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TECHNICAL PAPER

RAMAN AND SURFACE ENHANCED RAMAN SPECTRA OF 7-HYDROXYFLAVONE AND 3',4'-DIHYDROXYFLAVONE

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The FT-Raman and surface-enhanced Raman (SER) spectra of two hydroxyl derivatives of flavone, namely 7-hydroxyflavone, 3',4'-dihydroxyflavone, have been obtained. The importance of these compounds lies in the fact that they are simple precursors to the most important of the flavonoid dyes, such as quercetin. The SERS spectra were obtained on citrate reduced Ag colloids. Assignments of the experimentally obtained normal vibrational modes were aided by density functional theory (DFT) calculations using the B3LYP functional and the 6-31+G* basis set. Excellent fits were obtained for the observed spectra with little scaling. As in other flavone derivatives, the C=O stretching bands in the SERS spectra are diminished in intensity by proximity of the metal surface relatively compared to the normal Raman spectra. Additionally, the lines at lower wavenumbers, assigned to in-plane ring deformation, are strongly enhanced by the surface, indicating a perpendicular orientation of the flavonoids on the Ag surface. Finally, the influence of the 7 and 3',4' OH substitutions on the spectra of chrysin, apegenin, and luteolin are examined.

1 Introduction

Most flavone derivatives have been obtained from plants[1] and many of these flavonoids exist there as sugar derivatives (glycosides).[2] Flavone derivatives serve as ingredients for biochemical and pharmacological products used as human dietary supplements.[3-8] Due to their natural yellow color and unique chemical properties, flavones and flavonols are common chromophores in natural yellow dyes that have been used in the textile industry for over 100 years. When extracted from plants, they may easily be hydrolyzed from sugar derivatives to their parent flavonoid and can be applied to textiles as mordant dyes.[2] The parent compound, flavone, has only recently been studied in this laboratory with Raman spectroscopic techniques.[9] In addition to the spectral properties of flavone, we examined the 3-hydroxy derivative, the 5 hydroxy derivative and quercitin (the 3,5,7,3',4'-pentahydroxy derivative).[9] We have also recently exami-

ned the Raman and surface enhanced Raman spectra of the compounds chrysin (5,7-dihydroxyflavone), apigenin (5,7,4'-trihydroxyflavone) and luteolin (5,7,3',4'-tetrahydroxyflavone).[10] These latter compounds have the common feature that they lack a 3-hydroxy addition.

In recent years Raman spectroscopy has found increasing value as applied to the analysis of art objects as well as antiquities. For a valuable discussion of this topic, we suggest a recent review by Vandenabeele, Edwards and Moens,[11] in which over 300 recent references are cited and a discussion of recent international conferences and symposia are listed.

Examination of the possible hydroxyl derivatives of flavone indicates that there are ten available sites for substitution, leading to the possibility of 10! or 3,600,000 derivatives. Only a few of these have been studied, and it is likely that they will not all be studied in extensive detail. However, if we wish to understand these important compounds, and be able to predict the properties of their higher derivatives, it is best to understand the spectroscopy of the most basic of these compounds and to determine if we can extrapolate their properties to at least some of the more complex derivatives. With this in mind, in this study, we examine the two remaining simple precursors to the most important of the derivatives, namely 7-dihydroxyflavone and 3',4'-dihydroxyflavone (Figure 1). We then examine the influence of these substitutions on the spectra of chrysin, apigenin, and luteolin.

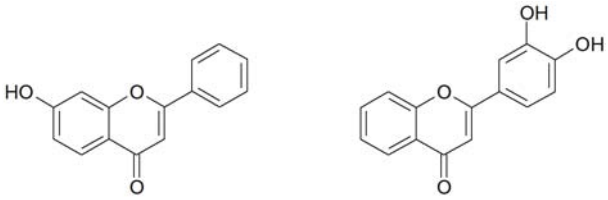

Figure 1: Structure of 7-hydroxyflavone (left) and 3',4'-dihydroxyflavone (right).

2 Materials and Methods

7-hydroxyflavone and 3',4'-dihydroxyflavone were purchased from Sigma. Stock solutions of the compounds were prepared in ethanol in a concentration 10^{-2} M. Then, a water/ethanol mixture (60/40 v/v) was added to prepare 10^{-4} M solution of the dye.

