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Dedicated to Prof. Ioan-Iovitz Popescu's 75th Anniversary

BIOPHYSICS. MEDICAL PHYSICS. ENVIRONMENTAL PHYSICS

RAMAN AND SURFACE ENHANCED RAMAN SPECTROSCOPY ON MOLECULES OF PHARMACEUTICAL AND BIOLOGICAL INTEREST

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Abstract. The molecules with antibacterial properties like, acridine derivatives, rivanol, furan derivatives, potassium benzylpenicillin, tranquilizers like, phenothiazine derivatives, anti-inflamatorry drugs like, diclofenac sodium and its complex with beta-cyclodextrin and some non natural β-amino acids were studied by theoretical (DFT) and experimental (Raman and Surface Enhanced Raman Spectroscopy (SERS)) point of view in order to have information about vibrational frequencies their assignment and the structure of the adsorbed molecular species and orientation of the molecules on metallic surface. These studies lead to a better understanding of their biological activity on different physico-chemical conditions.

Key words: Raman, SERS, pharmaceutical products.

1. INTRODUCTION

The observation of greatly enhanced Raman intensities for molecules after adsorption on certain rouged metal (silver, gold, copper) surface, with roughness of the nanometer size, has been both an experimentally as well as theoretically interesting and intriguing phenomenon, known as surface-enhanced Raman scattering (SERS), since its discovery in 1974 by Fleischmann *et al.* [1]. The intensity observed in SERS usually exceeds by a factor of 10^5–10^6 and, in special cases by 10^{12}–10^{14} 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). Due to high intensity of the Raman signal SERS is a very useful technique in surface science, electrochemistry and analytical

chemistry [2–6]. Because of the fluorescence suppression, this non-radiative transfer being determined by metal surface roughness, this technique is widely employed for molecules of biological interest that present this phenomenon [7, 8].

Unfortunately, a full theoretical and quantitative understanding of the SERS mechanisms is lacking even after more than three decades of investigation. However, the enhancement of the Raman scattering cross-section of molecules adsorbed on rough metal surfaces has been attributed to two major effects [5, 9]. The main and foremost contribution to SERS comes from the enhancement of electromagnetic field close to the surface through interaction with surface plasmon excitation. The local electromagnetic field depends on the microscopic shape of the metallic surface, such as the presence of a sharp edge. Metal colloids and colloidal aggregates provide a particularly rich example of such local electromagnetic enhancement. Several authors [10–12] suggest that protrusions on the surface of a colloidal particles, as well as "cavities" between adjacent particles in an aggregate, lead to a giant enhancement of the local field, up to a factor of 10^{14}–10^{15}.

The second contribution to the enhancement of the Raman scattering is a specific interaction of the adsorbed molecule with the metal surface [9]. In some instances, this molecular chemisorption effect leads to a charge-transfer interaction, whereby an electron is transferred from the molecule into the empty levels of the metal or from occupied metal levels to the molecule. This electron transfer can be regarded as an electron excitation of the coupled molecule-surface system (complex) and should therefore lead to the appearance of a new band in the electronic spectrum of the molecule, excitation light being in resonance with this new band. The charge transfer mechanism is restricted by its nature to molecules directly adsorbed on the metal, as opposed to the electromagnetic effect, which extends a certain distance beyond the surface.

The recent progress in the SERS field can be summarized in three parts: SERS substrate, technique, theory and application.

Several procedures have been proposed for preparing suitable SERS active supports. Noble metal (Ag, Au) colloids have been widely employed in SERS, since the aggregation of metal particles leads to the formation of aggregates with roughness and fractal morphology necessary to render intense Raman spectra [13, 14]. Among the methods employed to obtain metal colloids, the chemical reduction of silver nitrate with citrate [15] produces a more uniform distribution of particle sizes. It was found [16] that the SERS intensity increases as the size of the Ag particles becomes larger (average diameter about 50 nm). However, a further increase of the particle size (100–130 nm) leads to lower SERS intensity signal. Thus, one of the advantages of metal colloids is the possibility to control and modify the particle size and shape by choosing adequate experimental conditions [17].

One possible application of the silver colloid deposits in bacteria is the obtaining of intense SERS spectra [18] to probe the immediate biochemical

environment near the metal cores. SERS spectra obtained in this way are particularly sensitive to one specific component from the cell wall that is riboflavin (or flavinadenine dinucleotide), which is a co-factor of major importance in a variety of live-sustaining processes in living cell.

Recently there has been a renewed interest in the enhancement of isolate nanoparticles deposited on planar surfaces [19, 20]. Oldenberg *et al.* [21] obtained a high enhancement factor in SERS by using gold nanoshell as a support. A gold nanoshell is a composite nanoparticle consisting of a dielectric core coated by a thin metal shell, its peak plasmon resonance wavelength being determined by the ratio of the core diameter to the shell thickness.

Lithography technique can be exploited to fabricate topographically predictable SERS substrates. In this case, a colloidal crystal monolayer of size-monodisperse nanospheres is grown on a flat substrate, and then the desired nanoparticle material is thermally evaporated through the nanosphere mask. Upon removing the nanospheres from the surface, homogenous arrays of truncated tetrahedral nanoparticles remain on the surface [22].

Nanostructure preparation via self-assembly rout offer a new possibility as SERS support with sufficient stable building blocks which have to be well-characterized and uniform in size and shape [23].

The occurrence of giant SERS cross section on colloids facilitated the measurement of SER spectra of single molecule (SM) [24, 25]. Probably SM SERS will offer new possibility to comprehend the mechanisms of enhancement in SERS. Kneipp *et al.* [24] have reported the detection of single molecule of crystal violet adsorbed on aggregated cluster of colloidal silver. They used near-infrared excitation which is not resonant with any intramolecular optical transitions of dye but efficiently excite the plasmons of the fractal aggregated silver colloids. Nie and Emory [25] combined surface and resonance enhancement (SERRS) to produce the requirement sensitivity to detect rhodamine 6G molecule adsorbed on the surface of a single particles. A small number of these particles showed extraordinary high enhancements and they have been labelled “hot” particles. Information from SM can be obtained also from fluorescence spectra but SM SERS offer, by its vibrational aspects, more details about structure of adsorbed molecule. SM spectroscopy illustrates high potential of SERS, not only for its high sensitivity, but also its capability of characterizing the molecule orientation and dynamics.

In many cases is very important to know the orientation of the adsorbed molecule with respect to the metal surface. Surface selection rules for Raman scattering allow us to obtain such information [26, 27]. According to these rules, the vibrations of the adsorbed molecules, which have the polarizability tensor component normal to the surface, will be preferentially enhanced.

In this work, we present a few of our recent results concerning SERS investigations on molecules of pharmaceutical and biological interest. The following question appears: Way SERS on pharmaceutical and biological relevant

molecules? From pharmacological studies it is known that each drug is specific to a certain human organ in which it is adsorbed on some special centers. As a mimic of this adsorption process can be considered the adsorption of the molecules on a metal surface. In these investigations the silver surface serves as an artificial biological interface [28]. Moreover, for a complete understanding of the action of potential drugs, such as our derivatives, it is very important to know if the structure of the adsorbed molecules is the same as that of the free species. On the other hand, the analysis of the SER spectra of biological molecules like β-amino acids may lead to a better understanding of the interaction mechanism with other biological molecules and of their biological activity on different physico-chemical conditions.

2. RAMAN AND SERS STUDY

For all molecular species discussed in the present work SER spectra were recorded by using a Lee-Meisel [15] silver colloid as SERS support and employing visible laser lines for excitation. The SER spectra of the β-amino acids were obtained with the 1064 nm excitation line by using also a solid SERS substrate. Prior to the SERS analyses complete vibrational investigations of the molecules have been carried out from a theoretical (*ab initio* and density functional theory calculations (DFT) [29]) and experimental (IR and Raman spectroscopy) point of view. The DFT calculations allow to obtain the spatial configuration of the molecule, the frequencies value and their assignment.

2.1. MOLECULES WITH ANTIBACTERIAL PROPERTIES

2.1.1. Acridine derivatives

9-phenylacridine [30] and 9-methyl acridine [31], as well as most acridine derivatives, have antibacterial properties being used in the treatment of trichomonasis and protozoal diseases. Among the acridine derivatives we present here only SERS data for 9-methylacridine (9MA). Fig. 1A shows the SER spectra of 9MA at different pH values. At pH value lower than pK_a value of 3.8, acridine exists as 9 methylacridinium ion ($9MAH^+$) form while, in environments with pH higher than the pK_a value the neutral form of 9MA is present. These molecular species can be evidenced in SER spectra presented in Fig. 1A. Some of the bands specific to these molecular species can be observed in Fig. 1B. Thus the 1270, 1354 and 1582 cm^{-1} bands, which are present in the SER spectrum at pH value of 0.7, are assigned to the protonated $9MAH^+$ form, whereas the 1256, 1364 and 1562 cm^{-1} bands are determined by the neutral 9MA form. The neutral form is chemisorbed on silver surface *via* nitrogen lone pair electrons, while the $9MAH^+$

