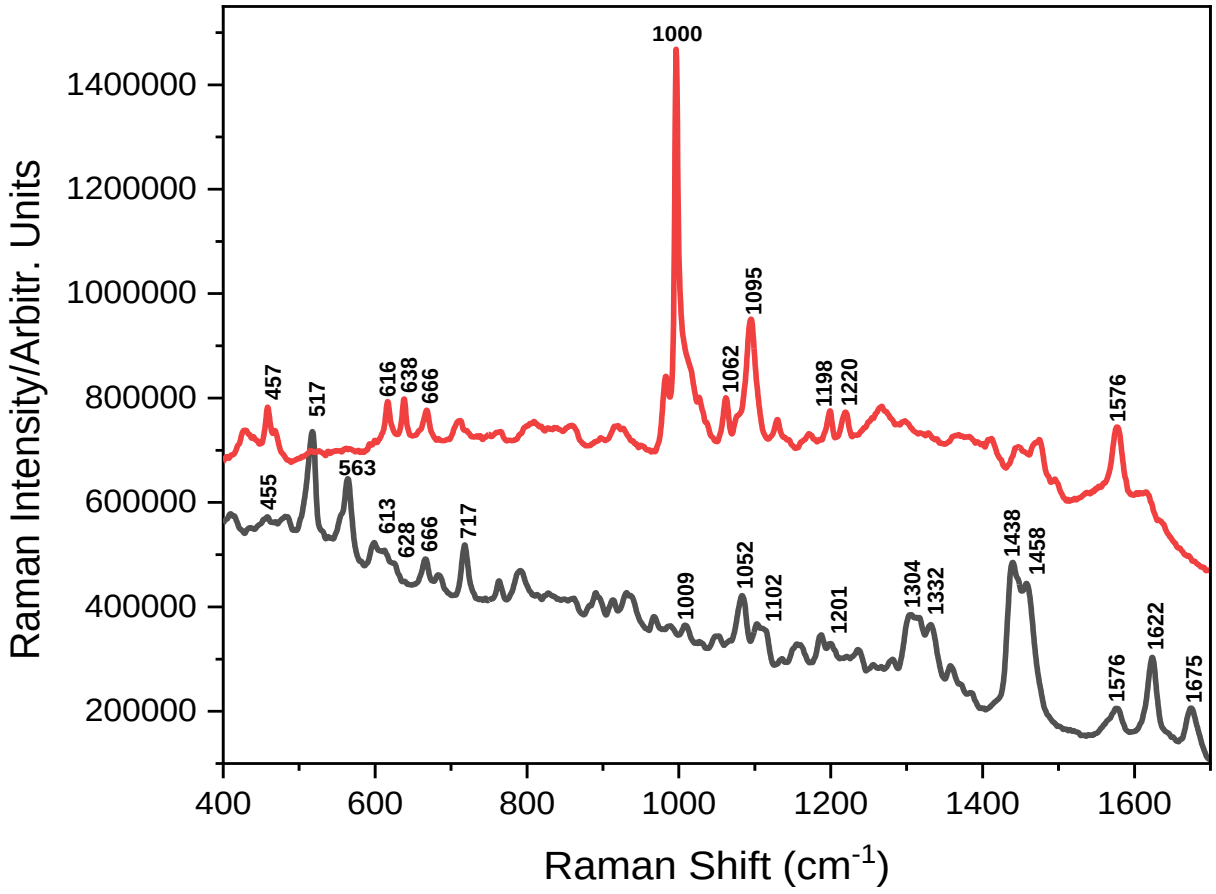

Figure 2d.

Figure 2. SERS spectrum of 0.1mM concentration of THC (fig. 2a), cannabinol (fig. 2b), cannabidiol (fig. 2c) and HU-210 (fig. 2d) (red) compared to normal Raman spectrum (black). The spectra differ mainly by the relative intensities of the spectral lines. Excitation wavelength 633 nm. Note, best overall sensitive SERS spectra were obtained for THC, cannabinol and cannabidiol using $MgCl_2$ salt. For HU-210, better sensitivity was collected using Na_2SO_4 salt. The intensity of the normal Raman spectrum was adjusted to highlight the difference between the SERS and the normal Raman. The red and the black lines are offset for clarity.

SERS Peaks with Diminished Enhancements				SERS Peaks with Strong Enhancements			
THC	**Cannabinol**	**Cannabidiol**	**HU-210**	**THC**	**Cannabinol**	**Cannabidiol**	**HU-210**
			517	415	420		
			563				457
		602					616
			717				638
		852					666
1108		1108		708	706		
1155	1156					807	
1304	1302	1301	1304	1004	1004	1005	1000
			1332	1060	1062	1062	1062
1444			1438	1090	1094	1093	1095
1501	1507		1458	1200	1202	1204	1198
	1623						1220
		1630	1622			1580	1576
			1675			1609	

Table 1. Peaks in the normal Raman spectra for all four cannabinoids which are either diminished in intensity or disappeared in the SERS spectra (left). Peaks with strong Raman signal enhancements appear in the SERS spectra of the four cannabinoids (right).

