

The development of DFT has provided an alternative means of including electron correlation in the study of the vibrational wavenumbers of moderately large molecules (Hutter et al. 1994, Barone et al. 1995). The DFT hybrid B3LYP functional tends also to overestimate the fundamental modes in comparison to the BPW91 method, therefore scaling factors have to be used for obtaining a considerable better agreement with the experimental data (Scott and Radom 1996, Wong 1996). Thus, according to the work of Rauhut and Pulay (Rauhut and Pulay 1995), a scaling factor of 0.963 has been uniformly applied to the B3LYP calculated wavenumber values from Table 4.2. The observed disagreement between the theory and experiment could be a consequence of the anharmonicity and of the general tendency of the quantum chemical methods to overestimate the force constants at the exact equilibrium geometry (Scott and Radom 1996). Nevertheless, as one can see from Table 4.2, the theoretical calculations reproduce well the experimental data and allow the assignment of the vibrational modes. The calculated Raman intensities presented in Fig. 4.4 are also in good agreement with the experimental data.

As one can see from Fig. 4.4, the dominant bands of the FT-Raman spectrum of polycrystalline DCFNa appear at 1605 (calc. 1615 cm^{-1}), 1585 (calc. 1588 cm^{-1}), and 1578 cm^{-1} (calc. 1572 cm^{-1}) and are given by phenyl ring stretching vibrations and the asymmetric OCO stretching modes, respectively. The ring breathing vibrations determine also intense bands at 1073 (calc. 1081 cm^{-1}) and 1046 cm^{-1} (calc. 1051 cm^{-1}) (see Table 4.2). The in-plane deformation vibrations of the CH groups of both rings give rise to Raman bands at 1160 (calc. 1167 cm^{-1}) and 1150 cm^{-1} (calc. 1149 cm^{-1}) (bending vibrations) and 1281 (calc. 1284 cm^{-1}), 1250 (calc. 1269 cm^{-1}) and 1235 cm^{-1} (calc. 1243 cm^{-1}) (rocking vibrations). The medium intense Raman bands at 517 (calc. 524 cm^{-1}) and 533 cm^{-1} (calc. 537 cm^{-1}) are determined by the out-of-plane deformation vibrations of the phenyl rings. The bands attributed to the out-of-plane deformation vibrations of the CH groups occur in the 840–950 cm^{-1} spectral range of the Raman spectrum. In the high wavenumber region between 3069 and 2890 cm^{-1} six bands assigned to the NH and CH stretching modes are observed. Weak bands at 1398 (calc. 1385 cm^{-1}) and 637 cm^{-1} (calc. 630 cm^{-1}) assigned to the symmetric stretching and in-plane deformation vibration of the carboxylate group can be also seen in the Raman spectrum. The absence of the carbonyl stretching band in the 1800–1600 cm^{-1} spectral range confirms the presence of the carboxylate group in the DCFNa species in the solid state (Iliescu et al. 2004a, Iliescu et al. 2003).

4.1.2 Adsorption on the Silver Surface

SERS spectra of diclofenac on the silver colloid at different pH values, together with the Raman spectrum of the polycrystalline sample, are presented in Fig. 4.5. The assignment of the vibrational modes of DCFNa to the SERS bands at different pH values is summarized in Table 4.3.

Having in view that for recording the SERS spectra, a DCFNa ethanol solution has been employed, and that in the solution the sodium atom is dissociated, we will further discuss the adsorption behavior of the diclofenac anion, which will be denoted as DCF.

As one can see from Fig. 4.5, good SERS spectra were obtained in acidic and neutral medium, while at alkaline pHs the spectra presented very broad bands. The shift in the peak position and the change in the relative intensities of the SERS bands with respect to the corresponding Raman bands, indicates a chemisorption process on the silver surface (Iliescu et al. 2004a, Iliescu et al. 2003).

By looking at the SERS spectra one can notice that the C=O stretching mode is absent in all spectra. The lack of this band evidences the presence of the carboxylate group not only in the solid state but also in the DCF adsorbed state.