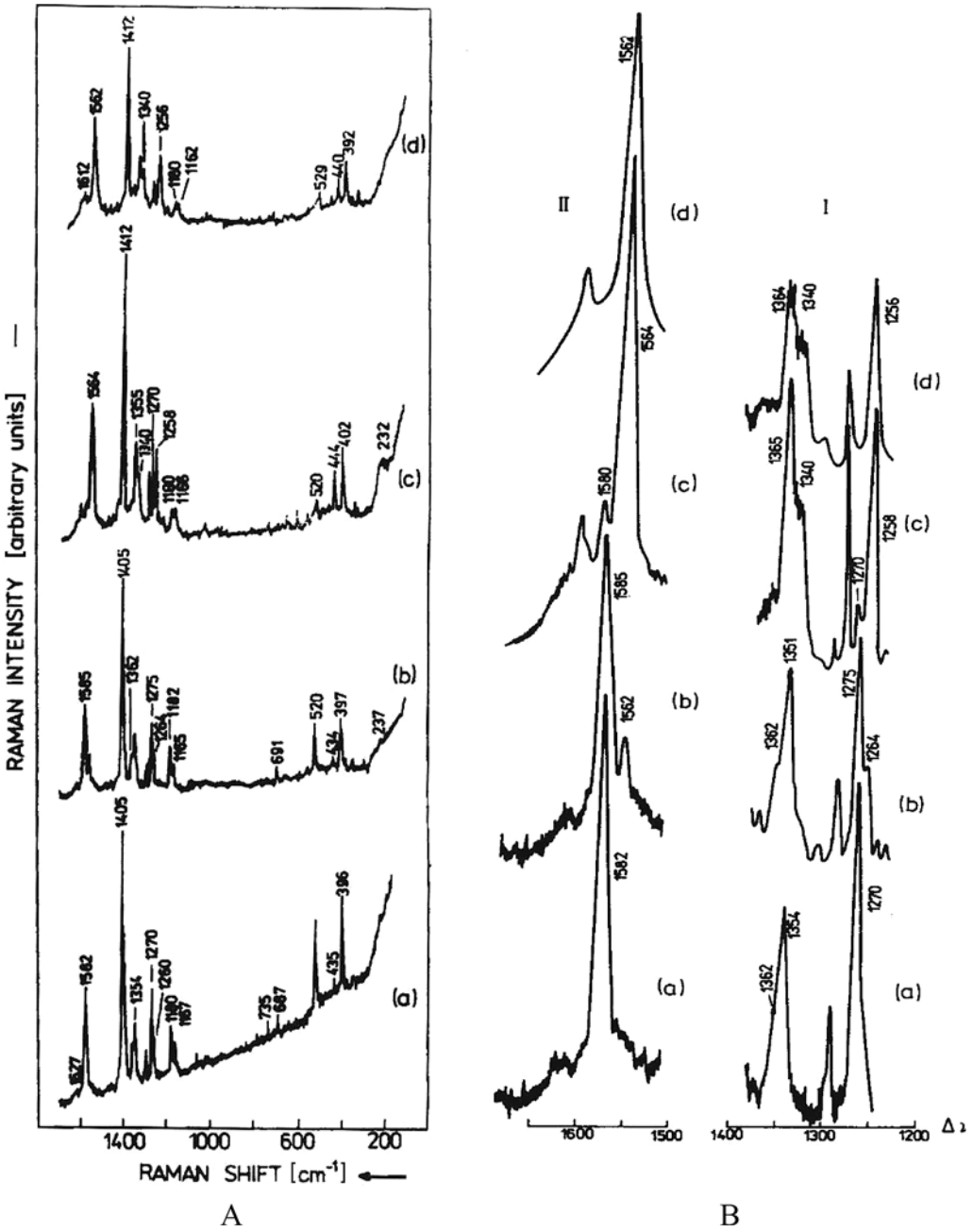

Fig. 1 – A) SER spectra of 7.5×10^{-4} M 9MA in silver sol solution containing 2.5×10^{-3} KCl at (a); pH 0.7; (b); pH 1.1; (c) pH 7.8; (d) pH 12.9. Laser output: 488 nm, 150 mW. B) Details of the spectral change showed by the SER spectra of 9MA due to pH variation. The concentration and pH values are the same as in Fig. 1A.

forme a pair with chloride ions that is adsorbed on the metal surface. According to selection rules for SER spectra [26, 27], both neutral and cationic forms were found to be perpendicular or at least tilted stance oriented with respect to the silver surface.

2.1.2. Rivanol

Rivanol is a potent antimicrobian agent that has been employed as an amebicide in the treatment of human dysentery and as bactericide in the treatment of bovine streptomastites. A special condition was necessary for the SERS study of the rivanol molecule [32]. Rivanol has three basic nitrogen atoms, and is capable of participating in three protolytic equilibrium involving four distinct species, neutral, monocation, dication and trication, depending of the solution pH value (Fig. 2). In order to have only one preponderant specie, the pH value of 5.5 was choused, when the concentration of monocationic specie is theoretically five orders of magnitude greater than that of dicationic or neutral species.

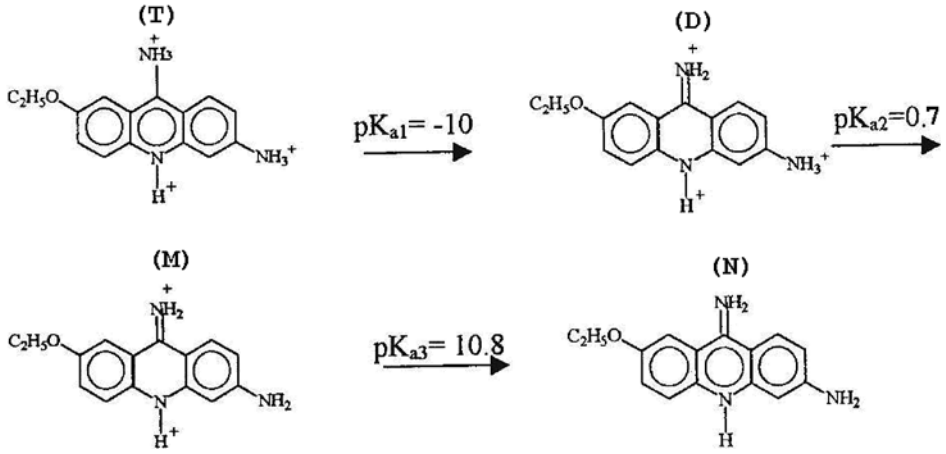

Fig. 2 – The protolitic equilibrium involving four species of rivanol: T, trication; D, dication; M, monocation; N, neutral.

There are two possibilities of molecule adsorption on the metal surface, namely physisorption and chemisorption. The spectrum of physisorbed molecule is practically the same as that of the free molecule, small changes being observed only for the bandwidths. This situation corresponds to a relatively larger distance between metal surface and adsorbed molecules. In chemisorption process the molecule is bonded to metal surface, a molecule-metal complex is formed, and the changes in SER spectra are dramatically. In solid state rivanol, only monocation specie is present because of the first possible protonation of the nitrogen ring atom, the lactic acid being weak. The comparison between SERS and Raman bands positions of rivanol (Fig. 3) shows a very good coincidence. Therefore, we conclude that there is a

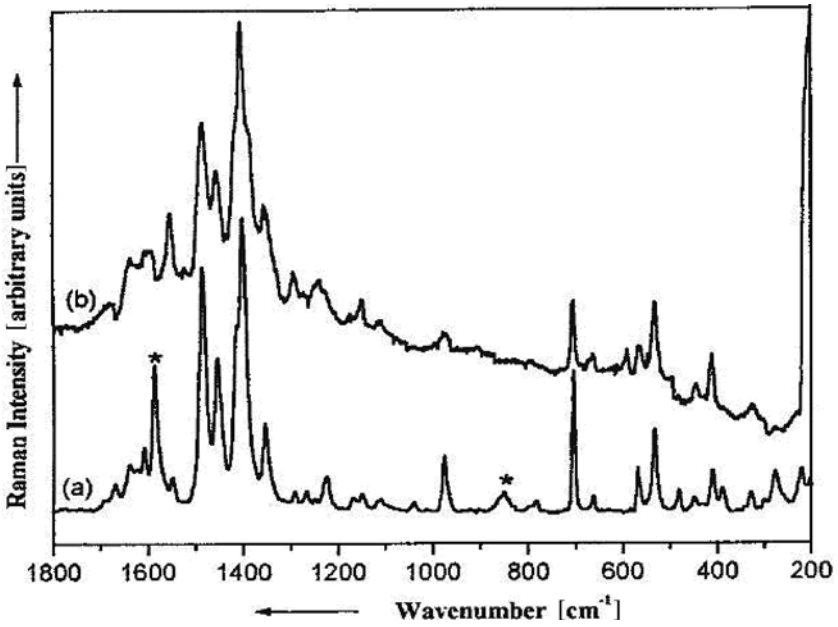

Fig. 3 – FT-Raman spectrum of rivanol solid state (a) and SER spectrum in silver sol at pH 5.5 (b). Laser output: 1064 nm, 200 mW (a), 514 nm, 250 mW (b). Asterisks denote lactate bands.

physisorption of rivanol monocationic species on the silver surface. As a consequence the electromagnetic mechanism is the main mechanism of the Raman enhancement.