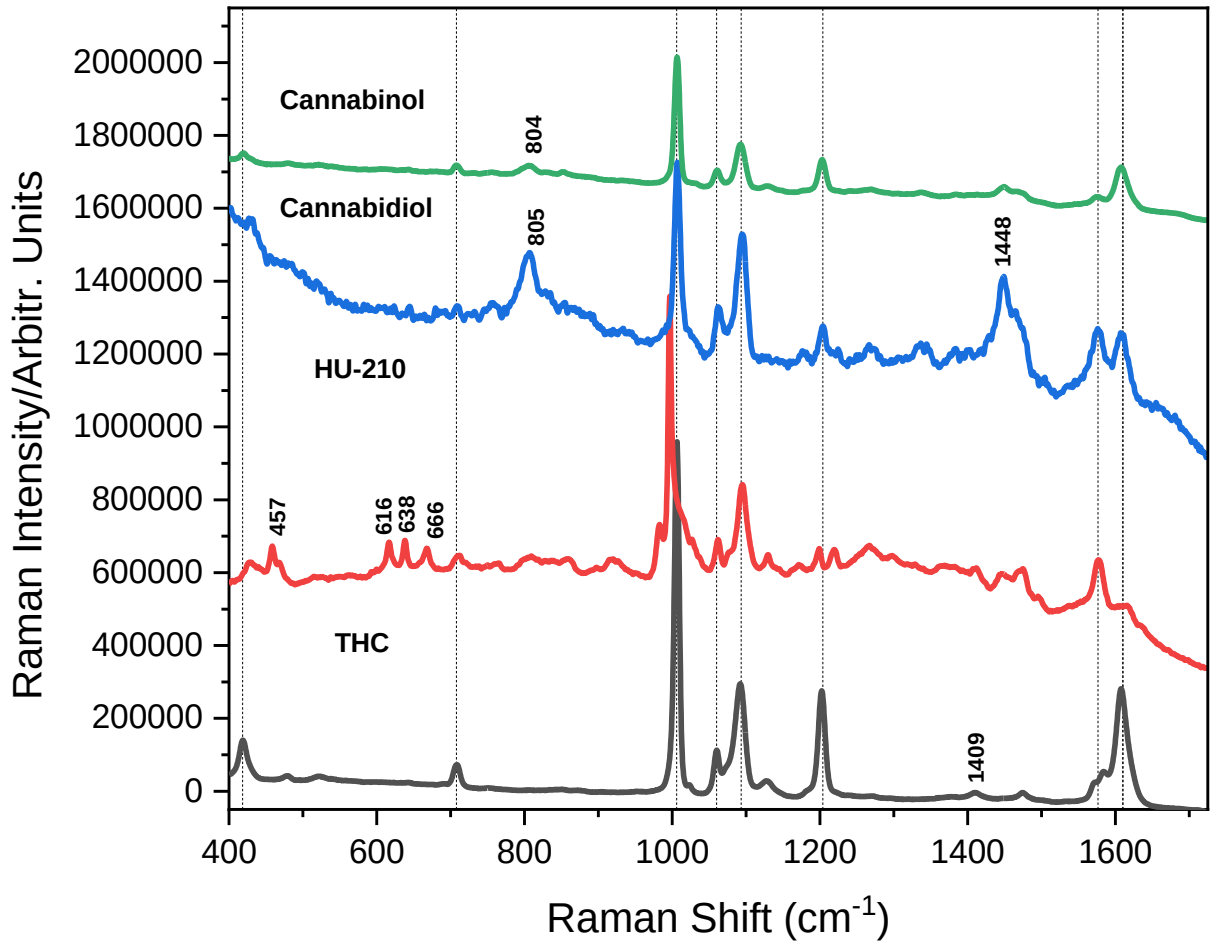

Figure 3. Comparison of the SERS spectra of THC, HU-210, cannabidiol and cannabinol. Excitation wavelength 633 nm.

THC			
Normal Raman	SERS	DFT	Description
412	415	474.82	CH_3 wag [2nd methyl]; CH_3 wag [3rd methyl]; ring stretch (sym) [cyclohexene]; CH & C-OH bend & ring bend [benzene] (ip)
	708	739.05	Ring twist [benzene]; CH_2 rock, ring stretch (sym) [cyclohexene]
1002	1004	1059.44	Ring stretch (sym) [benzene]; CH_2, CH_3 wag [alkyl chain]
1068	1060	1104.13	CH_3 wag [1st Methyl]; CH_2 twist, CH bend (ip), ring stretch (sym) [cyclohexene]
	1090	1127.40	C-chain stretch (sym), CH_3 wag, CH_2 wag, CH bend [alkyl chain]

1108		1152.42	Ring stretch (sym), CH bend (ip) [benzene ring]; CO stretch (sym); CH_3 twist [1st & 2nd methyl groups]
1155		1235.49	CH bending & OH bending (ip) [Benzene ring]; CH & CH_2 rocking [cyclohexene]; CH_3 wag (2nd and 3rd); C-H twist (alkyl chain)
	1200	1259.23	CH3 wag [2nd & 3rd methyl]; CH_2 twist, CH bend, ring stretch (sym) [Cyclohexene]; CH bend, CO stretch (sym) [benzene]; OH bend
1304		1353.79	Ring stretch and twist [benzene]; C-OH bend [benzene]; CH & CH_2 bend [cyclohexene]; CH_2 wag, [alkyl chain]; C-O stretch [tetrahydropyran ring]
1444		1491.82	CH_3 twist [1st]; CH_3 bend [2nd, 3rd]; CH & CH2 bend [Cyclohexene]
1501		1535.10	CH & C-OH bend, ring stretch (sym) [benzene]
Cannabinol			
410	420	429.29	Benzene ring bend (ip); CH_3 wag (1st, 2nd & 3rd); CH_2 & CH_3 wag [alkyl chain]
704	706	723.41	Stretch (sym) [1st & 2nd benzene ring]; CH_2 & CH_3 [alkyl chain]
997	1004	1003.42	Ring stretch (sym) [benzene]; CH_2 & CH_3 sci [alkyl chain]
1060	1062	1069.73	Bend (ip) [1st & 2nd benzene rings]; CO stretch (sym) [pyran ring]; OH bend (ip); CH_2 twist [alkyl chain]
1102	1094	1125.79	CH_2 & CH_3 wag [alkyl chain]
1156		1176.04	CH bend (ip) [1st & 2nd benzene ring]; OH bend (ip); CH_3 wag [2nd & 3rd methyl groups]
1196	1202	1218.21	CH bend (ip) [1st & 2nd benzene rings]; OH bend; CH_2 twist [alkyl chain]
1302		1321.11	Stretch (sym) [1st & 2nd benzene rings], CH bend (ip) [1st benzene ring]; CH_2 twist [alkyl chain]
1507		1525.10	C=C stretching (sym), C-H bending (ip) [1st benzene ring]; CH_3 (1st) & CH_3 (2nd) twist [pyran ring]; C=C stretch (sym) & O-H bending (ip) [2nd benzene ring]
1623		1645.12	C=C (ip) stretch, C-H bend [1st & 2nd benzene rings]; O-H bend
Cannabidiol			
602		628	Benzene ring stretch; cyclohexene ring twist; (=CH_2) & CH_3 (1st) twist
	807	849.63	CH_2 & CH_3 twist [alkyl chain]
852		896.97	Ch & CH_2 bend (ip) [cyclohexene ring], CH_3 (1st and 2nd) & (=CH_2) wagging; CH_2 & CH_3 wag [alkyl chain]
1003	1005	1036.69	Ring stretch (sym) [benzene]; CH_2 & CH_3 wag [alkyl chain]
1060	1062	1100.12	Cyclohexene ring stretch (asym) & twist; CH_3 wag (1st & 2nd); (=CH_2) twist
	1093	1125.78	CH_2 & CH_3 wag [alkyl chain]
1108		1157.26	Cyclohexene ring stretch (ip) & bend (oop) (sym); benzene ring stretch (sym); CH_3 wag (1st & 2nd); 1st OH and 2nd OH bending
1301		1354.67	Benzene ring stretch (sym); CH_2 & CH Wag [cyclohexene ring]; CH_2 wag [alkyl chain]
	1580	1611.19	Benzene ring stretch (sym); OH (1st & 2nd) bend (ip)