Ag colloid was prepared following the method of Lee and Meisel[12] by reduction of silver nitrate (Aldrich 209139 Silver Nitrate 99.9%) with sodium citrate (Aldrich W302600 Sodium Citrate Dihydrate). The colloid thus prepared shows an absorption maximum at 406 nm and FWHM of 106 nm, as measured with a Cary 50 UV-Vis Spectrophotometer (after a 1:4 dilution with ultrapure water to keep maximum absorbance within the instrumental range). To further concentrate the colloid for use, a volume of 10 ml of the original colloid was centrifuged at 5000 rpm for 2 min. The supernatant was discarded and the settled portion was resuspended in 1 ml of ultrapure water. All glassware was cleaned with Pierce PC54 cleaning solution, rinsed with ultrapure water and finally in acetone and methanol. This method proved to be as effective as the use of aggressive cleaning agents such as aqua regia or piranha solution, and was preferred for health and safety reasons. Only ultrapure water was used for the preparation of the various solutions. SERS measurement were made simply by adding 1 l of dye solution to a 2 μl drop of colloid deposited on a gold coated microscope slide, followed by addition of 2 μl of a 0.2 M KNO_3 solution. Raman measurements were taken directly from the drop using a 50 or 100x microscope objective and focusing on the microscope slide surface. SERS spectra could be obtained two or three minutes after addition of the KNO_3 and remained constant in quality until evaporation of the liquid.

The experimental set up for normal Raman and electrochemical SERS studies has been described in previous papers.[9,10] A Spectra Physics Model 2020 BeamLock argon ion laser line at 488 nm was used as a Raman excitation source. Spectra were recorded with a Spex Model 1401 double monochromator with a resolution of 2 cm^{-1}. Photon-counting detection was used. The laser power was approximately 30 mW in the SERS experiment and only 5 mW in the NR experiment. Chemicals were purchased from the Aldrich Chemical Company Inc., and used as received.

The NR spectra of solids were obtained in the region of 100 to 4000 cm^{-1} directly from pure powder samples. Since the fluorescence of the dyes prevented the acquisition of a Raman spectrum, FT-Raman spectroscopy was carried out using a Bruker Ram II FT-Raman-Vertex 70 FTIR Micro spectrometer. The 1064 nm line of an Nd:YAG laser was used as the excitation line. The resolution was set to 4 cm^{-1} in back scattering mode. A liquid nitrogen cooled Ge detector was used to collect 100 scans for a good Raman spectrum. The laser output was kept at 150 mW for the SERS spectra and 50 mW for the solid samples.

Additionally, some SERS work on Ag colloids was carried out using a Bruker Senterra Raman microscope using 785 nm excitation, a 1200 rulings/mm holographic grating, a CCD detector and power at the sample ranging from 8 to 80 mW.

SERS spectra in an electrochemical cell were obtained at different applied potentials with an activated Ag electrode, which had various molecules adsorbed on it. In SERS experiments, the sample cell consisted of a 99.999% pure silver working electrode, a Pt counter electrode, and a saturated calomel electrode (SCE) as the reference. All potentials reported in this paper are quoted vs. SCE. For activating a Ag electrode, the polished Ag electrode was roughened by an oxidation-reduction cycle (ORC) pretreatment, which was accomplished in the solution of the flavone derivatives ($2x10^{-5}$ M) in 0.1 M K_2SO_4 aqueous solution by applying a potential pulse from -0.4 V to 0.5 V for 2 seconds. These solutions were made with doubly-deionized, quartz distilled water. The molecule was adsorbed on the Ag electrode surface during the ORC. Non-adsorbed molecules were then washed from the electrode by distilled water. After the *ex-situ* ORC pretreatment, the activated Ag electrode was placed in 0.1 M K_2SO_4 aqueous solution for carrying out SERS experiments at various potentials. The same spectra were also obtained with *in-situ* ORC and direct recording of SERS spectra in the solutions. ORC pretreatment and potential control during the SERS experiments were carried out by using an EG&G PARC Model 175 universal programmer and an EG&G PARC Model 173 potentiostat.