Arancibia and Escadar (Arancibia and Escadar 1999) have determined from potentiometric and spectrophotometric measurements the pK_a value for the carboxylic group in DCF ($pK_a=4.9$). Another deprotonation reaction of DCF was detected at a very acidic pH, with a pK_a value of 1.7. This value was attributed to the deprotonation of RNH_2^+ probably present in a strong acidic solution.

Taking into account the pK_a value of 4.9, an excess of DCF molecules with a carboxylic group (protonated form) is expected to be present at $pH=2$. As one

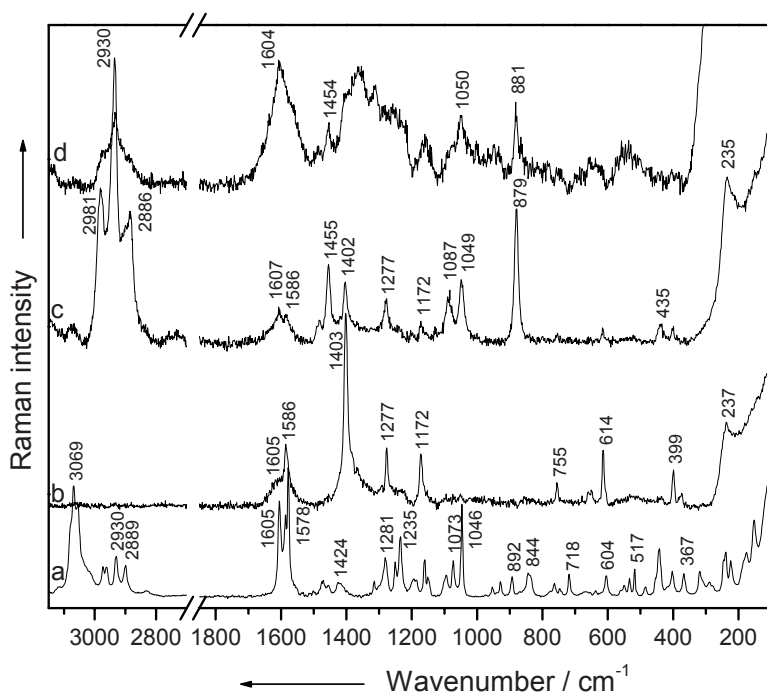


Fig. 4.5 FT-Raman spectrum of polycrystalline DCFNa (a) and SERS spectra of DCF on silver colloid at the pH values of 2 (b), 6 (c), and 10 (d). Reprinted from Chem. Phys. 298, Iliescu T, Baia M, Kiefer W, FT-Raman, surface-enhanced Raman spectroscopy and theoretical investigations of diclofenac sodium, 167–174, copyright 2004 with permission from Elsevier

can see from Fig. 4.5b the C=O stretching band typical for the carboxylic group is missing in the SERS spectrum at this pH value. The absence of this band could be due to the lowering of the pK_a value at the silver surface, and indicates the existence of a direct carboxylate-surface interaction. The high intensity of the symmetric and asymmetric COO^- stretching bands present in the SERS spectrum at 1403 and 1586 cm^{-1} is a proof of the existence of the carboxylate group in the DCF adsorbed state and of its proximity to the silver surface. In the SERS spectrum at $pH=2$ weak bands are also present at 647 and 399 cm^{-1} that contain contributions of the in-plane COO^- deformation vibrations (see Table 4.3).

Table 4.3 Wavenumbers (in cm^{-1}) and assignment of the vibrational modes of DCFNa to the SERS bands at different pH values

