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Study of Antiviral Diclofenac by Surface-Enhanced Raman Spectroscopy

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Abstract

Diclofenac is a medication that presents in its structure two benzene rings joined by an amine group presenting: The first ring two chlorine atoms, both in the ortho position with respect to the amino group and the second ring is bound to ethanoic acid, also in ortho position with respect to this amino group, thus showing the carboxylate group (COO-).

We have studied the Raman spectrum of solid Diclofenac (DCF), their spectra in solution in methanol at different concentrations and Surface-Enhanced Raman Spectroscopy (SERS) with additives that increase the profile of spectra. The interaction of this molecule with Ag (that is in the colloid used in SERS), is significant on the two rings, the COO- and amine group and Cl atom and C_7H_2 group, everyone in the structure of DCF.

Keywords: Diclofenac; Surface-Enhanced Raman Spectroscopy (SERS); FT-Raman spectrum

Introduction

Diclofenac Sodium (DCF-Na) or as IUPAC sodium salt of {2-[(2,6-dichlorophenyl)amino]-fenil}acetic acid, is a medication that shows properties anti-inflammatory, analgesic and antipyretic and binds with human proteins mainly with albumin and it is a well-known non-steroidal anti-inflammatory drug for the treatment of inflammatory conditions such as rheumatoid arthritis, osteoarthritis, and ankylosing spondilytis. Its formula is shown Figure 1 and its numbering 4.

There are several studies about this medication: The combination DCF-Na and tolperisone hydrochloride (chemically 2-methyl-1-(4-methylphenyl)-3-(1-piperidin-1-yl) propane-1-one), is used for the treatment of adult patients with acute muscle/musculoskeletal spasms [1-3].

Raman spectroscopy and Surface-Enhanced Raman Spectroscopy (SERS) are used to study of the vibrational characterization of diclofenac sodium and has been studied the photolytic transformation and its transformation products in aqueous solution [4,5]. Several analytical methods have been used for the quantification of DCF-Na, such as spectrofluorimetry, and chromatography and was investigated the biotransformation of diclofenac during wastewater treatment [6-8].

The inclusional complexation between the anti-inflammatory pharmaceutical diclofenac and beta-cyclodextrin (beta-CD) was studied by potentiometry, spectrophotometry and spectrofluorimetry, in both cases in acid and neutral pH. Guest-host 1:1 stoichiometries for the complexes in both media were determined and their equilibrium constants were calculated. There are Raman and SERS spectroscopy studies about the interaction DCF-Na-sodium-?-cyclodextrin [9,10].

Terahertz (THz) spectroscopy is used for analytical applications since this technique is sensitive to the intermolecular interactions of molecules in the solid state. Understanding the fundamental nature of the lattice vibrational motions leading to absorptions in THz spectra is challenging, but it can be achieved through computational approaches. In this study, the THz spectra of two diclofenac acid polymorphs were obtained by THz spectroscopy, and the vibrational characters of the observed absorptions were analyzed using solid-state Density Functional Theory (DFT). The results demonstrate the quantitative capacity of THz spectroscopy and the reliability and utility of solid-state DFT in the calculation of low-frequency vibrational motions [11].

Chemometrics-assisted UV spectrophotometric and RP-HPLC methods are presented for the simultaneous determination of Tolperisone Hydrochloride (TOL) and Diclofenac Sodium (DIC) from their combined pharmaceutical dosage form 3.

Several analytical methods are used to detect this drug also with other compounds [3,12,13]. Differential scanning calorimetry and thermogravimetric analysis are used to investigate the dispersed/dissolved state of DCF in preparation of diclofenac sodium and the water-insoluble ammonio methacrylate copolymer, the thermal stability and the properties of the films of this mixture were confirmed. Raman spectroscopy corroborates the possible interactions between the two products [14].

Surface-Enhanced Raman Spectroscopy (SERS) is a technique that obtains a very increase in the intensity of the Raman signal of a molecule physical or chemical absorbed on a rough metal surface (silver, gold, copper) of a microscopic size.