Density Functional Theory (DFT) calculations were performed as an aid in assigning the normal modes to which the spectral lines correspond. DFT has proven to be the best theoretical approach for the study of flavone and derivatives,[9,10] and for that reason we chose to use it here. Furthermore, good normal mode assignments are useful in extrapolating possible spectral changes to other flavone derivatives. The DFT calculations were carried out using the commercially available program Gaussian 03[13] at the B3LYP level of theory and employing the 6-31+G* basis set. The geometry optimization resulted in a planar geometry and no imaginary frequencies were observed in the calculated spectrum. This basis set was chosen to be consistent with earlier work, and because the fit obtained was excellent (see below). In general, vibrational normal mode assignments were based on the best-fit comparison of the calculated Raman spectrum with the observed normal Raman spectrum. Slight scaling of the calculated spectrum was utilized (usually 0.96-0.99). In instances where there was spectral congestion, such as in the carbonyl stretch region (near 1600 cm^{-1}), the relative intensities of the calculated spectra were matched to those of the observed spectra, so that the most intense calculated lines were assigned to the most intense observed lines.

3 Results

3.1 Raman and SERS spectra of 7-hydroxyflavone

Figure 2 shows the FT-Raman spectrum of 7-hydroxyflavone (Figure 1a) along with the results of the DFT calculation. The DFT frequencies below 1500 cm^{-1} were scaled by a factor of 0.98 to provide the best fit to the spectrum. In the region above 1500 cm^{-1} a slightly better fit is obtained using the factor of 0.97. As can be seen in the figure, the fit of the DFT calculations is excellent and we therefore can assign the normal modes with confidence. The results are listed in Table 1. Note the band at 1000 cm^{-1} (ν_{41}) consists almost entirely of the trigonal CC stretch of ring B. The prominent band at 1257 cm^{-1} (ν_{52}) involves mostly CH in-plane bends. The band predicted by DFT to be at 1659 cm^{-1} (ν_{68}), attributed to the C=O stretch is either too weak or blended with the more intense 1626 cm^{-1} (ν_{67}) peak to be observed. Both intense bands 1604 and 1626 cm^{-1} involve ring quinoid-like stretches in addition to C=O stretches. The weaker band at 1573 cm^{-1} (ν_{63}) also involves considerable OH in-plane bending. The OH stretch predicted to be around 3636 cm^{-1} by DFT is not observed in our spectra.

In Figure 3 we show a comparison of the Raman spectrum with that observed on the colloid. Notice, as with other flavones the relative intensity of the higher frequency region near 1600 cm^{-1} is some what diminished, while the lines around 500-800 cm^{-1} are relatively enhanced. These lines are mainly involved with in-plane vibrations of the various ring carbon atoms. These observations are consistent with previous observations in other flavone derivatives.[9,10]

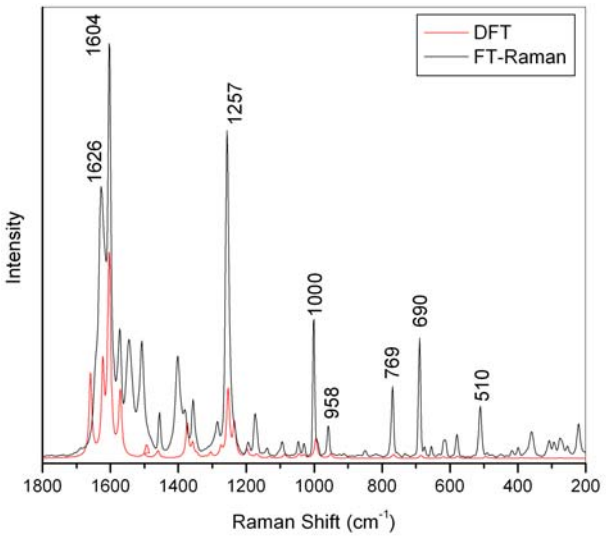

Figure 2: FT-Raman spectrum of 7-hydroxyflavone (powder) and DFT calculation.