Raman	SERS			Vibrational assignment
	pH 2	pH 6	pH 10	
–	237 s	235 s	–	AgCl + AgO stretch
367 m	373 w	–	–	C_1NC_1' def + $C_{6,7,8}$ def + CCl def
402 m	399 m	399 m	–	$O_1C_8O_2$ def + $Cl_1C_{2,3}$, $Cl_2C_{6,5}$ def + C_7H_2 def
442 ms	–	435 w	–	Ring 2 out-of-plane def
533 mw	–	–	537 br	Ring 2 + 1 out-of-plane def
604 m	614 m	616 w	–	Ring 1 + 2 in-plane def
637 w	647 w	–	649 wbr	$O_1C_8O_2$ def + C_7H_2 def
653 w	651 w	–	649 wbr	C_1NC_1' def + ring 1 out-of-plane def
747 vw	755 w	754 vw	–	CH wag (ring 2 + 1)
868 w	–	879 vs	882 s	CH twist (ring 2 + 1)
1046 s	–	1049 s	1050 s	Ring 2 breathing
1073 ms	–	1087 ms	–	Ring 1 breathing
1160 ms	1172 m	1172 w	1155 mbr	CH bend (ring 1 + 2)
1281 ms	1277 m	1277 m	–	CH rock (ring 1 + 2) + C_7H_2 wag
1327 vw	–	–	1316 m	Ring 1 stretch + C_7H_2 wag
1398 vw	1403 vs	1402 m	1392 br	$C_{7,8}$ stretch + $O_1C_8O_2$ s. stretch
1454 m	–	1455 s	1454 mw	C_1N stretch + CH rock (ring 1)
1470 sh	–	1480 w	1484 w	C_1N stretch + CH rock (ring 2)
1578 vs	1586 ms	1586 m	–	$O_1C_8O_2$ as. stretch
1605 vs	1606 sh	1607 m	1604 sbr	Ring 1 + 2 stretch
2890 mw	–	2886 s	2888 sh	CH stretch (C_7)
2930 m	–	2934 vs	2931 s	CH stretch (ring 2)
2973 w	–	2981	2970 sh	CH stretch (ring 1)
3069 s	–	–	3062 wbr	NH stretch

Abbreviations: w = weak, m = medium, s = strong, v = very, sh = shoulder, br = broad, stretch = stretching, bend = bending, def = deformation, wag = wagging, rock = rocking, twist = twisting, ring 1 = phenyl ring with Cl atoms, ring 2 = phenyl ring.

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Many molecular species, which possess a carboxyl group, can be bonded on the metallic surface as carboxylate groups. Kwon et al. (Kwon et al. 1994a) concluded from SERS spectra of 4-(methylthio)-benzoic acid that the symmetric stretching band of the COO^- group appeared very distinctly with a broad bandwidth, when this molecular species was bonded to the silver surface via the π system of the carboxylate group. Its peak position in the adsorbed state was shifted to lower wavenumbers by as much as 6 cm^{-1} compared to that of the free molecule. The asymmetric stretching mode of the carboxylate group was barely discernible in the SERS spectra, when adsorption occurred via the π system. Park et al. (Park et al. 1990) reported the adsorption of a 4-amino-benzoic acid on the silver surface via the π system of the carboxylate group. They observed a red shift of the symmetric stretching mode of the carboxylate group by approximately 10 cm^{-1} , compared to that of the free molecule and a very distinct SERS intensity. In contrast, the asymmetric stretching mode became weaker upon π coordination without a noticeable peak shift. On the other hand, a blue shift of the symmetric COO^- stretching mode upon surface adsorption was observed in the SERS spectrum of benzoic acid on silver electrode surface (Kwon et al. 1994b). This behavior was interpreted as a consequence of the coordination of the carboxylate group to the silver surface via its oxygen lone pair electrons.

By inspecting Fig. 4.5 and Table 4.3, blue shifts by 5 and 8 cm^{-1} of the symmetric and asymmetric COO^- stretching bands were observed in the SERS spectrum of the DCF molecule in an acidic medium in comparison with the corresponding bands from the Raman spectrum, which confirm (Iliescu et al. 2004a, Iliescu et al. 2003) the binding of this molecular species on the silver surface via oxygen lone pair electrons of the carboxylate group. According to the surface selection rules for Raman scattering (Creighton 1983, Moskovits and DiLella 1980), the vibration of the adsorbed molecules, which has a polarizability tensor component normal to the surface, will be preferentially enhanced. Stretching vibrations are assumed to have a large component of the polarizability along the bond axis. The very high intensity of the symmetric and asymmetric stretching bands of the COO^- group observed in the SERS spectrum of DCF at $\text{pH}=2$ indicates the perpendicular or at least tilted orientation of this group with respect to the silver surface.