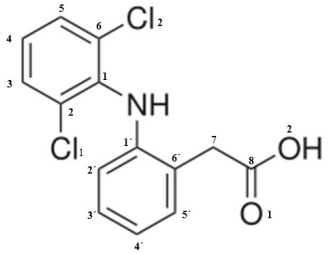

Figure 1: Structure of diclofenac.

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When an electromagnetic wave interacts with metal surface, the field next this surface change in regard the far field and, if it is rough, the plasmons on the surface are exited and the electromagnetic field is amplified. The intensity observed in SERS usually exceeds by a factor of 105-106 and in special cases by 1012-1014 the signal observed in normal Raman scattering. Moreover, the SERS effect becomes even stronger if the frequency of the excitation light is in resonance with the main absorption band of the molecule being illuminated Surface-Enhanced Resonance Raman Scattering (SERRS) [15-18].

This technique is widely used for study the molecules absorbed on metallic surfaces that are mainly in colloids of silver or copper. SERS allows obtain spectra of substances at very low concentration [19,20]. Two theories explain this technique: The electromagnetic theory: the increase in intensity of the Raman signal for adsorbates on particular surfaces occurs because of an enhancement in the electric field provided by the surface and the chemical theory explains the effect through the formation of charge-transfer complexes [21].

There are currently several studies about the SERS enhancement factor of silver sphere nanoparticles depended on temperature and instant synthesis of gold nanoparticles at room temperature [22,23]. The wide application of this drug in medicine makes us think that it is important to know its behavior in all possible ways and in the presence of different substances. This DCF study on silver colloid shows the interaction between this drug and silver of the colloid. We thought it would be interesting to study the SERS of this drug on others metals.

Materials and Methods

Diclofenac was purchased from Sigma Co. (Seelze, Germany). All other reagents were of analytical degree. The solutions were prepared by using tridistilled water. Preparation of the Ag Colloid for Surface-Enhanced Raman Spectroscopy (SERS) by method of Lee and Meisel [24]. 4 mL of a 1% trisodium citrate solution were added to 200 mL of a 10^{-3} M $AgNO_3$ boiling aqueous solution and all mixing was boiling for one hour.

The solutions of diclofenac were prepared in methanol at different concentrations and the samples for study were prepared by addition the 10^{-2} M KNO_3 solution for induced the necessary aggregation of the Ag nanoparticles.

Instrumentation

The absorption spectrum was recorded with a double beam Cintra 5 UV visible absorption spectrophotometer using a 10 mm silica cell. FT-Raman spectra were recorded with Bruker RFS 100/S by using a ND: YAG laser source at 1064 nm, the output power was 150 mW. The sample was placed into a 10 mm silica cell. The final spectra were the result of 1000 scans accumulations and power about 300 mW. Raman spectra were recorded with a Renishaw R. M. 1000 by using a laser source at 782 nm and placing the liquid sample on a glass sheet and evaporating until obtain a solid residue and objective 100. We have obtained also microscopic pictures of internal structure of samples.

Results and Discussion

Figure 1 shows the structure of the molecule of this medication which is constituted by a secondary amine having two benzene groups as substitutes, one with two Cl atoms in ortho position and the second shows a carboxylate group also in the ortho position and the numbering 5. Figure 2 shows: a) The FT-Raman spectrum of solid diclofenac, b) Diclofenac in methanol 0.1 M, c) SERS of diclofenac (in methanol) 10^{-3} M+Na_2SO_4 10^{-2} M and d) SERS of diclofenac 10^{-3} M+KNO_3 10^{-2} M as clustered.