2.1.3. Furan derivatives

2-formyl furan [33] is a very important intermediate in organic synthesis. Some furan-base derivatives are muscarime antagonists, and show inhibitory activity toward cholesterol O-acyltransferase. The bacteriostatic effects of 5-(4-fluor-phenyl)-furan-2 carbaldehide [34] were checked up with good results. The interest for studying 2-formylfuran (2FF) was due to the fact that in liquid phase its Raman

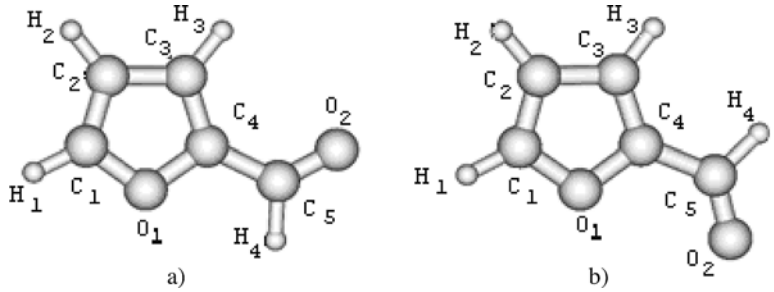

Fig. 4 – Structural formulas of two isomers of 2-formylfuran: a) *trans* and b) *cis.*

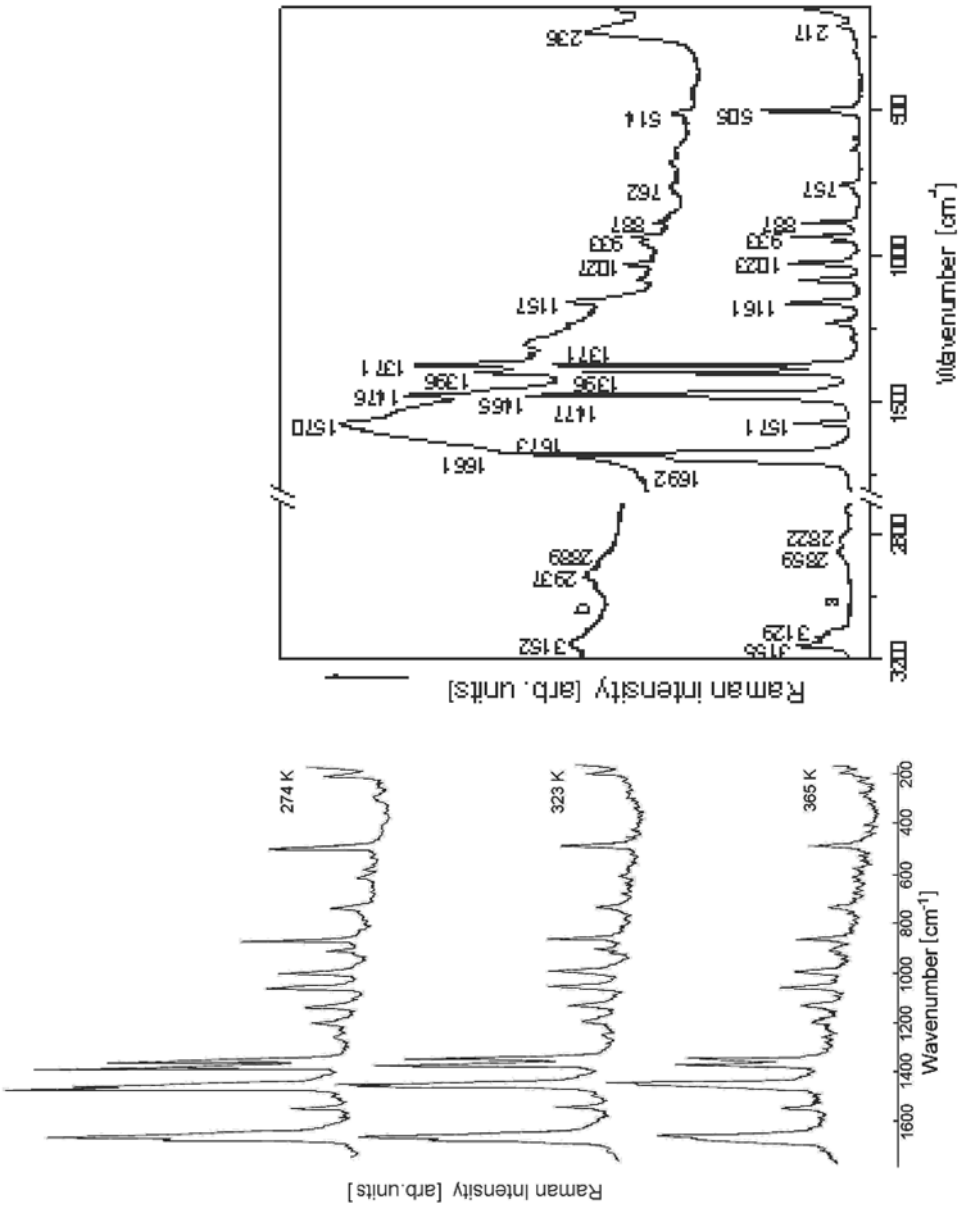

Fig. 5 – A) Raman spectra of 2FF at different temperatures as indicated. B) FT-Raman (a) and SER spectrum (b) of 2FF in silver sol at pH 6. Laser output: 1064 nm, 200 mW (a), 514 nm, 200 mW (b).

spectrum shows pairs of bands whose temperature and solvent dependence behavior can be interpreted in terms of *cis-trans* conformational equilibrium [33] (Fig. 4).

Density functional theory (DFT) calculations performed at the BPW91/6-311+G* and B3LYP/6-311+G* theoretical levels have been indicated that the *trans*-isomer is more stable than the *cis*-isomer by 2.81 and 3.12 kJ mol^{-1}, respectively. Raman spectra of 2FF at different temperatures are presented in Fig. 5A.

Theoretical ΔH values obtained from DFT calculations 2.99 and 3.1 kJ mol^{-1} (BPW91, B3LYP) are very close to the experimental value ΔH = 2.53 kJ mol^{-1} determined from the plots of logarithmic relative intensities of the band pairs 1673/1692, 1477/1465 and 1396/1371 cm^{-1} of these isomers against reciprocal temperature.

Anti and *syn*-form rotational isomers were also found to be present in the 5-(4-fluor-phenyl)-furan-carbaldehyde as a derivative of 2FF [34]. The SER spectrum of 2FF in silver colloid is illustrated in Fig. 5B. The selective enhancement of vibrational modes in SER spectrum was used as a probe for the determination of the adsorbate mode of 2FF molecule on the silver surface. Thus, the band at 236 cm^{-1} assigned to the Ag-Cl^- stretching mode present an asymmetryat low wavenumbers. This asymmetry can be determined by the contribution of the Ag-O stretching mode. These observations suggest that 2FF molecules are adsorbed in the metal surface through the oxygen atom. The enhancement of the band at 1157 cm^{-1} assigned to the $C_1O_1C_4$ stretching mode (see Fig. 4) in the SER spectrum further demonstrates the adsorption of 2FF molecules on the silver surface through the ring oxygen atom. The in plane ring deformation modes at 887 and 933 cm^{-1} and ring stretching modes at 1459, 1476 and 1570 cm^{-1} are enhanced also in SER spectrum, which suggest the perpendicular or least tilted orientation of 2FF molecules with respect to the solver surface. The enhancement and the strong shift of the carbonyl stretching mode present in SER spectrum at 1661 cm^{-1} suggest the existence of a strong interaction of between this group and silver surface and the perpendicular orientation of this bond with respect to the metal surface. Having in view the changes in the peak position and of the relative intensities of the bands in SER spectrum by comparison to the normal Raman spectrum a chemisorption process is present in this case, 2FF molecules being bonded to the silver surface through both the ring oxygen and the oxygen atom of the substituent group, the *cis*-form being preferred in the adsorbate state.

2.1.4. Potassium benzilpenicilin

Potassium benzylpenicillin (KBP) is a well-known component of the big family of antibacterial drugs, its biological activity being due to the presence of the beta-lactam-thiazoline ring system. The study of the interaction between metals and antibiotics, like benzylpenicillins, is important because of the metal strong influence upon susceptibility of bacteria to penicillin in vitro. The structure of KBP molecule

consists of amide, carbonyl and carboxylate groups, and phenyl, β-lactam and thyazolidine rings. FT Raman spectra of polycrystalline KBP and its water solution at pH value of 6 (resulted after dissolution) are illustrated in Fig. 6 [35]. A close analysis of Fig. 6 reveals no dramatic changes in Raman spectrum of the KBP solution in comparison with the solid-state Raman spectrum beside the well-known characteristic of the solution spectra concerning the broadening and change in the peak position of the bands.

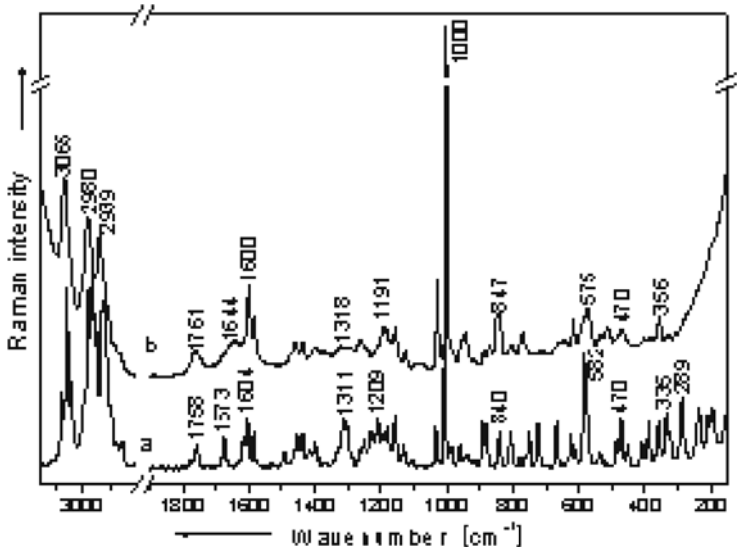

Fig. 6 – Raman spectra of the benzylpenicilin potassium salt: solid state (a) and solution (b).

