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Short Review

Identification and characterization of pharmaceuticals using Raman and surface-enhanced Raman scattering

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Many recent papers reflect ongoing research and development concerning pharmaceutical applications of Raman techniques. This short review highlights different Raman techniques (dispersive, Fourier transform, resonance Raman, SERS, SERRS, FT-SERS) employed in pharmaceutical investigations. Several Raman applications such as fundamental structural investigations, quantitative analysis, drug–excipient interaction, formulation, limit of detection, pH-dependent pharmaceutical species, adsorption geometry at a given surface and functional groups involved in adsorption for several widely used pharmaceutical compounds are presented. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: SERS; pharmaceuticals; drugs; vitamins; alkaloids

INTRODUCTION

In the last few years, the investigation of pharmaceutical compounds by means of Raman spectroscopy has attracted much interest.[1] This spectroscopic technique represents one of the most useful tools for obtaining information about the structure and properties of molecules from their vibrational structure despite the fact that the direct assignment of the Raman bands of relatively complex molecules is complicated. Theoretical simulations can certainly assist to obtain a deeper understanding of the vibrational spectra of complicated molecules. Recently, it was shown that density functional theory (DFT) methods are a powerful computational alternative to the conventional quantum chemical methods, since they are much less computationally demanding and take account of the effects of electron correlation.[2,3]

An exhaustive review written by Williams[1] pointed out well the pharmaceutical applications of Raman spectroscopy, covering the period 1978–99. The author described the pre- and post-renaissance applications of Raman spectroscopy in the pharmaceutical field, starting with the characterization of drug molecules, revealing the solid-state properties arising from polymorphism, quantitative applications in pharmaceutical systems, formulation, finishing with some predictions concerning the possibility of expanding the range of applications using Raman techniques in conjunction with the newest technological devices.

Raman spectroscopy is gaining popularity in different areas of the pharmaceutical industry. Like infrared spectroscopy, Raman spectroscopy also provides information on the fundamental vibrational bands (the fingerprint region), offering a high degree of specificity in analysis. It also forms an ideal complement for existing methods of analysis such as nuclear magnetic resonance and mass spectrometry and elemental analysis. The application of Raman spectroscopy in the field of pharmaceuticals is showing immense potential.[4] The rapid identification of compounds in the analysis of drug mixtures, active ingredients and excipients,[5] the identification of contaminants, the characterization of formulated materials and the understanding of the blending processes involved in pharmaceutical formations are now accessible using Raman techniques.[1,4–6]

Since we have moved into the 21st century, advanced nanotechnology and biotechnology applications will become increasingly common. This emerging field of nano-biotechnology has the potential to enhance healthcare significantly in the future. It also represents a new challenge for methods such as Raman spectroscopy, especially in relation to the sensitivity of the instruments, as we move from the micro- to the nano-level in many different areas of the healthcare industry.

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TECHNICAL CONSIDERATIONS

A significant factor in considering any Raman investigation of unpurified or mixed samples is the possibility of recording a broad fluorescent signal, which can completely cover the Raman bands. The fluorescence background could be generated either by the fluorophores contained in the sample or fluorescent impurities that may be present at very low concentrations. Different Raman techniques have been developed to overcome such problems in dispersive Raman spectroscopy where a visible excitation line is currently used, including Fourier transform (FT)-Raman, resonance Raman, surface-enhanced Raman scattering (SERS) and their combinations (FT-SERS, SERRS).

We review here some of the common applications of Raman techniques in the pharmaceutical industry.

FUNDAMENTAL STRUCTURAL INVESTIGATIONS

Raman spectroscopy has been used for band assignment and the study of the fundamental structural details of various families of compounds[7] such as hormones,[8] proteins,[9] narcotics,[10] illicit drugs like ecstasy and related phenethylamines,[11] chiral β-blockers (propanolol, alprenolol, acebutolol, atenolol),[12] tranquilizers (diazepam, nitrazepam,[13] oxazepam,[14] delorazepam, fludiazepam, flurazepam and tetrazepam[15]), etc. For example, in the case of 1,4-benzodiazepine-2-ones,[13–15] they differ from diazepam in the substituents at positions 1 and 5 of the diazepine ring, showing in the Raman spectra characteristic features associated with both the diazepine ring and substituents. The strong line near 1610 cm^{-1} in the Raman spectra is assigned to the C═N stretching vibration of the diazepine ring, and the medium-intensity Raman band near 1690 cm^{-1} (very strong IR absorption) is attributed to the C═O stretching mode. Various IR and Raman vibrational features serve to characterize and differentiate these molecules.[13–15]