The main peaks of these spectra are in Table 1. The following changes are observed between the solid DCF spectrum (and the methanol solution) and SERS on silver colloid: the strong band at 1277 cm^{-1}, in the solid and in the solution, which belongs to CH rock ring (1+2)+C_7H_2 (wagging), in the SERS is observed as medium at 1274 cm^{-1}. The shoulder at 1247 cm^{-1}, also in the solid and in the solution, which is assigned to C6`7 stretching+CH rock (ring 1+2) in SERS, disappears. The very strong peak in the solid at 1237 cm^{-1}, which is strong in the dissolution and that corresponds to C1NC1´s. stretching+CH rock (ring1+2)+C_7H_2 wagging, is observed in SERS strong at 1235 cm^{-1} in solution 10^{-3} M, weak in solution 10^{-4} M, very weak unfolded at 1241 cm^{-1} and 1227 cm^{-1} in solution 10^{-5} M and, at last, as shoulder at 1230 cm^{-1} and very weak at 1207 cm^{-1} in solution 10^{-6} M. It is observed in SERS, a decrease in the intensity (in the second case is canceled) and a small displacement of the peaks towards lower values. There is an alteration in the peaks corresponding to the two rings. The foregoing indicates that the interaction of the molecule with the metal is on two rings (Tables 1 and 2).

Other changes confirm this interaction: The very strong peak at 1604 cm^{-1} in solid and strong in solution, assigned to ring (1+2) stretching, disappears in SERS; the medium peak at 1161 cm^{-1} in solid and in solution, assigned to CH bend ring (1+2), appears weak at 1152 cm^{-1} in SERS 10^{-3} M and unfolded at weak 1144 cm^{-1} and very weak at 1123 cm^{-1} in SERS 10^{-5} M and also unfolded as a shoulder at 1152 cm^{-1} and strong broad at 1120 cm^{-1} in solution 10^{-6} M. The weak peak at 1093 in the solid and medium in the solution, corresponding to C_7H_2 wagging+CH bend (ring 1+2), only appears in SERS 10^{-5} M in Table 2. The weak peak at 717 cm^{-1} in the solid, corresponding to CH wagging (ring 2+1), in the solution is displaced at 721 cm^{-1} and in SERS is observed as a weak peak at 718 cm^{-1} only at concentration 10^{-3} M. The weak peak in the solid at 606 cm^{-1}, corresponding to ring (1+2) in plane deformation, and which appears also weak at 603 cm^{-1} in SERS in the solution 10^{-3} M and as a shoulder at 600 cm^{-1} in the solution 10^{-4} M and at 609 cm^{-1} in the solution 10^{-5} and at 10^{-6} M disappears. On the other

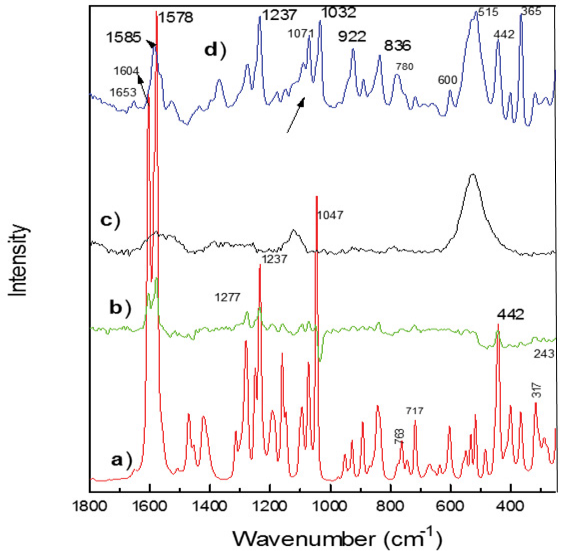

Figure 2: a) Solid diclofenac; b) diclofenac in methanol 0.1 M; c) SERS of diclofenac 10^{-3} M+Na_2SO_4 10^{-2} M; and d) SERS of diclofenac 10^{-3} M+KNO_3 10^{-2} M.