It is known (Iliescu et al. 1995) that molecules with a nitrogen ring atom can form a pair with the chloride ion and this pair is bonded to the silver surface. By looking at the SERS spectrum recorded at a pH value of 2 one can infer that the carboxylate group of the DCF is directly bound to the silver surface, otherwise a strong change in the peak position of the AgCl stretching mode would occur.

The vibrations specific to phenyl rings are also present in the SERS spectrum in an acidic medium. The stretching vibration of both rings gives rise to a broad shoulder at 1606 cm^{-1} . The in-plane CH and rings deformation vibrations were observed in the SERS spectrum at 1277, 1172, and 614 cm^{-1} (see Table 4.3). The shifts of these bands compared to their corresponding Raman bands confirm the interaction between phenyl rings and the silver surface. The out-of-plane deformation vibrations of CH groups of both rings present in the Raman spectrum in the spectral range between 850 and 950 cm^{-1} are not present in the SERS spectrum at

the pH value of 2. If one closely examines the conformation of the DCF molecule (Fig. 4.2) and the enhancement of the in-plane vibrations of phenyl rings, one can assume a tilted close to flat orientation of these rings with respect to the silver surface (Fig. 4.6a) (Iliescu et al. 2004a). According to the surface selection rules (Creighton 1983, Moskovits and DiLella 1980) one would expect the CH ring stretching modes to be present in the adsorbed state of DCF molecules with weak intensity. The absence of these bands in the SERS spectrum can be explained by the marginal contribution of these modes to the α_{zz} polarizability component (z being the axis perpendicular to the surface). A similar situation was found for the adsorbed phthalazine (Moskovits and Suh 1984), where CH stretching modes are very weak, even though the molecule stands up on the surface. A deformation of the DCF molecule in an adsorbed state could also occur.

SERS spectra recorded at close to neutral and alkaline pH values (Fig. 4.5c and d) show new bands in comparison to the spectrum obtained in an acidic environment. Very intense bands are developed in the high wavenumber region around 2900 cm^{-1} . The peak at 1607 cm^{-1} that appears as a shoulder in the spectrum recorded at the pH value of 2, became, in the SERS spectrum at pH=6, even more intense than the band at 1586 cm^{-1} . New peaks are also developed at 1480 , 1455 , 1087 , 1049 , 879 , and 435 cm^{-1} . These bands arise also in the SERS spectrum at the pH value of 10 but with a broader shape, probably determined by different adsorption sites in an alkaline medium. By considering the pK_a value for the carboxyl group ($\text{pK}_a=4.9$), one supposes that the carboxylate form is present in both neutral and alkaline environments, therefore it will be further analyzed only the SERS spectrum at the pH value of 6.

The appearance of new bands in the SERS spectrum at pH=6 indicates the change in orientation of the adsorbed DCF molecule with respect to the silver surface. The bands attributed to the symmetric and asymmetric stretching modes

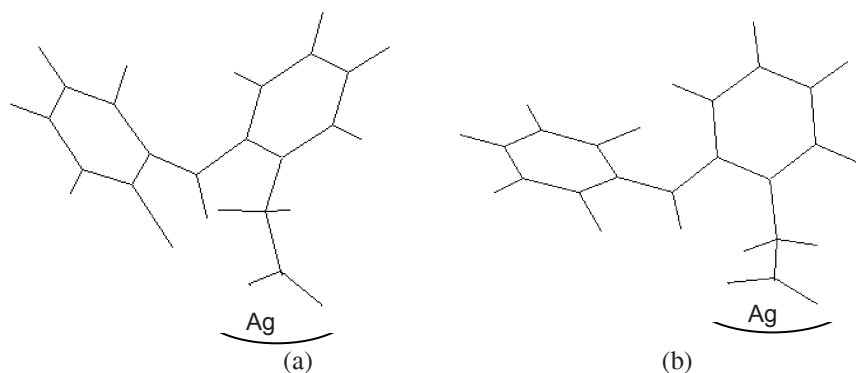


Fig. 4.6. Schematic model for the adsorption geometry of diclofenac on a colloidal silver surface at pH values (a) below 6 and (b) pH values above 6. Reprinted from Chem. Phys. 298, Iliescu T, Baia M, Kiefer W, FT-Raman, surface-enhanced Raman spectroscopy and theoretical investigations of diclofenac sodium, 167–174, copyright 2004 with permission from Elsevier