Mode no.	Description of modes	DFT (cm^{-1})	Intensity	FT-NR (cm^{-1})	Colloid (cm^{-1})
				360	
18	Ring C def	492	9	510	509
19	Ring A, CC def	570	10	580	580
22	Ring B, CC def	619	7	614	618
26	Ring A, B, C CC ip def	684	15	690	690
					740
29	Ring A CC def	763	17	769	
35	Ring C; CC and COC str ip	900	3		917
37	Ring A CC def,C5H,C6H,C8H bend ip	951	20	958	
41	Ring B trigonal str	995	96	1000	1000
43	CH ip bend	1046	20		1047
44	C5H, C6H ip bend (out of phase)	1086	18	1093	
46	C5H, C6H ip bend (out of phase); OH bend	1130	8		
47	OH bend; C6H, C8H ip bend	1168	11	1174	1172
50	Ring B, CH ip bend	1197	26	1193	
51	C3-H, C5-H, C8-H ip bend (out of phase)	1235	161		
52	C3-H, C5-H, C8-H ip bend (in phase)	1253	332	1257	1246
53	C3-H ip bend	1273	45		
54	OH bend CH ip bend	1302	25	1286	1291
57	OH bend CH ip bend	1357	64	1357	1347
58	OH bend, Ring A, CC def	1373	166	1401	1387
59	Ring B, CH ip bend	1460	34	1455	1439
62	Ring B, CH ip bend	1509	50	1507	1492
				1544	1533
63	OH bend, Ring A,B,C quinoid str	1571	308	1573	
66/65	Ring B quiniod str/C=Ostr-RingA,B,C	1603	597	1604	1584
67	Ring A, C quinoid str	1622	420	1626	1617
68	C=O str	1659	403		
69-77	CH str	3100		3066	
78	OH str	3636			

Table 1: Wavenumbers (in cm^{-1}) and assignments of the Raman and SERS spectra of 7-hydroxyflavone. The DFT wavenumbers are scaled by a factor of 0.98 (below 1500 cm^{-1}) and 0.97 (above 1500 cm^{-1}).

3.2 Raman and SERS spectra of 3',4'-dihydroxyflavone

Figure 4 shows the results of a comparison of the FT-Raman with the DFT calculated spectrum of 3',4'-DHF (Figure 1b). Note that although the calculated spectrum is very similar to that of all the other flavones in this study, the observed Raman spectrum is not. However, it does resemble quite closely a spectrum in solution previously published[14] (see Table 2). In all the previously studied flavones the most intense lines were those near 1600 cm^{-1}, which represented the C=O and C2=C3 stretching region. Note in this spectrum, these lines are surprisingly weak. Instead the lines at 1461 and 1027 cm^{-1} dominate the observed spectrum. These both involve in-plane ring CH bending vibrations and the former also includes strong contributions from the OH in-plane bends.

On the other hand, the SERS spectra (Figures 5-7) strongly resemble the SERS spectra of the other flavones. This is illustrated by the rather strong enhancement of the lines between 472 and 648 cm^{-1}, which can be assigned to the ring C-C in-plane deformations. The lines at 1221 and 1256 cm^{-1} are also strongly enhanced. These involve 3'OH in-plane bends. As with other flavones the C=O stretch region is not especially strong in SERS, but since it is not strong in the powder, the contrast between SERS and normal Raman spectra is not as great as in other flavones.

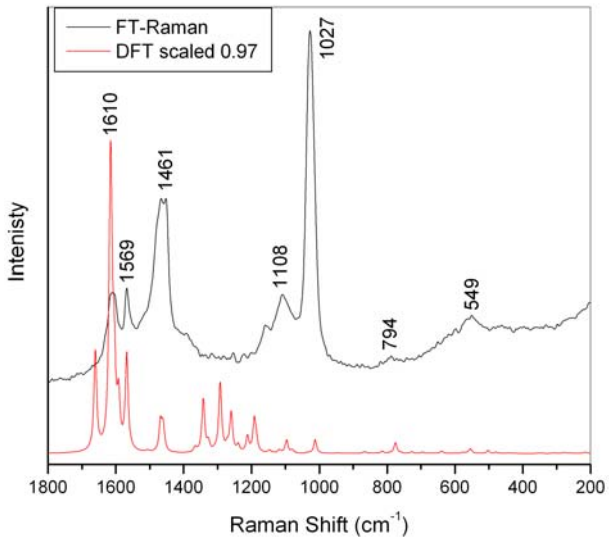

Figure 4: FT-Raman spectrum of 3',4'-dihydroxyflavone (powder) and DFT calculation.