Solid diclofenac	Diclofenac in methanol 0.1M	SERS over Ag colloid 10^{-3} M+KNO_3 10^{-2} M pH=12	Assignments
		1655 cm^{-1}(w)	Amines (NH_2 scissoring)
1604 cm^{-1} (vs)	1604 cm^{-1}(s)		Ring (1+2) stretching
1578´´(vs)	1578´´(vs)	1585´´(s)	$O_1C_8O_2$ as. stretching
		1562´´ (sh)	Ring 1stretching[a]
	1517´´(w)	1527´´(w)	Aromatic ν (C=C)
1509´´(w)	1506´´(w)		Aromatic ν (C=C)
1472´´(w)	1472´´(w)		C_1N stretching+CH rock (ring 2)[a]
1455´´ (sh)			C_1N stretching+CH rock (ring 1)[a]
1421´´ (w)	1444-1410´´(w)	1435´´ (w)	C_7H_2 bend[a]
		1395´´(vw)	$C_{7,8}$ stretching+$O_1C_8O_2$ s. stretching[a]
		1369´´(w)	Ring 2 stretching+C_7H_2 wagging[a]
1313´´(sh)	1307´´(vw)		Ring 1 stretching+C_7H_2 wagging[a]
1277´´(s)	1277´´ (s)	1274´´(m)	CH rock (ring 1+2)+C_7H_2 (wagging)[a]
1247´´(sh)	1247´´(sh)		C_{6-7} stretching+CH rock (ring 1+2)[a]
1237´´(vs)	1237´´(s)	1235´´(s)	C_1NC_1 s. stretching+CH rock (ring 1+2)+C_7H_2wagging[a].
1192´´(w)	1192´´(w)	1180´´ (w)	CH rock (ring 1)[a]
1161´´(m)	1161´´(m)	1152´´ (w)	CH bend ring (1+2)[a]
1093´´(w)	1093´´ (m)		C_7H_2 wagging+CH bend (ring 1+2)[a]
		1085´´ (w)	Ring 1 breathing[a]
1071´´(m)	1071´´(m)	1071´´(m)	Ring 1 breathing[a]
1047´´(vs)	1047´´ (m)	1033´´(s)	Ring 2 breathing[a]
973´´ (vw)	967´´(vw)	973´´(vw)	Aromatic δ_{ip}(C-H)
953´´(vw)	953´´(vw)		Aromatic δ_{ip}(C-H)
		944´´ (sh)	CH twist (ring 2+C_7H_2)[a]
928´´(vw)	920´´(vw)	924´´(m)	$C_{7,8}$ stretching
892´´(w)	894´´(w)	890´´(w)	
844´´(m)	866´´(w)	837´´(m)	CH twist (ring 2+1)[a]
763´´(w)	760´´(w)	776´´(m)	C-Cl stretching+ring (1+2) in plane def.[a]
743´´(vw)	759´´ (vw)	754´´(sh)	
717´´(w)	721´´(w)	718´´(w)	CH wagging (ring 2+1)[a]
670´´ (vw)	698´´ (vw)	690´´(vw)	
640´´(vw)		661´´ (vw)	$O_1C_8O_2$ def+C_7H_2 def.[a]
606´´(w)	608´´(vw)	603´´(w)	Ring (1+2)in plane def.[a]
550´´(w)	572´´(w)		$C_{6-7,7,8}$ def+C_7H_2 def.[a]
		530´´(s) 515´´(s)	Ring 2+1 out–of-plane def.
482´´(vw)			NH def.[a]
		442´´(s)	Ring 2 out-of-plane def.[a].
		401´´(w)	$O_1C_8O_2$ def.+$Cl_1C_{2,3}$, $Cl_2C_{6,5}$ def+C_7H_2 def[a]
		320´´(w)	$C_1NC_{1.}$ def+$C_{6-7,8}$ def+C-Cl def[a]
		282´´(w)	Ring 1 out-of-plane def+$C_1NC_{1.}$def.[a]
		216´´(sh)	Ring 2 out-of-plane def[a].
		106´´(vw)	

Table 1: FT Raman spectrum of solid diclofenac, diclofenac in methanol and SERS (w=weak, m=medium, sh=shoulder, s=strong, br=broad) [4].

hand the strong peaks at 530 cm^{-1} and 515 cm^{-1}, corresponding to ring (2+1) out-of-plane deformation, do not appear in the solid or in the dissolution and also the strong peak at 442 cm^{-1}, which also corresponds to ring 2 out-of-plane deformations, is observed only in SERS.