The SER spectrum of KBP at pH value of 6 compared with its solution Raman spectrum at the same pH value is presented in Fig. 7. By inspecting Fig. 7 we observe dramatic change in the relative intensities of some bands in the SER spectrum as compared to the normal Raman spectrum. These spectral features confirm the chemisorption of KBP molecule through some of its constituent groups, while other groups are located at relatively large distance from the metal surface and therefore their vibrations are not influenced by adsorption. The bonding of KBP molecule to the silver surface can be realized through different atoms. Thus, sulfur atom can form a stable metal-sulfur bond. Moreover, the KBP molecule could be adsorbed on the silver surface either through the nitrogen atoms of the amide, carbonyl or carboxylate groups, respectively.

According the prediction of surface selection rules and analyzing the intensity of the SERS bands, the adsorption of the KBP molecule on silver surface takes place in such a way that the phenyl, β-lactam and thyazolidine rings are situated at large distance to the silver surface and are oriented approximately perpendicular to it. For these groups, the electromagnetic mechanisms seem to be the main mechanism.

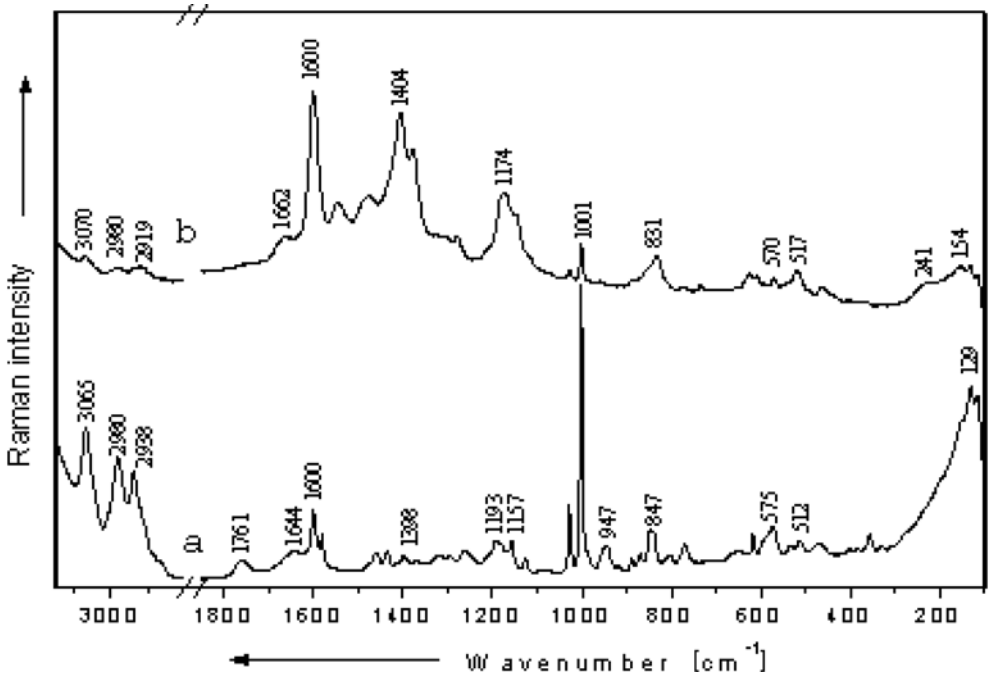

Fig. 7 – Raman spectrum of potassium benzylpenicillin salt 5×10^{-1} M water solution (a) and SER spectrum at pH 6 (b).

2.2. TRANQUILIZERS

2.2.1. Phenothiazine derivatives

Phenothiazine and related compounds, 10-isopentyl-10H-phenotiazine-5 oxide [36] and 10-isopentyl-10H-phenotiazine-5, 5 dioxide [37] have significant physiological activity and can be used as tranquilizers. Due to the flexibility of the isopentyl group 10-isopentyl-10H-phenoyhiazine-5-oxide molecule allows for several conformers (Fig. 8). Theoretical investigations have been performed on six of the most probable conformers [36] in order to find to most stable. The optimized geometries of the conformers calculated at the BPW91/6-31G* level of theory are illustrated in Fig. 8. The most stable conformer was found to be conformer 1. For this conformer the obtained theoretical wavenumber values were found to be in good agreement with Raman and infrared data. The comparison of the SER spectra obtained on activated silver colloid (Fig. 9) with the corresponding Raman spectrum reveals small shifts ($\Delta \leq 5$ cm^{-1}) and proves the partial chemisorption of the molecule on the silver surface *via* the lone pair electrons of the oxygen atom. The electromagnetic mechanism is the main mechanism of the enhancement in this case. Variations of the SER spectra with the change of pH were attributed to the orientation

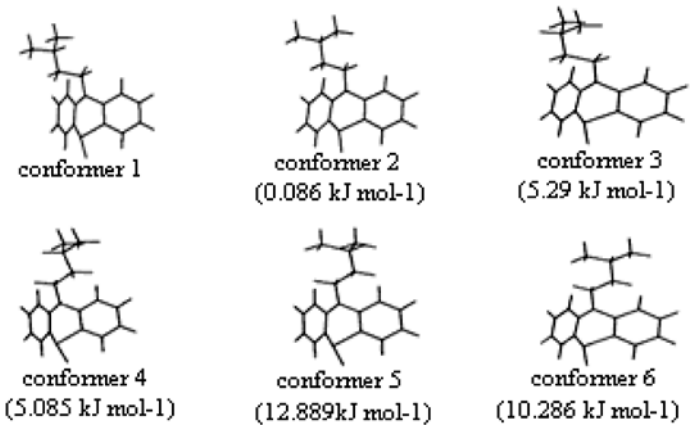

Fig. 8 – Optimized geometries of six most probable conformers of 10-isopentyl-10H-phenothiazine-5-oxide. The differences between the energy of the most stable conformer and energy of the conformers obtained at the BPW91-31G*level of theory are indicated in parentheses.

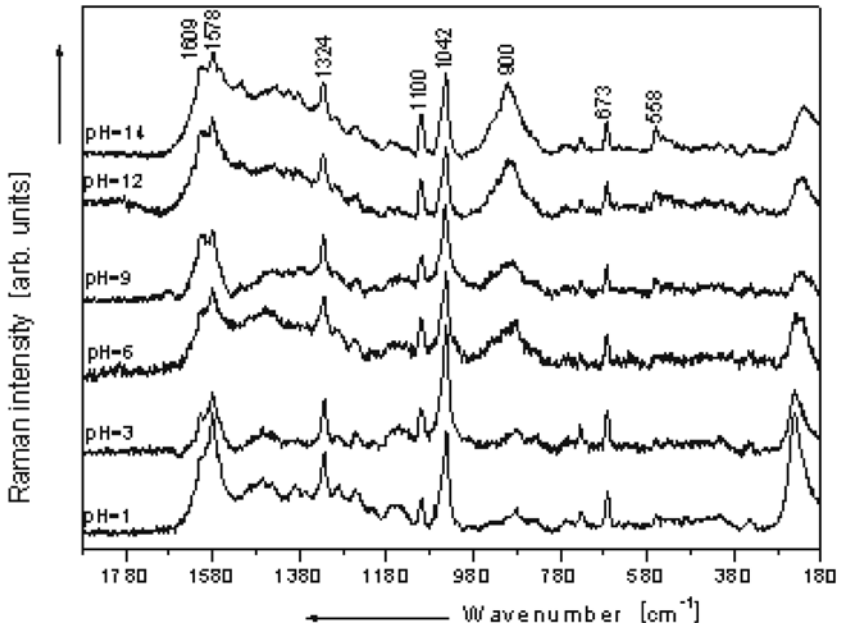

Fig. 9 – SER spectra of 10-isopentyl-10H-phenothiazine-5-oxide compound in silver colloid at different pH values, as indicated. Laser output: 514 nm, 200 mW.

change of the adsorbed molecule with respect to the silver surface. We assume that, at acidic pH, the molecules are oriented at the metal surface in such a way that the benzene rings are preponderantly perpendicular to the surface (Fig. 10a). At alkaline

pH, a reorientation of the molecules occurs, with the phenyl rings having a tilted orientation with respect to the metal surface as suggested in Fig. 10b.

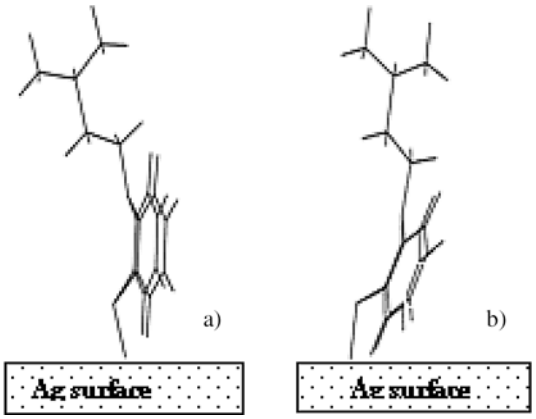

Fig. 10 – Schematic model for the adsorption geometry of 10-isopentyl-10H-phenothiazine.