	1609	1661.42	Benzene ring stretch (sym); OH (1^{st} & 2^{nd}) bend (ip)
1630		1684.16	C=C stretch (=CH_2), CH wag (=CH_2); CH_3 twist (2^{nd})
HU-210			
455	457	442.43	Hexane ring bend (ip); wag [alkyl chain]
517		526.35	Stretch (sym) [tetrahydropyran ring]; bend OH (2^{nd}) & benzene ring (ip); bend (ip) [Cyclohexene ring]
563		571.19	twist [cyclohexene]; bend (ip) benzene ring and OH (2^{nd})
613	616	620.83	Twist [benzene ring]
628	638	631.01	Wag 3^{rd} & 4^{th} CH_3; bend (oop) benzene ring
666	666	672.00	CH twist [benzene ring]
717		725.10	CH rock [benzene ring]; CH_2 rock [cyclohexene ring]
1009	1000	995.68	Ring stretch (sym) [benzene]; Wag CH_3 (1^{st} & 2^{nd}); Stretch (sym) [cyclohexene ring]
1052	1062	1065.89	Wag [alkyl chain]
1102	1095	1093.35	Stretch (sym) benzene ring; OH 2^{nd} bend (ip); CH_2 rock [cyclohexene ring]
1201	1198	1199.52	CH bend (iP) [benzene ring]; OH 2^{nd} bend (ip); rock [alkyl chain]
	1220	1210.83	CH bend (ip) [benzene ring]; OH 2^{nd} bend (ip)
1304		1308.58	Stretch (sym) [benzene ring]; OH 2^{nd} bend (ip); bend (ip) [cyclohexene ring]
1332		1323.02	Bend (ip) OH (2^{nd}); stretch (sym) [benzene ring]; bend (oop) [cyclohexene ring]
1438		1442.87	Ring stretch (sym), CH bend (ip) [benzene ring]; OH (2^{nd}) bend (ip)
1458		1486.50	CH_2 scissor [alkyl chain]
	1576		
1622		1604.71	Stretch (sym) [benzene ring]; CH & OH (2^{nd}) bend (ip)
1675		1655.27	

Table 2. Spectral assignments of some of the selected peaks of THC, cannabinol, cannabidiol and HU-210 observed in the normal Raman and SERS spectra of these drugs. Note, in plane (ip), out of plane (oop).

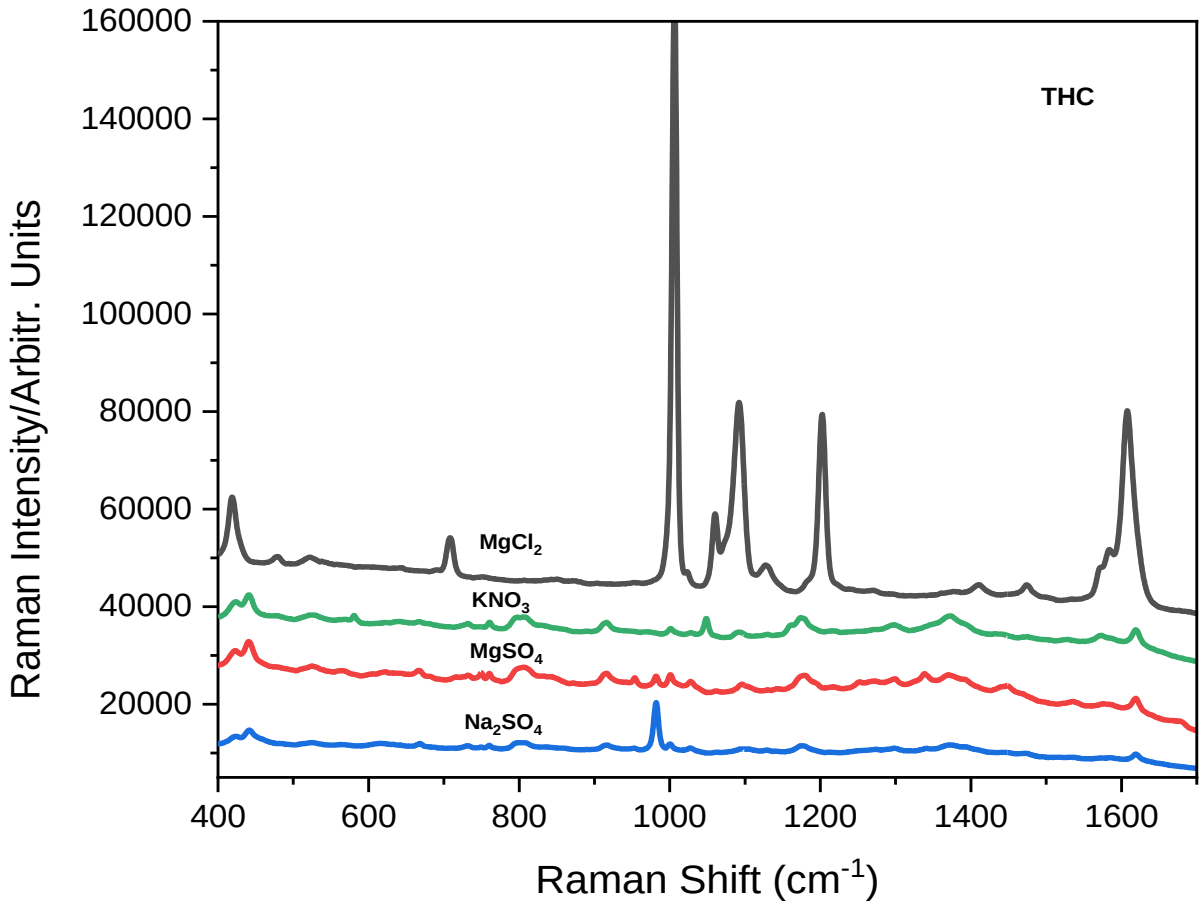
160000
140000
120000
100000
80000
60000
40000
20000
Raman Intensity/Arbitr. Units
400
600
800
1000
1200
1400
1600
Raman Shift (cm-1)
THC
MgCl2
KNO3
MgSO4
Na2SO4