Mode no.	Description of modes	DFT (cm^{-1})	Intensity	FT-NR Soln (cm^{-1})[13]	FT-NR Solid (cm^{-1})	Colloid (cm-1)	-0.5V (cm^{-1})
18	Ring B ip def	493	5			472	495
20	Ring A, B, C ip def	517	11			524	521
21	Ring A CH oop bend	535	0				
22	Ring A, B, C ip def	571	17		549	576	569
23	Ring B CC oop def	578	3				
24	Ring B ip def, 4'OH ip bend	594	4				
25	Ring C ip rock	623	1			619	
26	Ring A, B, C ip def	659	9			648	653
27	CH oop bend	679	2				
28	Ring B, C CH oop bend	697	1				
29	C3H, C2'H oop bend	718	5				703
30	Ring CC ip def	750	6			744	745
31	Ring A, C CH oop bend	764	0				
32	Ring A CH oop bend	786	2				765
33	Ring B CC str	799	42		794	790	787
34	Ring B, C CH oop bend	815	2				
35	Ring A, B, C ip def	840	9			842	846
36	C3H oop bend	873	1				
37	Ring A CH oop bend	883	0				
38	Ring A,B, C ip def	891	6			884	892
39	C2'H o op bend	897	2				
40	Ring B CH oop bend	935	1				
41	Ring B, C CC ip def	971	2			960	964
42	Ring A CH oop bend	977	0				
43	Ring A CH oop bend	1005	0				
44	CH ip bend	1043	53	~1015	1027	1048	1051
45	CH ip bend	1067	2				
46	Ring A CC def	1113	12		1108		
47	4'OH ip bend; Ring B CH ip bend	1130	51			1130	1135
48	Ring A CH ip bend	1153	12		1158		1157
49	4'OH ip bend	1182	8				
50	Ring A CH ip bend	1183	3				
51	3'OH, 4'OH ip bend	1222	39				
52	3'OH ip bend	1228	124			1221	
55	3'OH ip bend CH ip bend	1260	152			1256	1254
56	CH ip bend	1272	23				1273
57	Ring B CC ip str	1292	269				
58	OH ip bend; Ring B CH ip bend	1310	5				
59	OH ip bend	1327	36			1327	1337
60	4'OH ip bend	1342	206			1381	1385
61	OH ip bend	1366	19				
62	Ring A CH ip bend	1458	8				
63	OH ip bend CH ip bend	1461	95		1461	1466	1463
64	Ring A CH ip bend	1469	111				1484
65	Ring B CH ip bend	1506	6			1502	
66	Ring A quinoid str C2-C3 str	1568	368	1569	1569	1564	1559
67	Ring A, B, C str; C=O, C2-C3 str	1592	183				
68	Ring A, B, C str; C=O, C2-C3 str	1605	304	1602			
69	Ring A, B quinoid str	1614	559	1615	1610	1614	1614
70	Ring B quinoid str, 3'OH ip bend	1617	680	1615	1610	1614	1614
71	C=O str	1661	382	1634			
72	C5'H str	3080	139		2840		
73	Ring A CH str	3095	73				
74	Ring A CH str	3109	161		2948		
75	Ring A CH str	3120	79				
76	Ring A CH str	3124	240				
77	C3H, C2'H str (asym)	3128	23				
78	C5'H str	3140	50				
79	C3H, C2'H str (sym)	3148	85		3250		
80	3'OH str	3610	100				
81	4'OH str	3655	242				

Table 2: Wavenumbers (in cm^{-1}) and assignments of the Raman and SERS spectra of 3',4'-dihydroxyflavone. The DFT wavenumbers above 1250 cm^{-1} are scaled by a factor of 0.97.

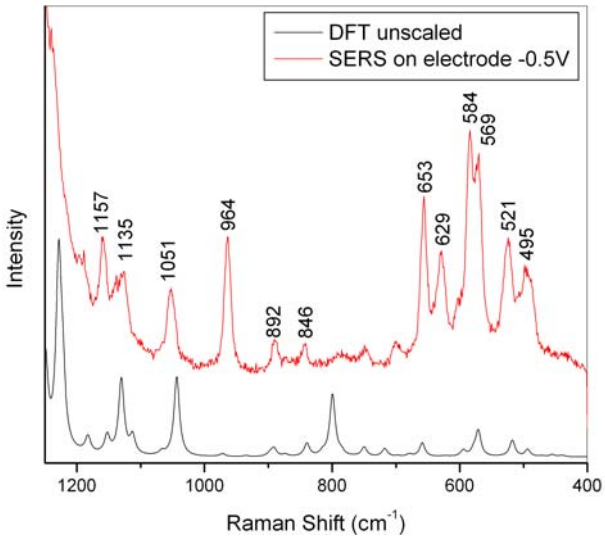

Figure 5: Comparison of the SERS spectrum on electrode of 3',4'-dihydroxyflavone with the DFT calculation in the region 400-1250 cm^{-1}.