2.3. ANTI-INFLAMATORY DRUGS

2.3.1. Diclofenac sodium

Diclofenac sodium (DCFNa) is a salt of an aminophenyl acid and is a well-known representative of nonsteroidal anti-inflammatory drugs. Theoretical calculations (DFT and *ab initio*) have been performed on DCFNa conformers, presented in Fig. 11, to find the optimized structure and computed vibrational wavenumbers of the most stable one [38]. The calculations performed on both isomers demonstrate, in agreement with the experimental data on tetrahydrate DCFNa crystals, that the conformer 2 is energetically more stable by an energy

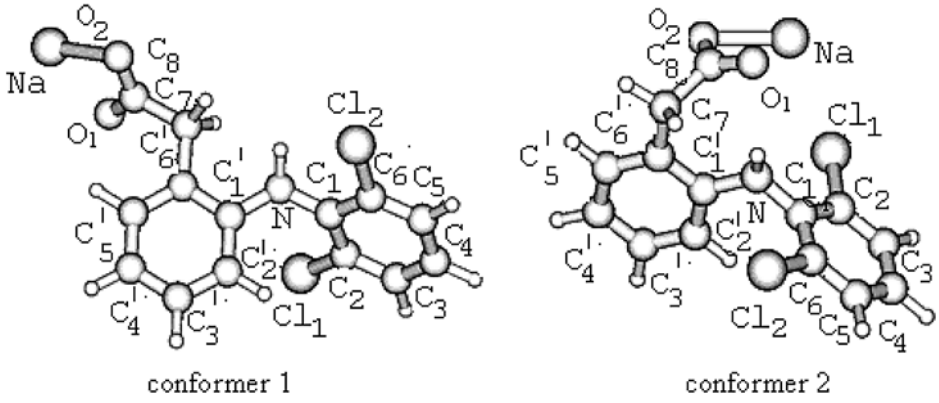

Fig. 11 – Optimized geometries of two conformational isomers of DCFNa obtained at BPW91/6-31G* level of theory.

difference of approximately 18 kJ/mol (RHF), 30 kJ/mol (BPW91) and 27 kJ/mol (B3LYP) respectively.

The calculated Raman intensities and wavenumber are in good agreement with the experimental data. The absence of the carbonyl stretching band in the 1800–1600 cm^{-1} spectral range confirms the presence of the carboxylate group in DCFNa specie in solid state. Good SER spectra were obtained in acidic and neutral environments indicating the chemisorption of the DCFNa molecule on the silver surface (Fig. 12). At all studied pH values the DCFNa 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 metal surface. By analyzing the SER spectra at different pH values a change of the phenyl ring's orientation with respect to the metal surface from tilted close to flat to a more perpendicular one was concluded (Fig. 13).

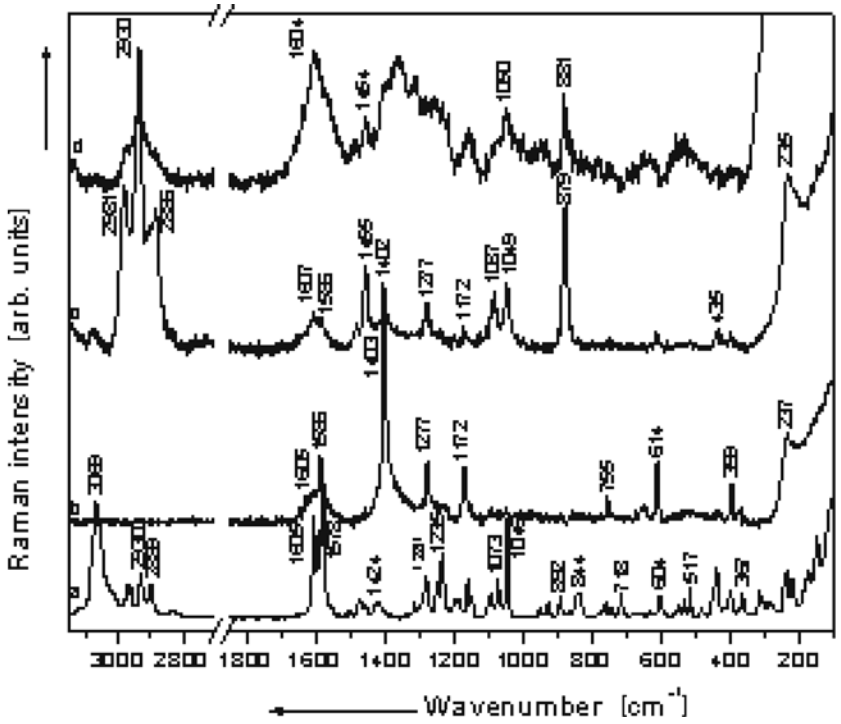

Fig. 12 – FT-Raman spectrum of polycrystalline DCFNa (a) and SER spectra of DCFNa on silver colloid at pH 2 (b); pH 6 (c); pH 10 (d). Laser output: 1064 nm, 200 mW (a), 514 nm, 200 mW (b–d).

The elucidation of the adsorption of the DCFNa molecule can be considered a start point in the characterization of adsorption of the diclofenac sodium-β cyclodextrin (DCFNa-βCD) complex on the silver surface.

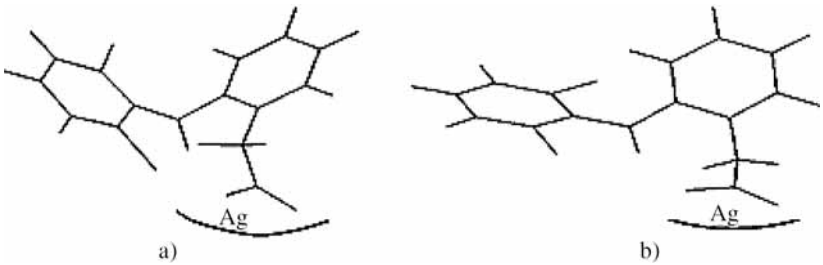

Fig. 13 – Schematic model for the adsorption geometry of DCFNa on colloidal silver surface at pH values below 6 (a) and pH values above 6 (b).

2.3.2. Diclofenac sodium-β-cyclodextrin complex

DCFNa that consists of a phenylacetate group, a secondary amino group and a dichlorophenyl ring (see Fig. 11) has limited water solubility especially in gastric juice. A possibility to overcome this limitation is the complexation with β-cyclodextrin. β-cyclodextrin is a cyclic oligosaccharide consisting of seven glucopyranose units that can be represented as truncated cone structure with the wide and narrow rims occupied by the secondary and primary hydroxyl group, respectively (Fig. 14). The central cavities of these molecules (host molecules) are hydrophobic and thus are able to encapsulate a wide variety of molecules (guest molecules).

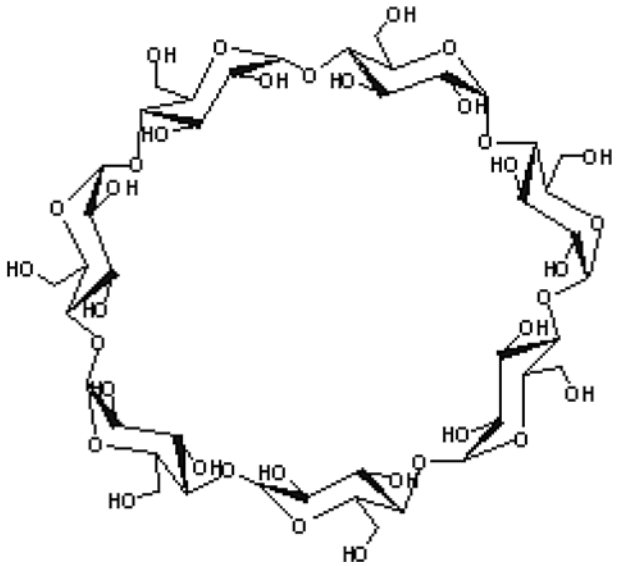

Fig. 14 – Schematic drawing of the β-cyclodextrin molecule.

By using different experimental techniques, like NMR spectroscopy, IR absorption spectroscopy and X-ray diffraction different inclusion ways of the guest molecule into the βCD cavities have been reported [44] depending on the aggregation

state and the preparation method of the DCFNa-βCD complex. Different guest-host complexes like 1:1, 1:2, 2:1 have been found [39, 40].

The different existence of the complex structure, determine us to examine the interaction between the DCFNa and βCD in solid state complex by using Raman spectroscopy [41]. The support of this study was the existence of some spectral range, where the Raman bands associated to atom group vibration directly involved in guest-host molecules interaction are not overlapped (see Fig. 15).

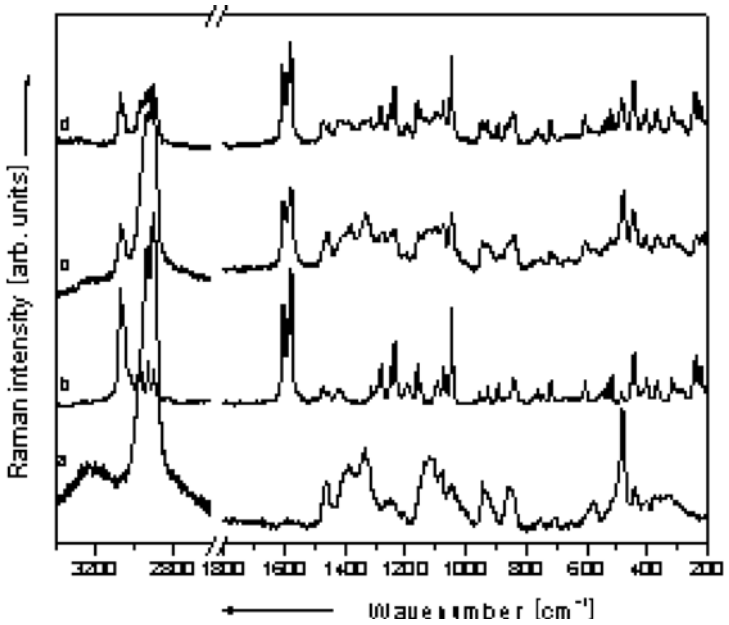

Fig. 15 – FT-Raman spectra of βCD (a); DCFNa (b); 1:1 DCFNa-βCD complex (c); 1:1 DCFNa-βCD physical mixture (d). Laser output: 1064 nm, 200 mW.