Other changes are significant: The peak at 1395 cm^{-1} (vw), corresponding to $O_1C_8O_2$ stretching and C7,8 stretching, are only in SERS, and the very weak peak in the solid DCF at 640 cm^{-1}, assigned to $O_1C_8O_2$ deformation, is moving in SERS to 661 cm-1. All these changes indicate that the carboxylic group interacts with metal.

The weak peak at 1472 cm^{-1}, corresponding to C_1N stretching+CH rock (ring 2) disappears in SERS 10^{-3} M and it appears as a shoulder in SERS 10^{-4}, 10^{-5} and 10^{-6} M at 1484 cm^{-1}, 1493 cm^{-1} and 1490 cm^{-1} respectively and the shoulder at 1455 cm^{-1} in the solid, corresponding to C_1N stretching+CH rock (ring 1) is not observed in the solution and in SERS 10^{-3} M. The shoulder at 1313 in the solid, and at 1307 in the solution, which corresponds to ring 1 stretching+C_7H_2 wagging, appears in SERS only as a shoulder at 1320 cm^{-1} in concentration 10^{-6} M. The very strong peak at 1047 cm^{-1} in the solid that is medium in the solution is shown strong at 1033 cm^{-1} in SERS and is assigned to ring 2

10^{-3} M	10^{-4} M	10^{-5} M	10^{-6} M	Assignements
1655 (cm^{-1}) (w)	1652 (cm^{-1}) (w)	1658 (cm^{-1}) (sh)	1649 (cm^{-1}) (sh)	Amines (NH_2 scissoring)
		1638´´ (vw)		Amines (NH_2 scissoring)
	1617´´ (sh)	1620´´ (vw)	1620´´ (sh)	Amines (NH_2 scissoring)
1585´´ (s)	1579´´ (s) {br}	1579´´ (s) {br}	1579´´ (s)	$O_1C_8O_2$ as. stretching[a]
1562 (sh)	1551´´ (s)			Ring 1 stretching[a]
1527´´ (w)	1527´´ (m)	1527´´ (m)	1527´´ (vs)	v (arom. C=C) // v (arom. C-C)
	1484´´ (sh)	1493´´ (sh)	1490´´ (sh)	C_1N stretching+CH rock (ring 2)[a]
		1458´´ (w)	1450´´ (m)	C_1N stretching+CH rock (ring 1)[a]
1435´´ (vw)	1433´´ (w)	1440´´(w)		C_7H_2 bend[a]
1395´´ (vw)	1398´´ (w)	1385´´ (w)	1391´´ (m)	$C_{7,8}$ stretching+$O_1C_6O_2$ stretching[a]
1369´´ (w)	1351´´ (w)	1346´´ (w)	1334´´ (m)	Ring 2 stretching+C_7H_2 wagging[a]
			1320´´ (sh)	Ring 1 stretching+C_7H_2 wagging[a]
	1291´´ (vw)	1300´´ (vw)	1300´´ (vw)	C_1NC_1a. stretching+CH rock (ring 1+2)+C_7H_2 wagging[a]
1274´´ (m)	1268´´ (w)	1276´´(w) 1264´´ (w) {br}	1268´´(s)	CH rock (ring 1+2)+C_7H_2 wagging[a]
1235´´ (s)	1235´´ (w)	1241´´ (vw) 1227´´(vw)	1230´´ (sh) 1207´´ (vw)	C_1NC_1s. stretching+CH rock (ring 1+2)+C_7H_2 wagging[a].
1180´´ (w)		1196´´ (vw)	1186´´ (vw) 1175´´ (vw) {br}	C_7H_2 twist[a].// CHrock (ring 1)[a]
1152´´(w)		1144´´(w) 1123´´(vw) {br}	1152´´(sh) 1120´´(s) {br}	CH bend (ring 1+2)[a]
		1094´´ (vw)		C_7H_2 wagging+CH bend (ring 1+2)[a]
1085´´ (w)		1063´´(vw)		Ring 1 breathing[a]