Figure 4a.

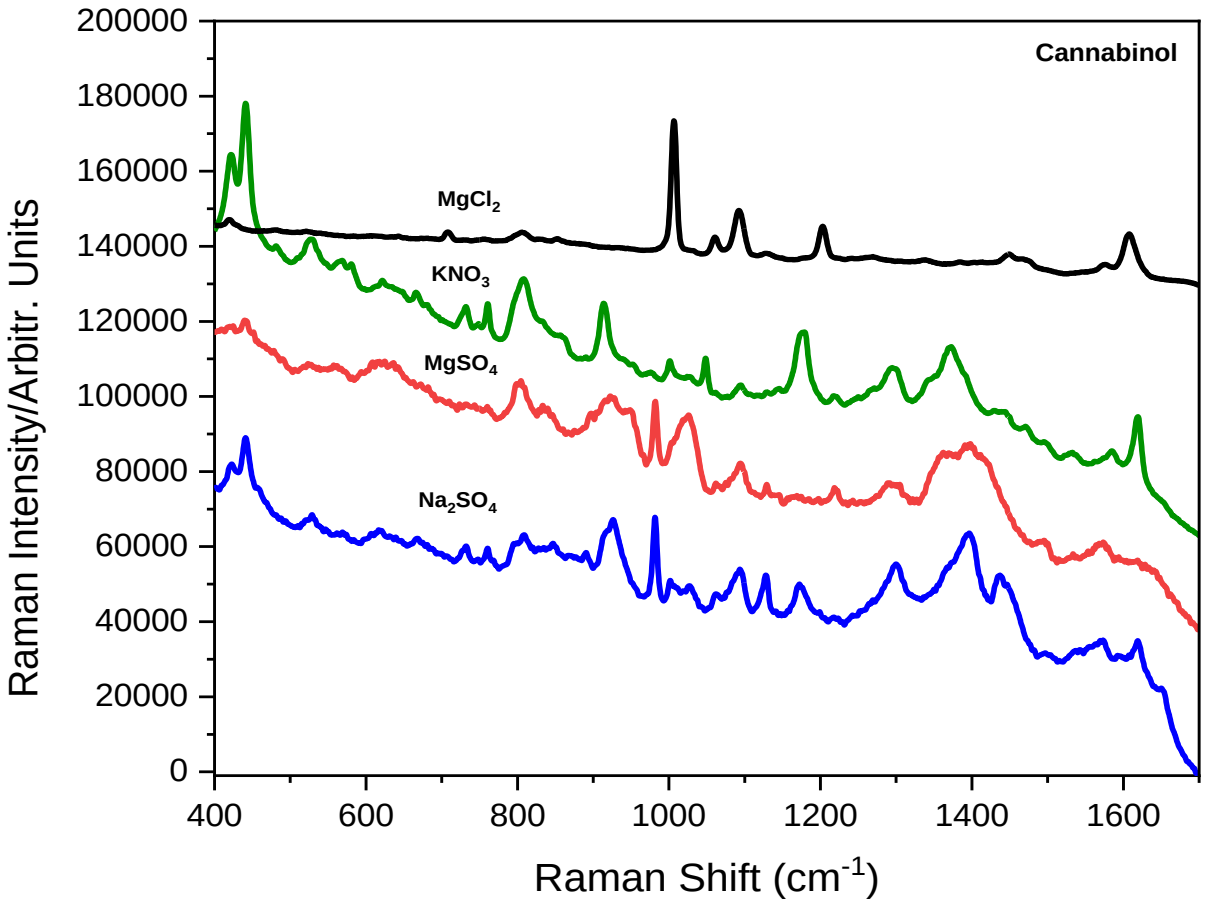
Cannabinol
MgCl2
KNO3
MgSO4
Na2SO4
Raman Intensity/Arbitr. Units
Raman Shift (cm-1)
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
400
600
800
1000
1200
1400
1600

Figure 4b.