From Fig. 15 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 βCD molecule vibrations in the following spectral ranges: 3050–3151, 1500–1650, 1220–1300 and 1000–1100 cm^{-1}. Fig. 16 presents detailed the spectral range between 1500 and 1650 cm^{-1}. The band at 1578 cm^{-1} was assigned to the $O_1C_8C_2$ asymmetric stretching vibration (see Fig. 11), while the bands at 1585 and 1604 cm^{-1} were attributed to dichlorophenyl and phenyl rings stretching vibration, respectively. The corresponding Raman bands of the inclusion complex are broader and their peak positions are changed in comparison to the bands of pure DCFNa and indicate the existence of the guest-host interaction. The deconvolutions of the bands from Fig. 16 done by using Lorentz functions are presented in Fig. 17.

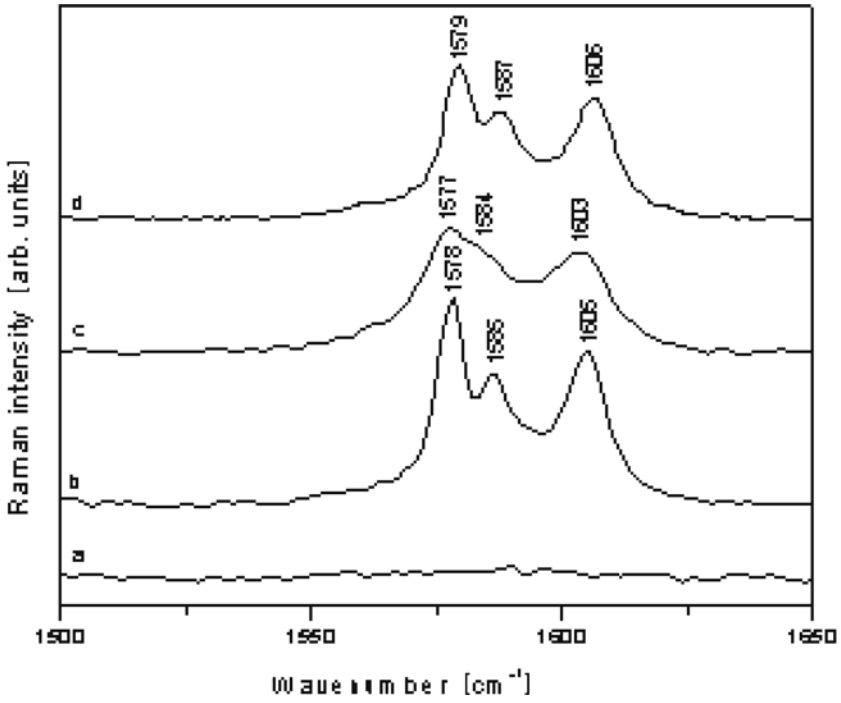

Fig. 16 – FT-Raman spectra of βCD (a); DCFNa (b); 1:1 DCFNa-βCD complex (c); 1:1 DCFNa-βCD physical mixture (d) in the 1500–1650 cm^{-1} spectral range.

The differences evidenced in the Raman spectra allow us to assume the existence of an interaction between both rings of DCFNa and βCD molecule and consequently the existence of two isomeric 1:1 DCFNa-βCD complexes. By comparing the changes between the bands attributed to the dichlorophenyl and phenylacetate rings vibrations we suppose the interaction of the first ring with the βCD molecule is stronger that than of phenylacetate group with host molecule.

SER spectra of DCFNa-βCD complex should provide insights about the complexation way. Having in view that for recording the SER spectrum a DCFNa-βCD solution was prepared and in consequence a equilibrium between the free and the encapsulated guest molecule could appear, we must first verify if the recorded SER spectrum belongs indeed to the encapsulated species. It should be mentioned that attempts to observe SER spectra from native βCD yielded to negative results.

The changes evidenced between the SER and Raman spectra of the complex (Fig. 18) reveal the existence of a strong interaction between the encapsulated DCFNa molecule and the metal surface. By looking at the spectra recorded for different pH values one remarks dramatic change on passing from acidic to alkaline environments. The behavior of the bands located at 1467, 1563, 622 and 407 cm^{-1} attributed to symmetric, asymmetric and deformation vibrations of the COO^- group

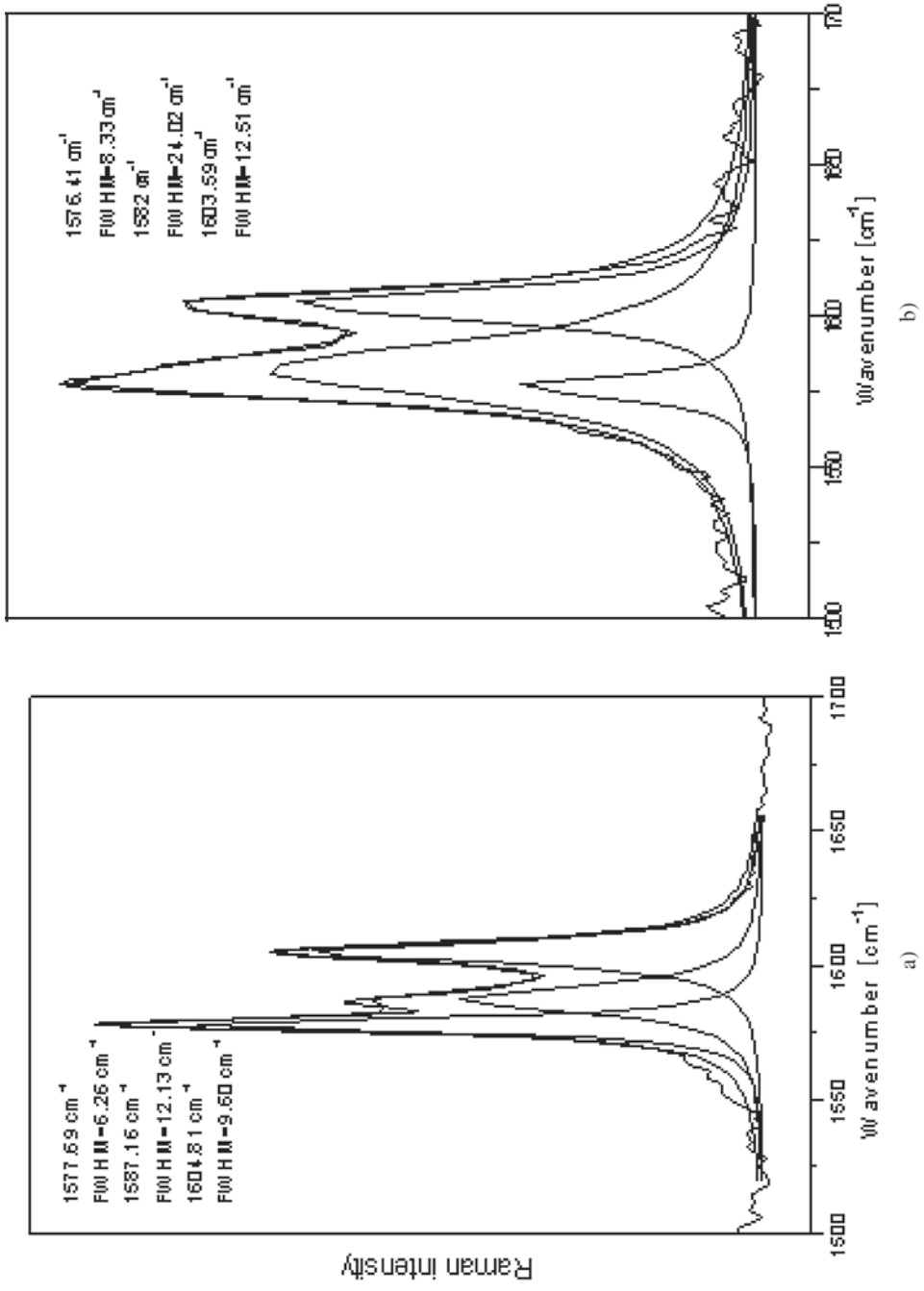

Fig. 17 – The fit of Raman bands around 1600 cm^{-1} of DCFNa (a) and DCFNa-βCD complex (b). The inset presents the bands characteristics.