1071´´(m)				Ring 1 breathing[a]
1033´´(s)	1033´´(m)	1033´´(vw) 1019´´(vw)	1033´´(w)	Ring 2 breathing[a]
			1004´´(w)	Aromatic in plane deformation/carboxilic acids(C-O)
973´´(vw)				Aromatic δ_{ip}(C-H)
944´´(sh)				CH twist (ring 2+C_7H_2)[a]
924´´(m)	924´´(w)	936´´(w) 926´´(w) {br}		$C_{7,8}$ stretching[a]
890´´(w)	890´´(vw)	886´´(vw)		NH_2 & NH wagging
837´´(m)	837´´(w)			CH twist (ring 2+1)[a]
		794´´(vw)	799´´(w) 782´´(w)	C-Cl or CH- out of plane deformation
776´´(m)	773´´(w) {br}			C-Cl stretching+ring (1+2) in plane def.[a]
754´´(sh)				CH wagging (ring 2+1)[a]
718´´(w)			724´´(w)	CH wagging (ring 2+1)[a]
		695´´(vw)	692´´(w)	NH_2 & NH wagging
661´´ (vw)	666´´(vw)	664´´(vw) {br}	661´´(w)	$O_1C_8O_2$ def+C_7H_2 def.[a]
603´´(w)	600´´(sh)	609´´(sh)		Ring (1+2) in plane def.[a]
530´´(s) 515´´(s)	530´´(s) 513´´(s) {br}	539´´(vs) {br}	521´´(vs) {br}	Ring (2+1) out–of-plane def.[a]
442´´(s)	440´´(m)			Ring 2 out of plane def.[a]
401´´(w)		401´´(w)	406´´(w)	$O_1C_8O_2$ def+$Cl_1C_{2,3}$, $Cl_2C_{6,5}$ def+C_7H_2 def[qa]
			380´´(w)	
320´´(w)	316´´(w)			C_1NC_1def+$C_{6',7,8}$def+C-Cl def[a]
282´´(w)				Ring 1 out-of-plane def+C_1NC_1.def[a]
216´´(sh)				Ring 2 out-of-plane def.[a]
106´´(vw)	106´´(vs)	111´´(vs)	115´´(vs)	

Table 2: SERS of diclofenac (in methanol) on Ag colloid+KNO_3 10^{-2} M pH=12 (w=weak, m=medium, sh=shoulder, s=strong, br=broad) [4].

breathing. In SERS there is a shoulder at 944 cm^{-1} which is not observed either in the solid or in the dissolution and which is assigned to CH twist (ring 2+C_7H_2). All these differences indicate a different behavior of two rings in SERS.

The weak peak in the solid at 1421 cm^{-1} and the wide band in the solution, between 1444 and 1410 cm^{-1}, assigned to C_7H_2 bend, in SERS moves to 1435 cm^{-1}, in the solution 10^{-3} M, to 1433 cm^{-1} in the solution 10^{-4} M and 1440 cm^{-1} at 10^{-5} M show the interaction between Ag and this chain.

The peaks of Cl in the molecule show some differences in regard to SERS: The weak peak in the solid at 763 cm^{-1} (at 760 cm^{-1} in the solution), which appears in the middle SERS at 776 cm^{-1} corresponds to C-Cl stretching (and ring 1+2 in plane deformation). And, at last, the two weak peaks at 401 and 320 cm^{-1}, assigned to C-Cl deformation, appear only in SERS.

Table 2 shows the main frequencies in the SERS spectra of the molecule of diclofenac, dissolved in methanol at pH=12, over Ag colloid obtained using hydroxylamine as reducing agent, at different concentrations (Figure 3).