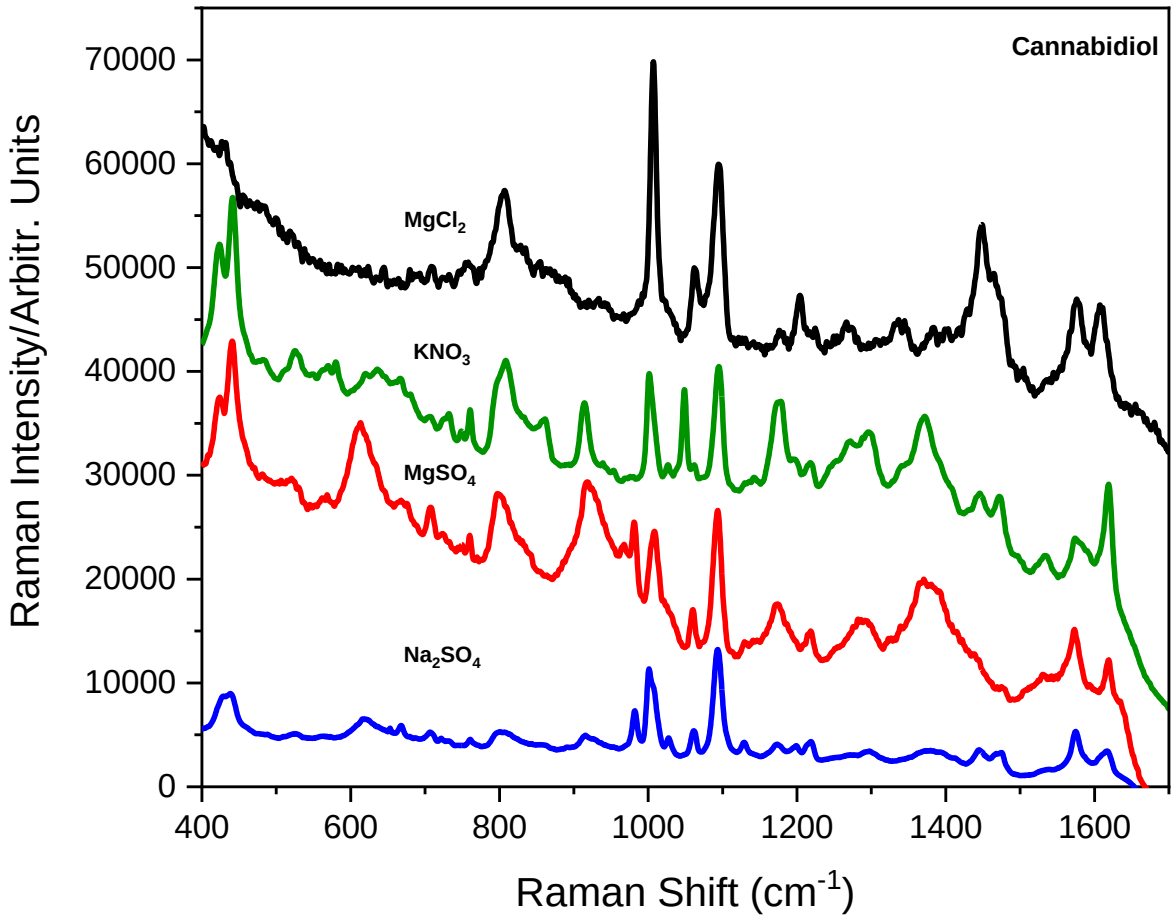
Cannabidiol
MgCl2
KNO3
MgSO4
Na2SO4
Raman Intensity/Arbitr. Units
Raman Shift (cm-1)
0
10000
20000
30000
40000
50000
60000
70000
400
600
800
1000
1200
1400
1600

Figure 4c.

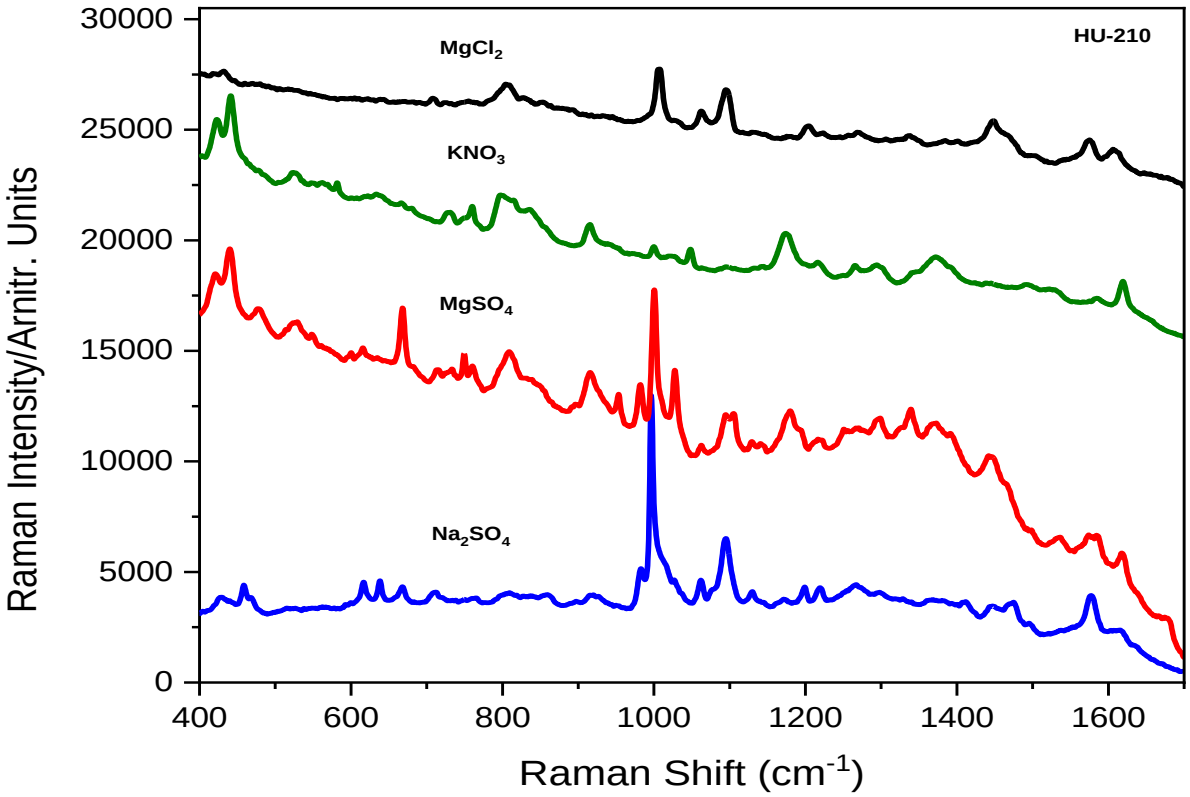

Figure 4d

SERS spectra of THC (fig. 4a), cannabinol (fig. 4b), cannabidiol (fig. 4c) and HU-210 (fig. 4d) using all four salts. Note, no adjustment has been made to the SERS intensities of the cannabinoids in order to highlight the differences of the SERS enhancements using all four salts.