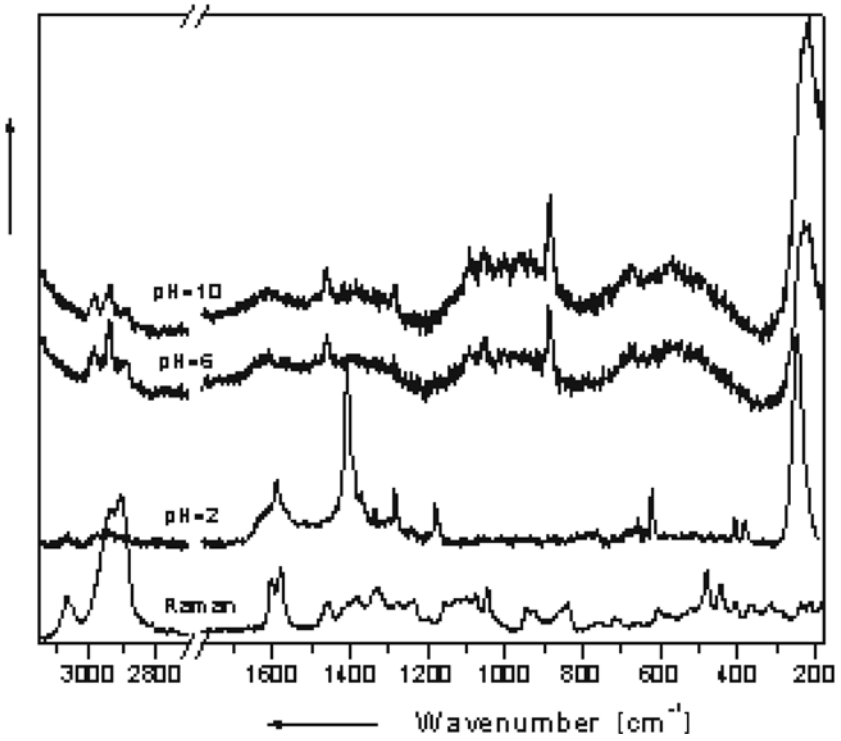

Fig. 18 – FT-Raman and SER spectra of DCFNa-βCD complex at different pH values as indicated. Laser output: 1064 nm, 200 mW (a); 514 nm, 200 mW (b).

indicates that this group directly interacts with the silver surface. Enhancement and shift of the bands attributed to the COO^- group vibrations evidenced in SER spectrum prove that in acidic pH the adsorption of the guest molecule on the silver surface is maintained through the lone pair electrons of the oxygen atom. In the spectral range around 1600 cm^{-1} one can notice the high intensity and the blue shift of the band due to the stretching vibration of the dichlorophenyl ring (1590 cm^{-1}), while the band given by the stretching vibration of phenylacetate ring appears as a shoulder at 1613 cm^{-1}. By considering the spectral features evidenced in the SER spectrum recorded at pH 2 we assume that the isomeric form of the DCFNa-βCD complex having the phenylacetate ring included into the βCD cavity is preferentially adsorbed on the metal surface in acidic environments, the COO^- group being perpendicular or least tilted oriented with respect to silver surface. Furthermore, the enhancement of the bands at 1284 and 1180 cm^{-1} attributed to the rocking and bending vibrations of the CH group demonstrates that the dichlorophenyl ring is tilted relative to the metal surface (Fig. 19a). In the SER spectrum recorded for pH 6 the bands attributed to the CH stretching vibrations of phenylacetate ring present

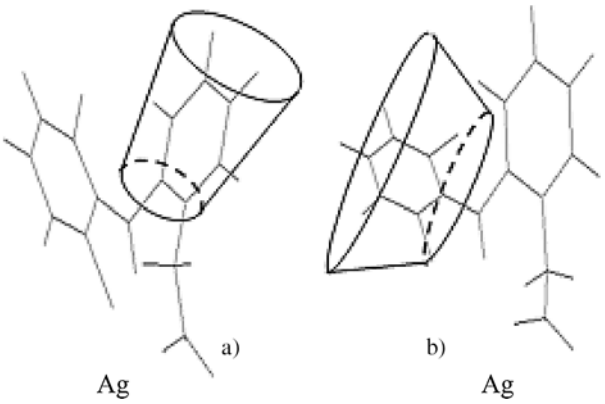

Fig. 19 – Schematic model for the adsorption geometry of DCFNa-βCD complex on colloidal silver surface at pH values bellow 6 (a) and above 6 (b).

around 2900 cm^{-1} are enhanced. The band ascribed to the stretching vibration of phenyl acetate group appears also weakly enhanced in the SER spectrum at 1609 cm^{-1}. The presence in SER spectrum at pH value of 6 of the Ag-O stretching vibration with high intensity proves that guest molecules are adsorbed on silver surface via non-bonding electrons of oxygen atom. All these spectral characteristic of the SER spectrum recorded for pH ≥ 6 allow us to suppose that at these pH values the DCFNa-βCD complex form with the dichlorophenyl ring included into the βCD cavity is mainly adsorbed on the metal surface (see Fig. 19b).

2.4. NON-NATURAL β-AMINO ACIDS

Many interesting investigations were performed on natural α-amino acids [42–45] but very few studies are present in the literature on β-amino acids. β-Amino acids are also present in peptides and their free forms and derivatives exhibit interesting pharmacological effects. By insertion of β-amino acids in the plane of an α-amino acid, the stability of the natural peptide can be increased since β-peptides are resistant to enzymatic degradation [46].

Theoretical calculations carried out on the non-natural β-amino acids 3-amino-3-(furan-2yl)-propionic acid (βAA1) and 3-amino-3-[(5-benzothiazole–2yl)-furan-2yl]-propionic acid (βAA2) at the B3LYP/6-31G* theoretical level (see Fig. 20) revealed that the plane determined by the COO^- group is perpendicular to the furan ring plane for the βAA1 molecule and perpendicular to the plane determined by phenyl, thiazole and furan rings, for the βAA2 amino acid [47]. Theoretical wavenumber values reproduce well the experimental data and allow the assignment of the vibrational modes. The pK_a values of βAA1 and βAA2 amino acids are: pK_{a1} 3.69 and 3.62 for the acidic group and pK_{a2} 9.91 and 9.61 for the basic group. Taking into account the indicated pK_a values the zwitterionic form of

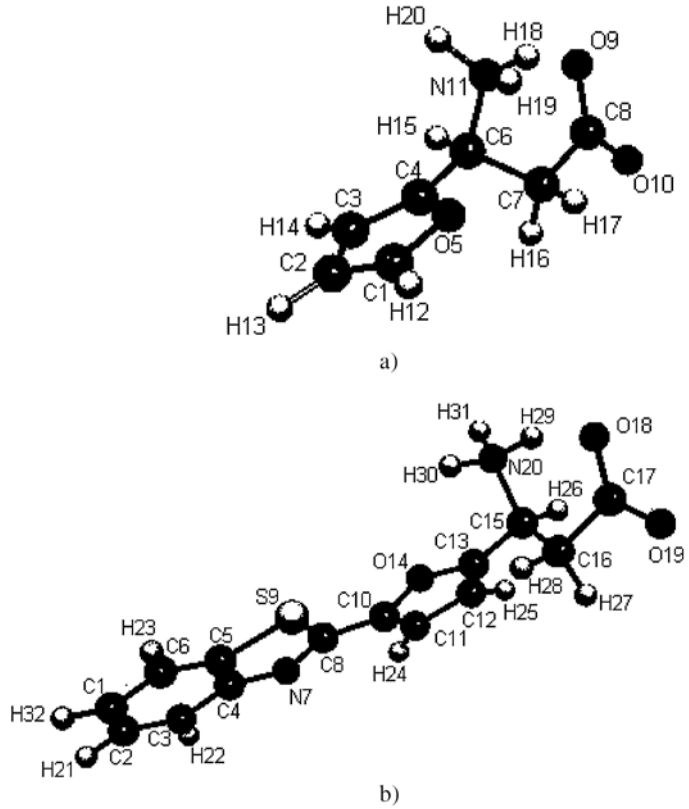

Fig. 20 – Optimized geometry of the βAA1 (a) and the βAA2 (b) amino acids at the B3LYP/6–31G* theoretical level.

amino acids is expected to be preponderant at the working pH values of 5 (for βAA1) and 6 (for βAA2). NIR-FT-SER spectra of βAA1 and βAA2 amino acids at indicated pH values are presented in Figs. 21A and 21B together with the corresponding solid state FT-Raman spectra.