QUANTITATIVE ANALYSIS

A procedure for the determination of aspirin and acetaminophen in pharmaceutical tablets by partial least-squares (PLS) and principal component regression (PCR) treatment of FT-Raman spectroscopic data has already been proposed.[16] By utilizing selected spectral ranges and by changing the chemometric conditions, it was possible to carry out fast and precise analyses of the active component content in medicines, with promising applications. The monoclinic and orthorhombic forms of paracetamol[17] and impurities in polyene antibiotics (nystatin, amphotericin A and amphotericin B)[18] were quantitatively determined using Raman techniques.

Raman spectroscopy can also be used to differentiate polymorph mixtures, commonly present in the manufacturing process of pharmaceutical tablets. For instance, ranitidine hydrochloride[19] exists as two polymorphs, forms I and II, both present in tablets. Raman spectroscopy can be used to differentiate the two forms, but univariate methods of quantitative analysis of one polymorph as an impurity in the other lack sensitivity. The study demonstrates that Raman spectroscopy represents a sensitive method for the quantitative analysis of polymorphic impurities of drugs in commercial tablets with a limit of quantitation of <2%.

DRUG–EXCIPIENT INTERACTION

The interaction between drugs and excipients can have an effect on drug solubility. Raman spectroscopy has routinely been used to study the chemical interaction/attachment of the two or the changes in the solid-state form of the drug as it interacts with the excipients.[20] The spectra can be processed to give unambiguous identification of both drugs and excipients (even when more than one compound has been used as bulking agent), and the relative intensities of drug and excipient bands can be used for quantitative or at least semi-quantitative analysis. In the case of tablet samples of *N*-methyl-3,4-methylenedioxyamphetamine (MDMA) and related compounds [3,4-methylenedioxyamphetamine (MDA), *N*-ethyl-3,4-methylenedioxyamphetamine (MDEA), *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB), 4-bromo-2,5-dimethoxyphenethylamine (2C-B) and amphetamine sulfate], as well as pure standards of these drugs,[11] it was found that by using far-red (785 nm) excitation the level of the fluorescence background even in untreated seized samples is sufficiently low. The spectra can be used to distinguish between even chemically similar substances, such as the geometric isomers MDEA and MBDB, and between different polymorphic/hydrated forms of the same drug. Moreover, these differences can be found even in directly recorded spectra of seized samples, which have been bulked with other materials, giving a rapid and non-destructive alternative for drug identification.

An interesting Raman application regarding a non-contact and non-invasive method for quantification of the local concentration of certain antibiotic and antifungal drugs in the eye was recently reported.[21] An integrated CCD-based Raman spectroscopic system designed specifically for ophthalmic applications was used to detect non-invasively the presence of ceftazidime and amphotericin B in ocular media. The characteristic Raman fingerprints of the drugs were evidenced for various concentrations that were injected through a needle into the aqueous humor of rabbit eyes *in vivo*. Raman spectra were subsequently acquired by focusing an argon ion laser beam within the anterior chamber of the eye. Compared with the ocular tissue, the ceftazidime bands appeared near 1028, 1506, 1586, and 1641 cm^{-1}. Amphotericin B exhibited its characteristic peaks at 1156 and 1556 cm^{-1}. The relative intensity of the bands was determined to be linearly dependent on their

local concentration in the anterior chamber of the eye. This potentially opens up Raman applications to the investigation of the pharmacokinetics of intraocular drugs *in vivo* from either a releasing implant or a direct injection.

Several studies have been reported which allow the simultaneous identification and quantitative analysis of mixtures without separating them into individual components. Such results were recently reported for cocaine in solid mixtures[22] using Raman spectroscopy in conjunction with chemometric methods, *in situ* identification of cocaine and selected adulterants[23] and a large number of narcotics including opium alkaloids[24] illicit drugs[25] and generally substances not only of pharmaceutical but also of forensic interest.[23–26]

RAMAN TECHNIQUES SUITABLE FOR PHARMACEUTICAL INVESTIGATIONS

FT-Raman

The FT-Raman method, typically employing an 1064 nm excitation laser, is particularly used in the pharmaceutical industry for process, quality and quantity control applications.[27,28] Comparing the accuracy of the results, FT-Raman spectroscopy proved to be a reliable alternative to the expensive and time-consuming HPLC method to quantify the amount of drugs in formulated tablets.[28] Such examples are the quantitative analysis of diltiazem hydrochloride in commercially available tablets (Tildiem)[28] and the identification and quantitation of orthorhombic and monoclinic paracetamol in powder mixes.[17,29]