It is observed: The weak peak at 1517 cm^{-1}, in the solution 0.1 M in Table 1, assigned to aromatic? (C=C), which appears weak at 1527 cm^{-1} in SERS at concentration 10^{-3} M, it is observed at medium intensity in SERS at concentrations 10^{-4} M and 10^{-5} M and very strong at 10^{-6} M. Similar, the middle peak at 1274 cm^{-1}, quoted in previous paragraphs, which corresponds to the C-H rock (ring 1+2), appears weak at 1268 cm^{-1} in the SERS and is split in two weak peaks at 1276 and 1264 cm^{-1} corresponding to respective 10^{-4} and 10^{-5} M concentrations and in the solution 10^{-6} M is observed a strong peak at 1268 cm^{-1}. The medium peak at 776 cm^{-1} in SERS 10^{-3} M, already cited as ring 1+2 in plane deformation, only is observed in this table in SERS 10^{-4} M.

The different interaction of each ring with the metal is observed in the shoulder at 1562 cm^{-1}, in SERS 10^{-3} M and strong at 1551 cm^{-1} in SERS 10^{-4} M that are not observed in solid and in solution and that corresponds to ring 1 stretching; the weak peak at 1458 cm^{-1}, that is seen in SERS 10^{-5} M, which is not observed in the two higher concentrations of SERS, and that is assigned to CH rock ring 1, also appears medium (1450 cm^{-1}) in solution 10^{-6} M; also the peaks at 1369 cm^{-1}-1351 cm^{-1}-1346 cm^{-1}-1334 cm^{-1} corresponding to SERS 10^{-3} M, 10^{-4} M, 10^{-5} M and 10^{-6} M and assigned to ring 2 stretching and C_7H_2 wagging, which appear neither in the solid nor in the solution and it is noted that the intensity of ring vibrations increases in the little concentrations; and the peak at 1320 cm^{-1} (only in SERS 10^{-6} M) corresponding to ring 1 stretching.

Also the weak peak at 1180 cm^{-1}, in SERS 10^{-3} M, is observed at 1196 cm^{-1}, in SERS 10^{-5} M, and at 1186 and 1175 cm^{-1} in SERS 10^{-6} M, corresponding to CH rock (ring 1) and C_7H_2 twist also is in solid and in solution at 1192 peak. The peak at 1085 cm^{-1} corresponding to ring 1 breathing is only observed in SERS; the peak at 1071 cm^{-1}, also corresponding to ring 1 breathing, is not observed at little concentrations of SERS, and the intensity of the strong peak at 1033 cm^{-1}, already cited, that corresponds to ring 2 breathing, is decreasing until see a weak peak at the concentration 10^{-6} M. The strong peak at 442 cm^{-1}, in SERS 10^{-3} and medium in SERS 10^{-4} M, which corresponds to ring 2 out of plane deformation and it does not appear in solid or dissolution. We can deduce that the interaction with the metal is not the same in the two rings.

Other peaks confirm this behavior: The shoulder at 944 cm^{-1} (ring 2) only appears in SERS at concentration 10^{-3} M. This behavior indicates that the two aromatic rings have a different interaction with the metal.

Also it is observed that the weak peak at 794 cm^{-1}, corresponding to C-Cl vibrations, at concentration 10^{-5} M and the double at 799 and 782 cm^{-1} at 10^{-6} M concentration and which corresponds to the same vibrations, are not observed in solid and in solution 0.1 M. Thus we can think that one Cl atom, that is aligned one-self with the -COO- and -NH_2 groups, interacts with the metal.

In Figure 4 it is showed the spectra of diclofenac in methanol and on Ag colloid, at concentration 10^{-3} M and at different pH values and the largest intensity corresponds to pH=12 with a large difference.