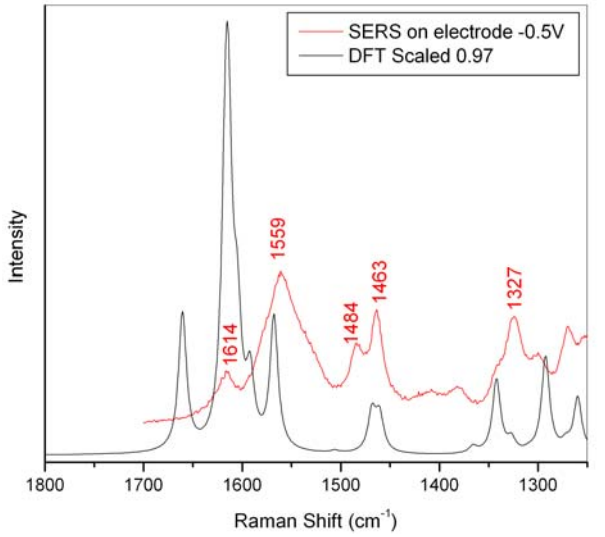

Figure 6: Comparison of the SERS spectrum on electrode of 3',4'-dihydroxy-flavone with the DFT calculation in the region 1250-1800 cm^{-1}.

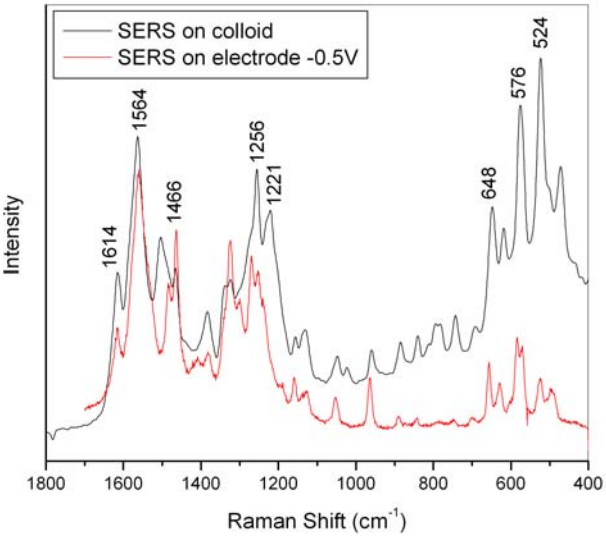

Figure 7: Comparison of the SERS spectrum of 3',4'-dihydroxyflavone on colloid and on electrode

3.3 Comparison of Raman and SERS spectra of chrysin, apigenin and luteolin

It is of interest to compare the effects of successive OH substitution on the observed wavenumbers of the various flavones studied. As a complete comparison of all modes would be a lengthy and complex exercise, we will only focus on a few regions of the spectrum. The most intense lines in the C=O stretching region are also of special interest. The FT-Raman spectra of chrysin, apigenin and luteolin are shown in Figure 8.

These spectra are formed by five bands, two or three intense ones together with two or three weak lines. In this region, the relative intensity of the bands corresponding to chrysin and apigenin appear quite similar, and different from the spectral profile of luteolin. In Table 3 we present the observed wavenumbers and their spectral assignments obtained from the DFT calculations. The band located at the lowest wavenumbers in this region (1559 and 1556 cm^{-1}) can be assigned to the C=C aromatic stretching of one or more rings (A, B and C) in the case of chrysin and luteolin and to C=O and C2=C3 stretching. It can be seen that the OH substitution does not affect to this vibra-

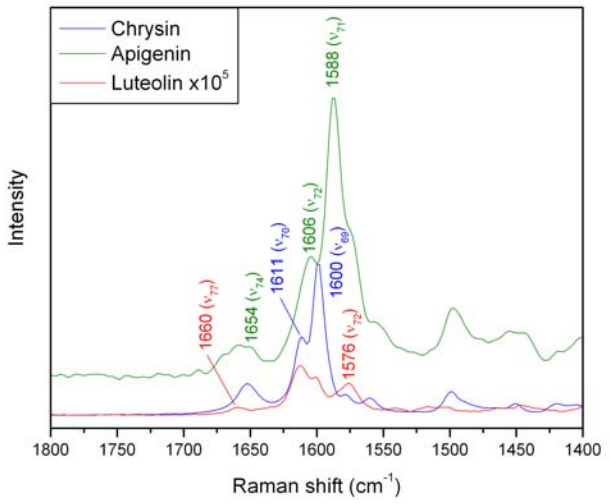

Figure 8: Comparison of the C=O stretching region of the FT-Raman spectra of chrysin, apigenin and luteolin.