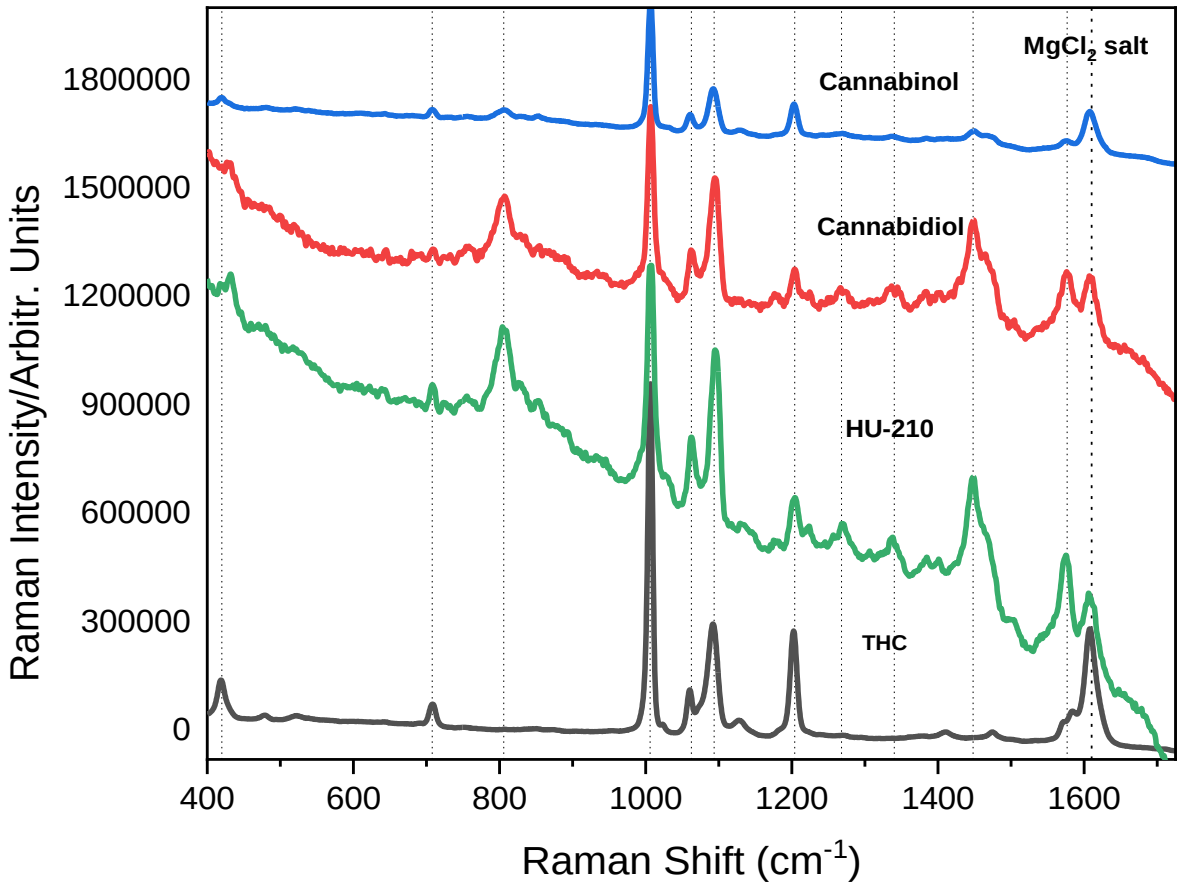
MgCl2 salt
Cannabinol
Cannabidiol
HU-210
THC
1800000
1500000
1200000
900000
600000
300000
0
Raman Intensity/Arbitr. Units
400
600
800
1000
1200
1400
1600
Raman Shift (cm-1)

Figure 5a.

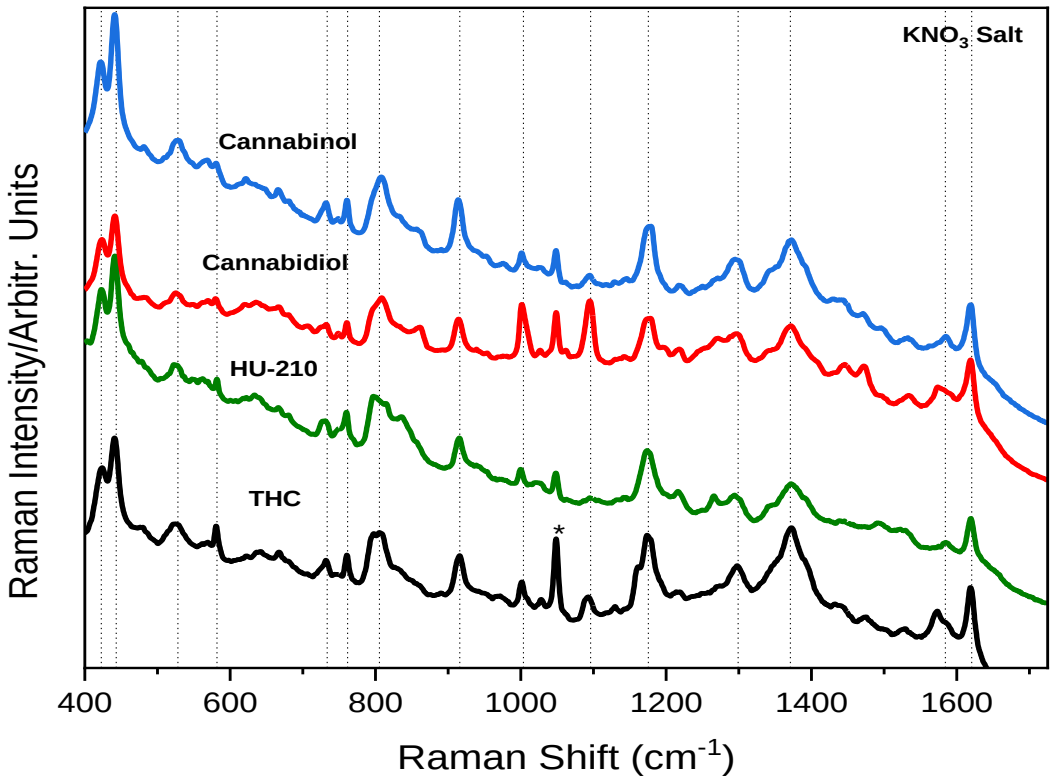
KNO3 Salt
Cannabinol
Cannabidiol
HU-210
THC
*
Raman Intensity/Arbitr. Units
400
600
800
1000
1200
1400
1600
Raman Shift (cm-1)