By looking at the SER spectrum of the βAA1 amino acid (Fig. 21A, b) one can notice the absence of the C=O stretching mode. The lack of this band evidences the presence of the carboxylate group (COO^-) also in βAA1 adsorbed state not only in the solid state (Fig. 21A, a). The chemisorption process of the βAA1 molecule on the silver surface was evidenced. The very low intensity in the SER spectrum (Fig. 23b) of the symmetric (1385 cm^{-1}) and asymmetric (1568 cm^{-1}) COO^- stretching bands indicates a tilted close to flat orientation of this group to the silver surface. The most intense bands in the SER spectrum of βAA1 amino acid are those at 1476 and 1632 cm^{-1}, assigned to furan ring in-plane deformation and NH_3^+ asymmetric bending vibration, respectively. The very high intensity of the $\delta_{as}\,NH_3^+$

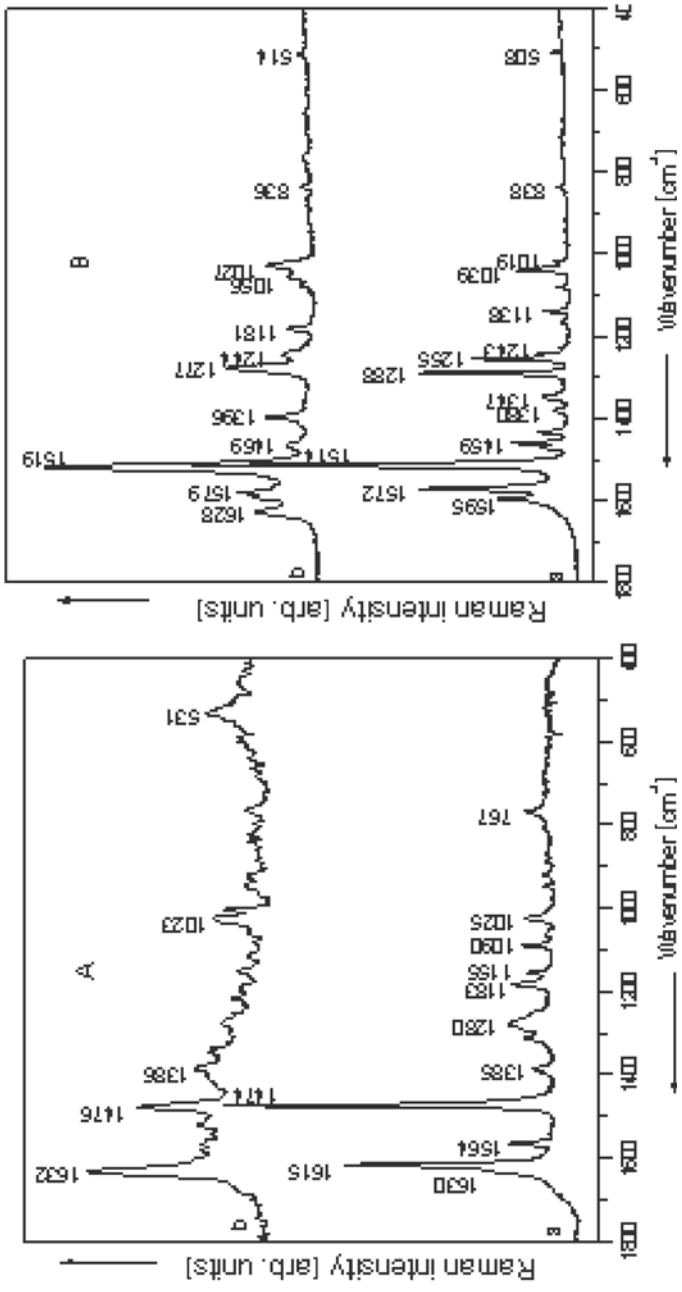

Fig. 21 – A) NIR-FT Raman (a) and NIR-FT SER spectra (b), pH 5, $c = 1.5 \times 10^{-3}$ M, of βAA1 amino acid. Laser output: a) 1064 nm, 30 mW, b) 1064 nm, 380 mW. B) NIR-FT Raman (a) and NIR-FT-SER spectra in silver sol (b), pH 6, $c = 5 \times 10^{-4}$, of βAA2 amino acid. Laser output: a) 1064 nm, 70 mW, b) 1064 nm, 400 mW.

vibration in the SER spectrum relative to that of the furan ring in-plane deformation mode (1476 cm^{-1}) indicates the proximity of this group on silver surface and their strong interaction.

According to surface selection rules we propose the tilted close to perpendicular orientation of the furan ring on the metal surface. The presence in the SER spectrum of the CH ring in-plane deformation mode at 1023 cm^{-1} reinforces the proposed orientation. This orientation of the adsorbed molecule presumes the proximity of NH_3^+ group to the silver surface and explains the high intensity of the $\delta_{as}NH_3^+$ (1632 cm^{-1}) band. In the FT-Raman spectrum of the solid state βAA2 amino acid (Fig. 21B, a) one can observe the absence of the band specific to NH_3^+ bending mode (around 1630 cm^{-1}), which is probably determined by the presence of the strong intramolecular hydrogen bonds. In the solid state FT-Raman spectrum of the corresponding chlorohydrate (not shown here), in which NH_3^+ group is present, this band was observed at 1628 cm^{-1}. FT-Raman spectrum of the βAA2 amino acid sodium salt shows in this spectral region a band at 1632 cm^{-1} specific to NH_2 group. The presence of the band at 1628 cm^{-1} in the SER spectrum at pH value of 6 (Fig. 21B, b) indicates that in the adsorbed state of the βAA2 molecule the intermolecular hydrogen bonds are weaker than in the solid state form. According to surface selection rules the strong intensities of the bands assigned to the in-plane deformation modes of phenyl, thiazole and furan rings (1519, 1579, 1593 cm^{-1}) and CH phenyl and furan ring in-plane deformation vibrations (1027, 1237 and 1469 cm^{-1}) imply both the proximity of the rings plane to the silver surface and their strong interaction with the surface as well as the perpendicular orientation of the rings plane with respect to the surface. The COO^- symmetric stretching vibration is present in the SER spectrum as a weak contribution to the 1396 cm^{-1} band. Having in view the geometry of the βAA2 molecule (see Fig. 20b) in which rings plane and the COO^- group are perpendicular to each other, we suppose that the COO^- group is approximately parallel to the silver surface.

The adsorption behavior of both amino acids was also studied on a solid SERS-substrate consisting of an Ag-coated filter paper. NIR-FT-SER spectra of βAA1 and βAA2 amino acids adsorbed on the Ag-coated filter paper are presented in Fig. 22. One should remark that for the same sample concentration both in silver sol and on the Ag-coated filter paper a very small laser power was necessary for the excitation of the SER spectra on the solid substrate (65–100 mW) relative to that employed for recording the spectra in silver sol (≈ 400 mW). The NIR-SER spectra on Ag-coated filter paper are similar with the corresponding spectra obtained in silver sol. Having in view the very low power used for excitation it becomes evident that no photodecomposition process of the β-amino acids adsorbed on the silver surface occurs when recording the SER spectra on different substrates and using different laser powers. A possible explanation of the high SERS band intensity on the

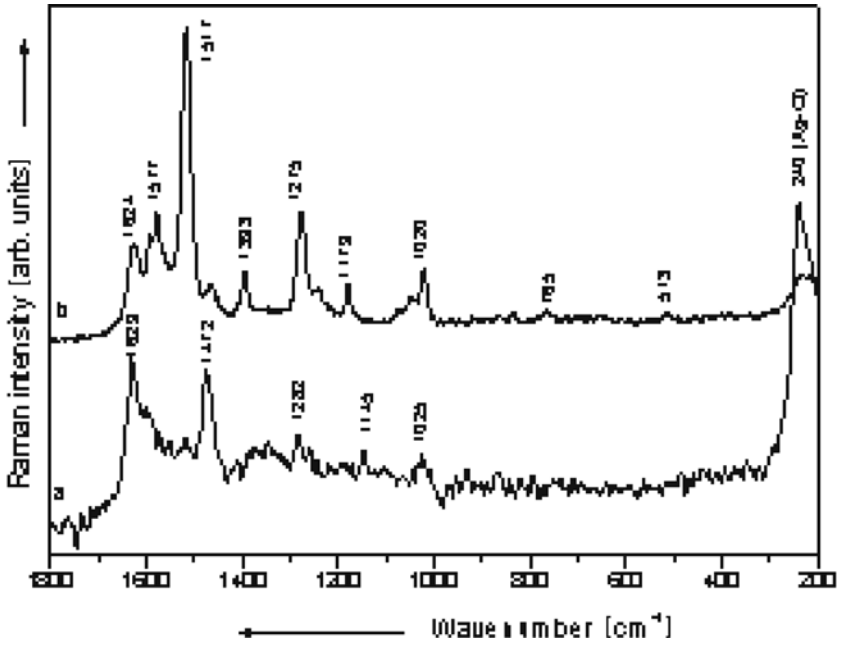

Fig. 22 – NIR-FT-SER spectra of βAA1 (a) and βAA2 (b) amino acid adsorbed on Ag-coated filter paper. Laser output 1064 nm: a) 65 mW, b) 100 mW.

solid support could be considered the existence of "hot area", where giant electric fields are known to appear.

3. CONCLUSIONS

Throughout of the review the results of our SERS investigations on a few representative molecules with antibacterial properties (9-methyl acridine, rivanol, 2-formyl furan and its derivatives, and potassium benzylpenicillin), tranquilizant (phenothiazine derivatives) and anti-inflammatory effects (diclofenac sodium and diclofenac sodium-β-cyclodextrin complex) as well as on some non natural β-amino acids have been presented. In order to understand the adsorption behavior of the molecules on the colloidal silver particles detailed vibrational investigations by using IR and Raman spectroscopy in conjunction with theoretical calculations were previously performed. The results of theoretical calculations established if necessary the most stable conformation of the molecular species and provided the theoretical wavenumber values employed for the assignment of the vibrational modes. The analyses of the SERS spectra provided information concerning the structure of the adsorbed species and their orientation relative to the metal surface. Furthermore, the pH influence on the adsorption behaviour of the investigated molecules has

been monitored. It was found that most of the investigated species are chemisorbed on the silver surface, the physisorption process beeing observed only for the rivanol molecules and for some constituent part of complex molecules.

Moreover, it was shown that the SERS technique is able to discriminate between the possible ways of complexation of DCFNa into the β-cyclodextrin host molecule cavities. These results prove that SERS is a versatil tool in elucidating the encapsulation way of different drugs into the cavities of host molecules, which obviously improve the drug qualities. On the other hand, the results of the SERS investigations of the non natural β-amino acids could be considered preliminary studies in establishing their complex interaction mechanism with peptides.

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