Chrysin (cm^{-1})	Apigenin (cm^{-1})	Luteolin (cm^{-1})	Mode description (DFT)
1559 ν_{66}(w)	1556 ν_{69}(w)	(1553) ν_{71}(vw)	Ring A, B and / or C stretch
1579 ν_{67}(w)	1588 ν_{71}(s)	1576 ν_{72}(s)	Ring A, B and / or C quinoid stretch
1600 ν_{69}(vs)	1606 ν_{72}(s)	1600 ν_{73}(sh)	Ring A, B quinoid stretch.
1611 ν_{70}(m)	(1614) ν_{73}(vw)	1612 ν_{75}(vs)	Ring A, B and / or C stretch
1652 ν_{71}(w)	1654 ν_{74}(w)	1660 ν_{77}(w)	C=O stretch

Table 3: Comparison of C=O stretching region for chrysin, apigenin and luteolin (Figure 8). The intensity of the bands is shown in braquets[a].

tion. However, the line at 1579 cm^{-1} in chrysin shifts up to 1588 cm^{-1} in apigenin, but remains nearly unchanged in luteolin. This vibration, as the previous one, is mainly assigned to the C=C aromatic stretching, with a component of C=O stretching. For this reason, only a small variation with substitution is expected. The next vibration, at 1600 cm^{-1}, consists of a C=C aromatic stretching. In chrysin that mode is coupled to the C=O stretching and, in apigenin, to the C=O and C2=C3 stretches. Thus, that band remains practically unshifted as the number of OH groups increases. The same happens to the last two bands, assigned to C=O stretching. In luteolin, this mode is coupled to the C2=C3 stretching.

Of importance are the two intense bands at 1000 and 1247 cm^{-1} in chrysin (Table 4). The first one is assigned to the ring B trigonal stretch in chrysin and apigenin. In the spectra of the former molecule, this bands shift down to 983 cm^{-1}. This is due to the presence of an extra OH group in the apigenin B ring. In the case of luteolin, the band does not change, and this is attributed to the CH in-plane bend, which is not affected by the presence of additional hydroxy groups. As in the case of chrysin, the band at 1000 cm^{-1} of 7-HF (Table 1) also has medium intensity and corresponds to the same vibrational mode. The second band is very strong in the spectra of the three flavonoids. It corresponds to the bending of the OH groups in C5 and C7, coupled to a C-H in-plane bend. The position

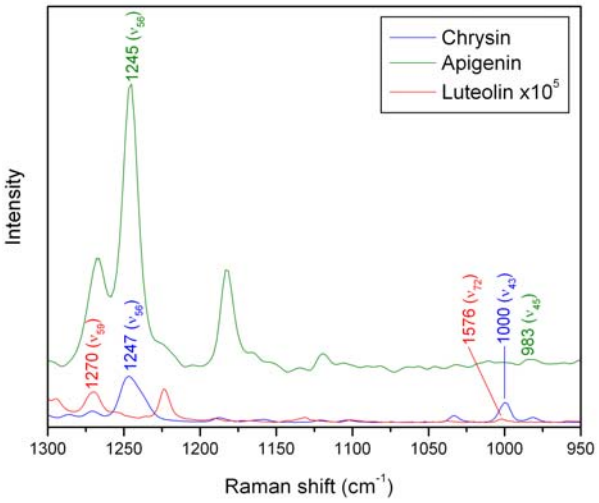

Figure 9. FT-Raman spectra of chrysin, apigenin and luteolin in the 1000-1250 cm^{-1} region.