Figure 5b.

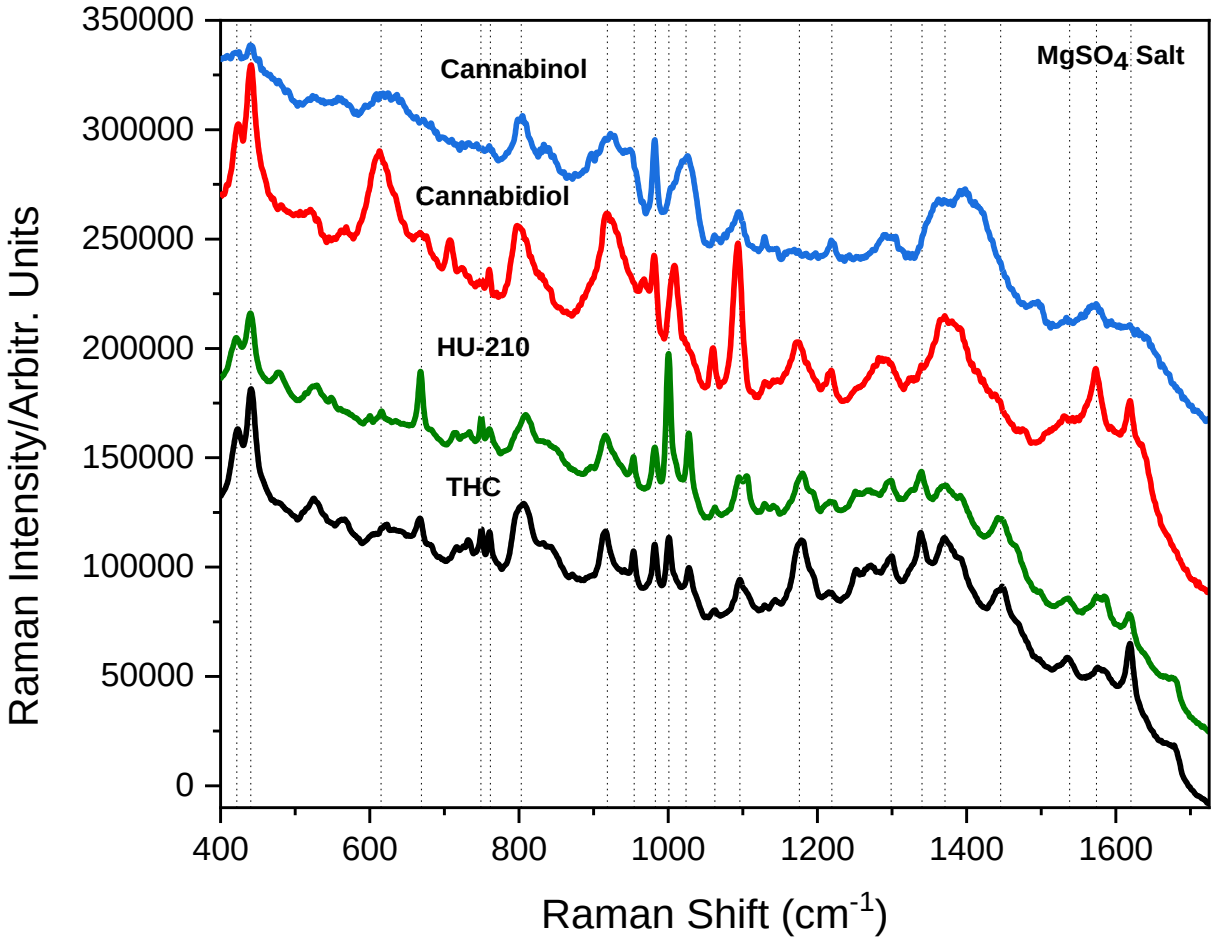
Cannabinol
Cannabidiol
HU-210
THC
MgSO4 Salt
Raman Intensity/Arbitr. Units
Raman Shift (cm-1)
350000
300000
250000
200000
150000
100000
50000
0
400
600
800
1000
1200
1400
1600

Figure 5c.