of the carboxylate group appear at 1586 and 1402 cm^{-1} in the SERS spectrum at this pH value, but with lower intensities compared to those observed in the SERS spectrum at pH=2 (see Figs. 4.5*b* and *c*). This feature of the above-mentioned bands is a proof that the interaction between the carboxylate group and the silver surface via lone pair electrons of the oxygen atom exists also in neutral and alkaline environments, but it is weaker than in an acidic medium (Iliescu et al. 2004a, Iliescu et al. 2003).

The bands at 1049 and 1087 cm^{-1} due to the ring breathing vibrations are intense and broadened in the SERS spectrum at pH=6 compared to the Raman spectrum. The band assigned to the C_1N stretching vibration appears at almost the same position as in the Raman spectrum (1454 cm^{-1}), but its intensity is substantially increased. The behavior of this band can be considered as an evidence of the large distance between this group and the metal surface. The $\text{C}'_1\text{N}$ stretching band is also observed in the SERS spectrum at 1480 cm^{-1} , but with a low intensity. The presence in the SERS spectrum of the very intense bands specific to the CH ring stretching modes (around 2950 cm^{-1}), and the breathing (1049 and 1087 cm^{-1}) and stretching (1607 cm^{-1}) vibrations of the phenyl rings shows that a more perpendicular orientation of phenyl rings with respect to the silver surface exists at the pH value of 6, as compared with that taken from the adsorbed molecule in an acidic medium (see Fig. 4.6*b*) (Iliescu et al. 2004a).

4.1.3 Conclusions

Ab initio and DFT calculations have been performed at the RHF/6-31G*, BPW91/6-31G*, and B3LYP/6-31G* levels of theory on the two most probable conformers of DCFNa. The conformer with the sodium atom closer to the phenyl ring substituted by chlorine atoms was found to be energetically more stable by an energy difference of approximately 18 kJ/mol (RHF), 30 kJ/mol (BPW91), and 27 kJ/mol (B3LYP), respectively. Optimized structural parameters of the DCFNa calculated by various theoretical methods agree well with experimental X-ray diffraction values. The assignment of the vibrational modes was also accomplished, and a good agreement was obtained between the theoretical vibrational wavenumbers and the experimental FT-Raman data.

Good SERS spectra were obtained in acidic and neutral environments, indicating the chemisorption of the DCF molecule on the silver surface. At all studied pH values, the DCF molecule was bonded to the silver surface through the lone pair oxygen electrons of the carboxylate group, which has a perpendicular or slightly tilted orientation with respect to the silver surface. By analyzing SERS spectra at different pH values, a change of the phenyl rings' orientation with respect to the metal surface from a tilted close to flat to a more perpendicular one was concluded.

4.2 Diclofenac Sodium – β -Cyclodextrin Complex

The elucidation of the adsorption behavior of the free diclofenac molecule can be considered the starting point in the characterization of the adsorption of the DCFNa- β CD complex on the silver surface. Having in view that different parts of the DCFNa molecule can be included into the β CD cavity, the results of the investigations carried out with the SERS technique could provide insights about the complexation way.

Thus, the purpose of this work was to examine the interaction between the DCFNa and β CD in the solid state complex by using Raman spectroscopy (reprinted from Eur. J. Pharma. Sci. 22, Iliescu T, Baia M, Miclaus V, A Raman spectroscopic study of the diclofenac sodium- β -cyclodextrin interaction, 487–495, copyright 2004 with permission from Elsevier). The support of this study was the existence of some spectral ranges, where the Raman bands associated to atom group vibrations directly involved in the interaction are not overlapped. Having in mind that previous studies reported about the possibility to detect inclusional complexation by cyclodextrins at the surface of a metal (Maeda and Kitano 1995, Hill et al. 1999) SERS spectra of the DCFNa- β CD inclusion complex were also recorded and analyzed in an attempt to elucidate the adsorption behavior of the guest-host complex on the silver surface, and thus to discriminate between the possible ways of complexation. The analysis of the SERS spectra of the guest-host complex was based on the results obtained for the free DCFNa molecule.