Chrysin (cm^{-1})	Apigenin (cm^{-1})	Luteolin (cm^{-1})	Mode description (DFT)
1000 ν_{43}(m)	983 ν_{45}(vw)	1002 ν_{48}(w)	Ring B trigonal stretch, C-H bend (ip)
1247 ν_{55}(s)	1245 ν_{56}(s)	1270 ν_{59}(s)	C-H bend (ip), OH bend (ip)

Table 4: Comparison of the most intense bands in the region 1000-1250 cm^{-1} for chrysin, apigenin and luteolin (Figure 9). The intensity of the bands is shown in braquets[a].

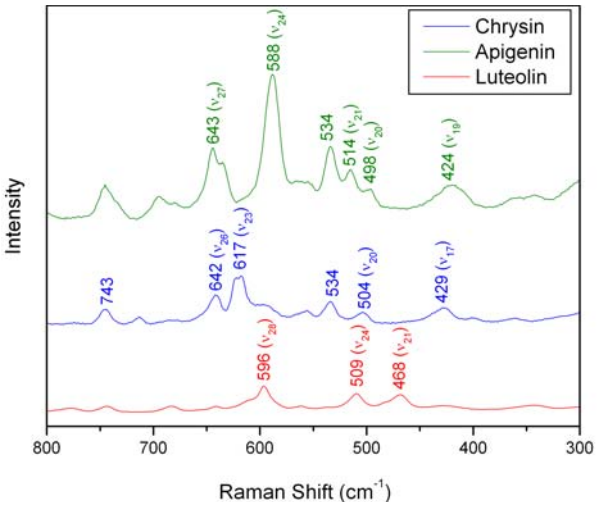

Figure 10: Low frequency region of the SERS spectra of chrysin, apigenin and luteolin. (Spectra were translated vertically in order to view them clearly.)

Chrysin (cm^{-1})	Apigenin (cm^{-1})	Luteolin (cm^{-1})	Mode description (DFT)
429 ν_{17}	424 ν_{19}	468 ν_{21}	Ring A, B and/or C C-C def (ip)
504 ν_{20}	498 ν_{20}	509 ν_{24}	Ring A, B and /or C, C-C def (ip)
	514 ν_{21}		Ring A, B and /or C, C-C def (ip)
534	534		
556 ν_{21}	588 ν_{24}	596 ν_{28}	Ring A, B and /or C, C-C def (ip)
617 ν_{23}			Ring A, B and /or C, C-C def (ip)
642 ν_{26}	643 ν_{27}		Ring A, B and/or C def (ip)
743			Ring A, C, C-H bending

Table 5: Modes of chrysin, apigenin and luteolin, which are strongly enhanced on the colloid surface (Figure 10).

of this mode is much higher in the luteolin spectrum, in which the component of C3-H bend is higher than the OH, in contrast to the other two flavonoids. In 5-HF[9] and 7-HF (Table 1) the equivalent bands (at 1253 and 1257 cm^{-1}, respectively) are also intense and correspond solely to the C-H in-plane deformation.

Several modes observed in the SERS spectra of the flavonoids studied are strongly enhanced compared to the FT-Raman spectra. In Figure 10 we show the region of 200-800 cm^{-1} of the chrysin, apigenin and luteolin SERS spectra. The positions and the assignments of the bands are listed is Table 5. Most of these modes correspond to ring C-C in-plane deformations, indicating that the molecules are oriented perpendicular to the silver surface, as seen in other flavonoids.[9,10] This is also consistent with the decrease in intensity of the modes in the C=O region in the SERS spectra.

4 Conclusion

We have obtained the FT-Raman and SERS spectra of two hydroxyl derivatives of flavone, namely 7-hydroxyflavone and 3',4'-dihydroxyflavone. The SERS technique enabled us to obtain intense

spectra from a small quantity of material while simultaneously suppressing fluorescence. We have compared the relative intensities from the FT-Raman spectra with both colloidal and potential-dependent SERS spectra and with DFT calculations. It is concluded that DFT calculations provide a source of accurate normal mode assignments, as well as a basis of comparison of the effects of successive OH substitutions on the normal modes of the parent flavone. Besides, the influence of the 7 and 3',4' OH substitutions on the most intense Raman and SERS bands of chrysin, apegenin, and luteolin were examined. None or small variations with OH substitution were observed in the C=O stretching region of the Raman spectra. On the contrary, the intense band at 1000 cm^{-1} in chrysin, assigned to the ring B trigonal stretch, shifts down to 983 cm^{-1} in apigenin. This is probably due to the presence of an extra OH group in the apigenin B ring.

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