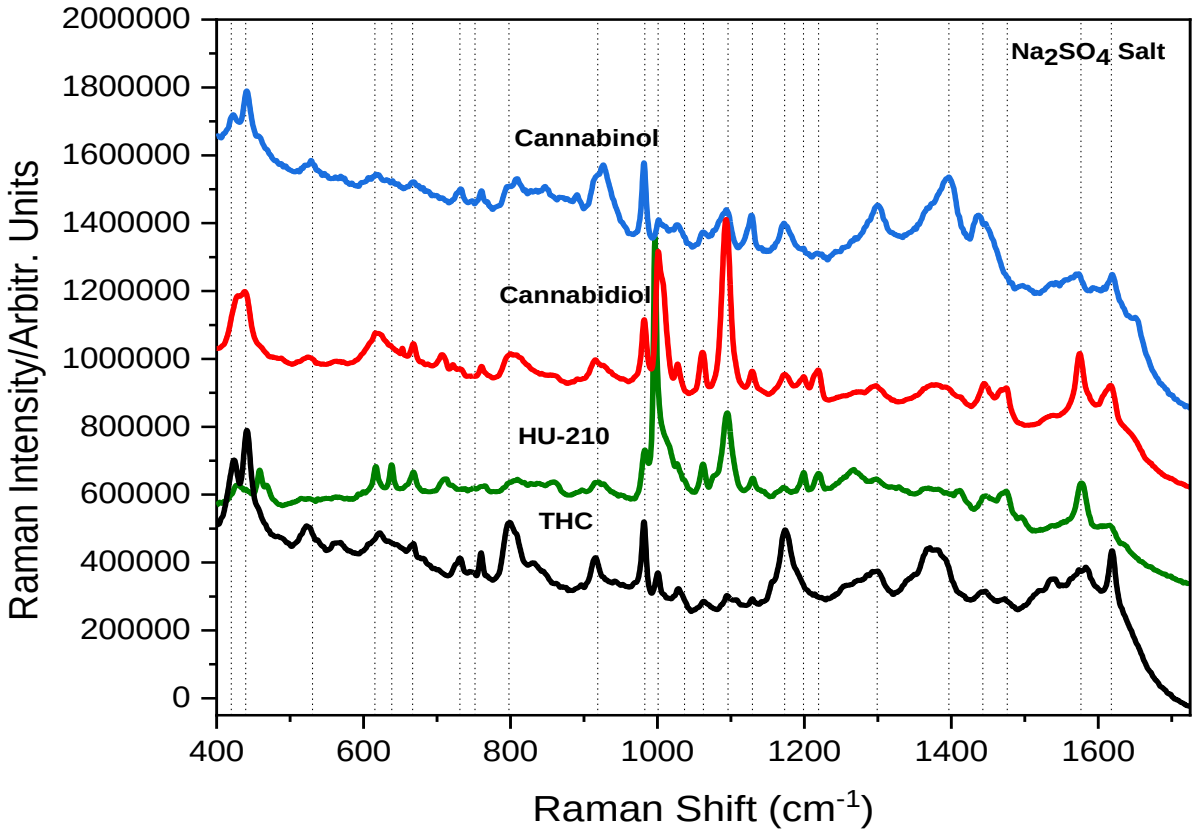

Figure 5d.

SERS spectra of all the four cannabinoids using $MgCl_2$ (fig. 5a), KNO_3 (fig. 5b), $MgSO_4$ (fig. 5c) and Na_2SO_4 (fig. 5d). Note, in (fig. 5b), the peak marked (*) might belong to NO_3^-. Note, no adjustment has been made to the SERS intensities of the cannabinoids in order to highlight the similarities of the SERS spectra of all four cannabinoids.

Supplemental Information

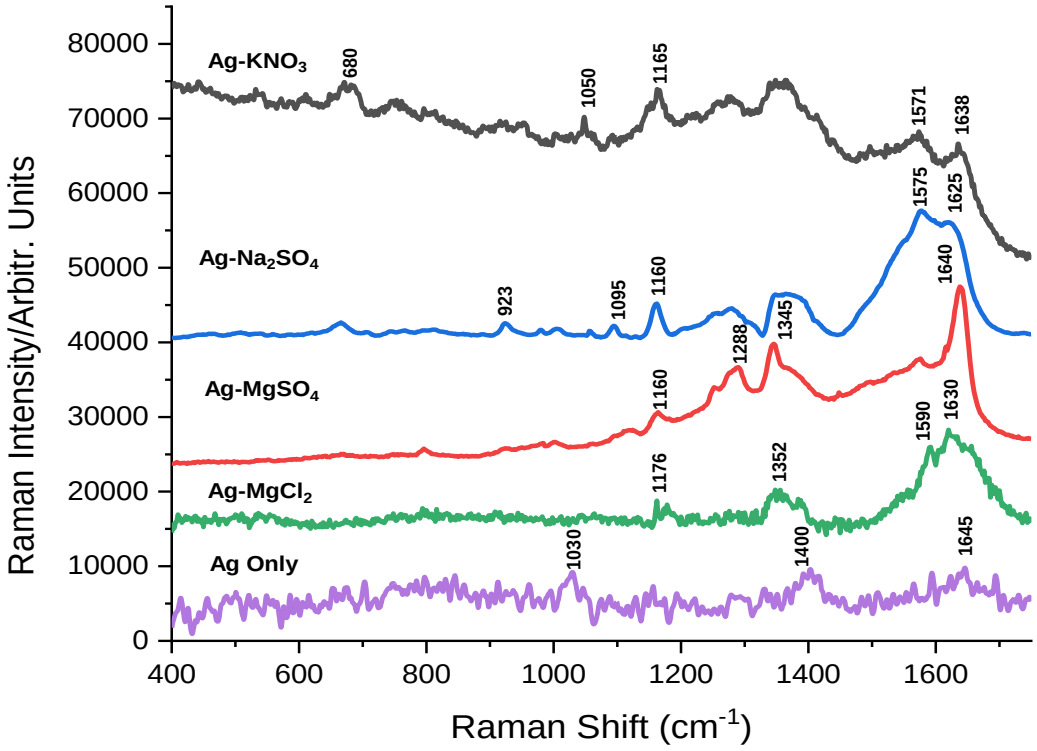

Figure 1. Raman spectra of silver and the four salts. The Raman measurements of salt solutions were conducted by mixing 4µL of silver nanoparticles and 2 µL of (0.5M) each of the different salts in an Eppendorf tube and then deposited on a glass surface for measurement. The excitation wavelength is 633 nm.

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