4.2.1 Vibrational Analysis

Figure 4.7 shows the Raman spectra of the β CD, DCFNa, their guest-host complex and the 1 : 1 DCFNa- β CD physical mixture in the spectral range between 200 and 3300 cm^{-1} and the assignment of the main Raman bands of the guest molecule is summarized in Table 4.4.

By comparing the spectra illustrated in this figure one can see that the Raman spectrum of the 1 : 1 physical mixture closely resembles the sum of the individual spectra of the guest and host molecules. Furthermore, one can notice the absence of the Raman bands given by the β CD molecule vibrations in the following spectral ranges: 3050–3150, 1500–1650, 1220–1300, and 1000–1100 cm^{-1} . These spectral regions will be analyzed in detail in order to evidence the changes caused by the guest-host interaction concerning the positions and widths of the DCFNa Raman bands (Iliescu et al. 2004b, Iliescu et al. 2004c).

The Raman spectra of the β CD, DCFNa, the inclusion complex, and the 1 : 1 DCFNa- β CD physical mixture in the spectral range between 1500 and 1650 cm^{-1} are illustrated in Fig. 4.8. In this spectral region, three bands can be seen in the Raman spectrum of the DCFNa molecule.

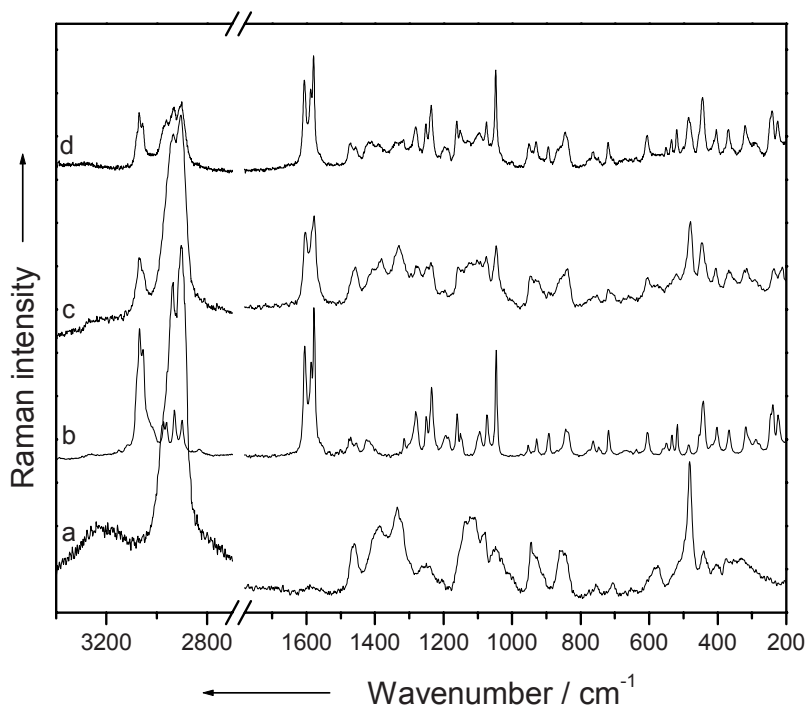


Fig. 4.7 FT-Raman spectra of β CD (a), DCFNa (b), 1:1 DCFNa- β CD complex (c), 1:1 DCFNa- β CD physical mixture (d). Reprinted from Eur. J. Pharma. Sci. 22, Iliescu T, Baia M, Miclaus V, A Raman spectroscopic study of the diclofenac sodium- β -cyclodextrin interaction, 487–495, copyright 2004 with permission from Elsevier

Thus, the band at 1578 cm^{-1} was assigned to the $\text{O}_1\text{C}_8\text{O}_2$ asymmetric stretching vibration (see Fig. 4.1), while the bands at 1585 and 1605 cm^{-1} were attributed to dichlorophenyl and phenylacetate rings stretching vibrations (see Table 4.4). As can be observed, the corresponding Raman bands of the inclusion complex are broader and their peak positions are changed in comparison to the bands of the pure DCFNa and indicate the existence of the guest-host interactions (Iliescu et al. 2004b, Iliescu et al. 2004c). One should emphasize the similitude of these Raman bands in the spectra of the pure DCFNa and 1:1 DCFNa- β CD physical mixture.