The mapping facility of an FT-Raman microscope was used to construct a profile of oestradiol distribution in a transdermal drug delivery device, demonstrating the advantages for determining the homogeneity of components in a complex system.[30]

Another comprehensive FT-Raman study[31] recently demonstrated the potential of this technique to probe the solid-state form of active substances present in tablets and capsules. FT-Raman spectra were obtained from intact tablets and capsules containing enalapril maleate, prednisolone, form I and form II polymorphs of ranitidine, anhydrous and monohydrate theophylline and warfarin sodium chlorhydrate. It was shown that it is possible to detect the active ingredients in the intact dosage form, even where the substance comprises <1% of the total mass of the tablet. Moreover, in some cases, FT-Raman spectroscopy can also be applied to investigate the solid-state form of a drug present in the dosage form and even to determine if a mixture of forms is present.[31]

In order to avoid absorption or sample heating or even decomposition during FT-Raman measurements, which often required high laser power, the pharmaceutical tablets could be placed into a rotating holder.[32] A tablet containing maleic acid and one made up of sub-millimetre silica particles with metoprolol succinate as active ingredient were rotated at different speeds, it being possible to record and analyse the FT-Raman spectra perfectly acceptably up to 1500 rpm.[32]

Resonance Raman technique

The resonance Raman (RR) technique has been widely applied in the pharmaceutical field, where appropriate, owing to its selectivity for certain chromophores and its sensitivity for monitoring, for instance, drug–DNA[33–36] or drug–protein[37] interactions.

The characterization of diffusion processes of pharmacologically relevant molecules through membranes was also possible using the confocal RR technique.[38]

For *in vivo* studies, where the physiological concentrations of drugs are $<10^{-6}$ mol l^{-1}, RR spectroscopy was not always sufficiently sensitive; further, not all the investigated drugs possess a resonant moiety.

Surface-enhanced Raman scattering

In spite of the huge area of Raman pharmaceutical applications, however, the conventional Raman techniques are limited by the weak intensity of the Raman scattered light and/or the appearance of fluorescence. One of the most usual ways to overcome these disadvantages is the use of surface-enhanced Raman scattering (SERS),[39–43] resulting in strongly increased Raman signals from molecules attached to or close to nanometre sized metallic structures. It is generally agreed that more than one effect contributes to the observed large effective SERS cross-section. The enhancement mechanisms are roughly divided into electromagnetic and chemical effects. The electromagnetic enhancement factor arises from enhanced optical fields due to the excitation of electromagnetic resonances in the metallic nanostructures. Chemical SERS enhancement results from a metal electron-mediated resonance Raman effect via a charge-transfer intermediate state,[44] which takes place at so-called 'active sites'.

Although the theoretical understanding of the mechanism of surface enhancement is not definite and still evolving, the experimental data accumulated in recent years have demonstrated SERS to be a sufficiently sensitive spectroscopic method for analytical applications to pharmaceuticals.[45,46]

Distinct advantages, such as detection limits at the parts-per-billion level or lower, real-time response, both qualitative and quantitative analysis capabilities, a high degree of specificity, simultaneous multi-component detection and trace analysis,[47] have to be pointed out when one performs SERS. The possibility of determining the orientation of a given molecule at the surface and the functional groups involved in adsorption arises from the surface selection rules, where the Raman modes having polarization components perpendicular to the surface would be mostly enhanced. This relates especially to the totally symmetric modes: depending on the molecule orientation with respect to the surface, they can be almost fully suppressed or strongly enhanced. By performing SERS analysis, valuable information about the geometry of the adsorbed molecule can be obtained. Under optimized conditions, SERS can provide spectral enhancements of 10^{14}-fold or even higher.[48] Further recent

developments have proved that SERS is the only technique capable of evidencing single molecule detection (SMD).[49–52]

SERS has been employed to analyse highly complex biological samples to probe selectively for certain drug components and to study the interaction between small organic molecules (drugs, dyes, intercalating agents) and various macromolecules such as proteins, nucleic acids, etc.[53] The analytical reasons of such studies are numerous and range from an understanding of the mechanisms involved in the interaction, to the synthesis and characterization of molecules with therapeutic properties, such as antitumoral, antibacterial and antiviral. A major challenge for the spectroscopy is the sequencing of the human genome, where SERS is already involved.[54]