Carboxylate group presents some variations in SERS in respect of the solid: The peak at 1395 cm^{-1} in SERS is not observed in solid and in solution; the peak at 640 cm^{-1} in solid, in SERS is observed at 661 cm^{-1}. There are some differences in C_7H_2 group between the solid and the solution with respect to SERS: The peak at 1369 cm^{-1} corresponding at C_7H_2 wagging, appears only in SERS; the peaks in the solid and in the solution at 1313 cm^{-1} and 1307 cm^{-1} respective, corresponding to C_7H_2 wagging, in SERS appear only at 10^{-6} M; the peaks at 1291 and 1300 cm^{-1}, corresponding to C_7H_2 wagging, only appear in SERS; the peak in the solid at 640 cm^{-1}, corresponding to C_7H_2 deformation, appears in SERS about 661/666 in SERS; the peak at 401 cm^{-1} only is showed in SERS.

The peaks in SERS at 1655/1658 cm^{-1}, 1617/1620 cm^{-1} corresponding to amines (NH_2 scissoring) only appear in SERS. All these differences show the interaction of the colloid with the groups studied.

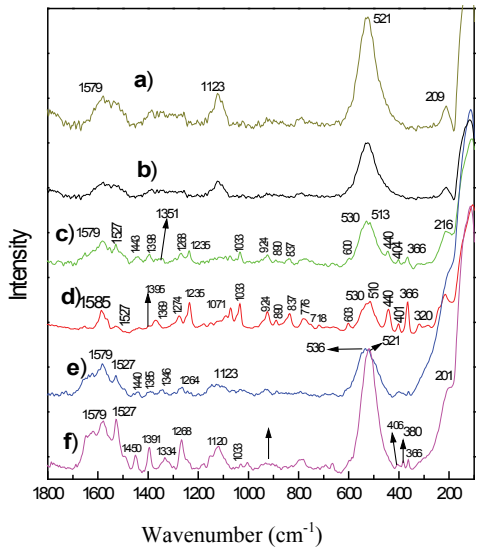

Figure 3: SERS of diclofenac (in methanol) on Ag colloid (pH=12): a) 10^{-3} M+Na_2SO_4 10^{-2} M (X2); b) 10^{-6} M+Na_2SO_4 10^{-2} M; c) 10^{-4} M+KNO_3 10^{-2} M; d) 10^{-3} M+KNO_3 10^{-2} M; e) 10^{-5} M+KNO_3 10^{-2} M (X4); f) 10^{-6} M+KNO_3 10^{-2} M (X3).

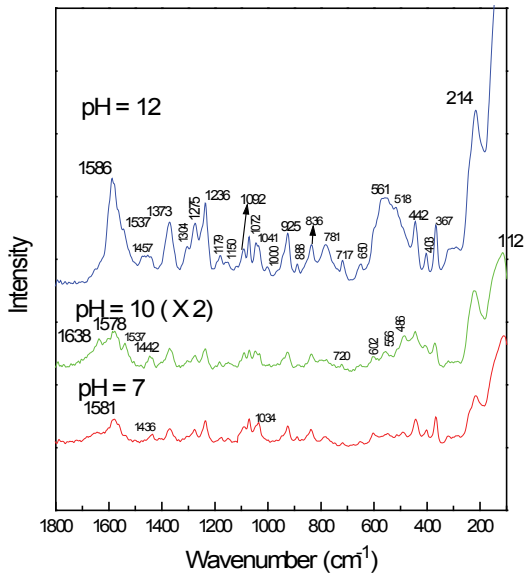

Figure 4: SERS of diclofenac (in methanol) 10^{-3} M on Ag colloid+KNO_3 10^{-2} M at different pH.

Of what was written above is deduced: The presence of silver colloid alters the spectrum of this molecule due mainly to the interaction between this molecule and the silver of the colloid: There is interaction between the molecule and the metal mainly on the rings of diclofenac and every ring interacts differently. Other changes in the spectrum indicate the interaction of carboxylate and Cl atom with the metal. The colloid interacts with the amine groups and C_7H_2.

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