In order to separate the unresolved bands present in this spectral region into several components, the curve-fitting technique was used. In order to fit as realistically as possible, the component Raman bands were approximated by Lorentz functions, and a minimum number of bands corresponding to the number of distinct features observed in the experimental spectrum such as resolved maxima and well-developed shoulders was used. The deconvolutions of the bands corresponding to the pure DCFNa molecule and the DCFNa- β CD complex are shown in Figs. 4.9a and b, and the deconvolution data are given in the insert of each figure.

Table 4.4 Selected FT-Raman and SERS bands of DCFNa- β CD complex with their vibrational assignment. The Raman bands of the pure DCFNa are also presented

FT-Raman			SERS		Vibrational assignment ¹
DCFNa	DCFNa- β CD	pH 2	pH 6	pH 10	
–	–	216 sh	216 sh	218 vs	AgO stretch +
223 m	220 m	221 sh	222 sh	223 sh	ring 2 out-of-plane def
239 m	236 m	233 sh	233 ms	233 sh	CONa def + C ₇ H ₂ rock +
–	–	246 s	240 sh	240 sh	AgCl stretch
–	–	381 m	–	–	O ₁ C ₈ O ₂ def + Cl ₁ C _{2,3} , Cl ₂ C _{6,5}
402 m	404 w	407 m	–	–	def + C ₇ H ₂ def
637 w	–	622 m	–	–	O ₁ C ₈ O ₂ def + C ₇ H ₂ def
653 w	658 vw	657 w	668 w	673 w	C ₁ NC _{1'} def + ring 1 out-of-plane def
844 m	838 ms	–	886 s	883 s	CH twist (rings)
892 m	893 w	–	–	–	
1046 s	1046 s	–	1058 w	1052 w	Ring 2 breathing
1073 ms	1075 ms	–	–	–	Ring 1 breathing
1094 m	1092 m	–	1093 w	1089 w	C ₇ H ₂ wag + CH bend (rings)
1150 m	–	–	–	–	CH bend (rings)
1160 ms	1158 m	1180 m	–	–	
1281 ms	1280 m	1284 m	1286 w	1281 w	CH rock (rings) + C ₇ H ₂ wag
1398 vw	1405 sh	1407 s	–	–	C _{7,8} stretch + O ₁ C ₈ O ₂ s. stretch
1454 m	1456 ms	–	1461 m	1461 m	C ₁ N str + CH rock (ring 1)
1578 vs	1577 s	1577 sh	–	–	O ₁ C ₈ O ₂ as. stretch
1585 s	1584 sh	1590 m	–	–	Ring 1 stretch
1605 s	1603 s	1613 sh	1609 mw	1609 mw	Ring 2 stretch
2829 m	–	–	2897 m	2897 m	CH stretch (C ₇)
2900 m	2904 vs	–	–	–	
2929 m	2933 s	–	2939 s	2937 s	CH stretch (ring 2)
2961 m	–	–	–	–	
2973 m	–	–	2988 m	2986 m	
3054 s	3054 sh	–	–	–	
3069 s	3070 m	–	–	–	CH stretch (ring 1)
3080 sh	3077 sh	–	–	–	

Abbreviations: w = weak, m = medium, s = strong, v = very, sh = shoulder, stretch = stretching, def = deformation, rock = rocking, twist = twisting, wag = wagging, bend = bending, ring 1 = dichlorophenyl ring, ring 2 = phenylacetate ring, ¹ Ref. (Iliescu et al. 2004a)
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