Basically, colloids, island films or roughened electrodes of noble metals exhibit the necessary morphological features that are essential for the enhancement of the Raman cross-section of the adsorbed molecule. The type and morphology of the metallic surface are responsible for the specific adsorption properties,[55] the enhancement of the Raman signal being largely dependent on the excitation frequency[56] and the chemical nature of the adsorbed species. Generally, silver colloids provide an optimal enhancement from the visible to the NIR region, silver island films are more suited for the visible region and gold island films and gold colloids work best from the red to the NIR region.[57] Important practical problems related to the use of SERS for different analytical applications arise from the difficulty of obtaining reproducible SERS substrates, mainly for multiple usage purposes. In this sense, several approaches have been developed, such as covering the active surfaces with organic monolayers to avoid degradation and memory effects or obtaining fresh colloids used in flow systems.[58] The results revealed that high-quality SERS spectra could be obtained using 9-aminoacridine, 6-mercaptopurine, acridine and thiamine down to the femtogram level.[58] Two different, commonly used procedures reported in the literature for obtaining Ag colloids, by using borohydride[56] or citrate[55] as reducing agent of silver ions, are dominant in SERS applications. Recently, a new method has been proposed using hydroxylamine hydrochloride.[59] The preparation method is an important factor in the identification and characterization of pharmaceuticals, because it strongly affects the properties of the metal substrates. Moreover, metal colloids usually undergo aggregation phenomena, which also play an important role in the stability of colloidal nanoparticles and in the reproducibility of SERS spectra.

PHARMACEUTICAL COMPOUNDS: RAMAN APPROACH

Concerning the large number of SERS publications dealing with pharmaceutical compounds or pharmaceutical applications on the one hand, and the different SERS/SERRS (surface-enhanced resonance Raman scattering) approaches in surfaces preparation, the nature of the surface and the particular experimental conditions on the other hand, it is difficult to classify SERS applications to pharmaceuticals. Recent Raman and/or SERS investigations on several classes of pharmaceuticals are summarized.

Vitamins

Of the water soluble vitamins, thiamine (B_1), niacin (B_3), also known as nicotinic acid and nicotinamide, pyridoxine (B_6) and ascorbic acid (C) were considered.

Vitamin B_1 (thiamine)

FT-Raman and FT-IR spectra of thiamine hydrochloride (Fig. 1) were recorded and the assignment of the vibrational modes was accomplished with the help of DFT calculations.[60] The pH influence was monitored by Raman and surface-enhanced Raman spectroscopy. The experimental details are largely described elsewhere.[60] The FT-Raman spectra of solid and aqueous saturated solutions of thiamine hydrochloride revealed the presence of the protonated molecular form. From the pH-dependent Raman spectra of thiamine aqueous solution, the pK_a value for the protonation of the N1′ atom was found to be slightly over 5. In a strongly alkaline environment (pH > 8), denaturation of the molecule was observed. Monitoring the adsorption of thiamine on a gold surface by means of the SERS technique, the coexistence of neutral and protonated molecules adsorbed on the metal surface at pH 3, 4 and 5 was revealed (Fig. 2). At pH 4 and 5 the adsorption of the unprotonated molecular species on the gold surface prevailed over the protonation at the N1′ position. The protonated molecular species of thiamine were assumed to be adsorbed through the N3′ atom of the pyrimidine moiety, whereas for the unprotonated species an additional adsorption through the N1′ atom probably occurs and confirms the presence of metal–thiamine bond at pH values >3. The adsorption geometry for the protonated thiamine was concluded to be with the pyrimidine ring closer to the surface, more or less tilted, depending on the deprotonation process.

Vitamin B_3

Niacin (nicotinic acid and nicotinamide) is also known as vitamin B_3. Both nicotinic acid and nicotinamide can serve as dietary source of vitamin B_3. Niacin is required for the synthesis of the active forms of vitamin B_3, nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$). Both NAD^+ and $NADP^+$ function as cofactors for numerous dehydrogenase (e.g. lactate), and malate dehydrogenases. Nicotinic acid[61] and

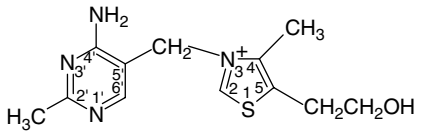

Figure 1. Structure of the thiamine molecule.

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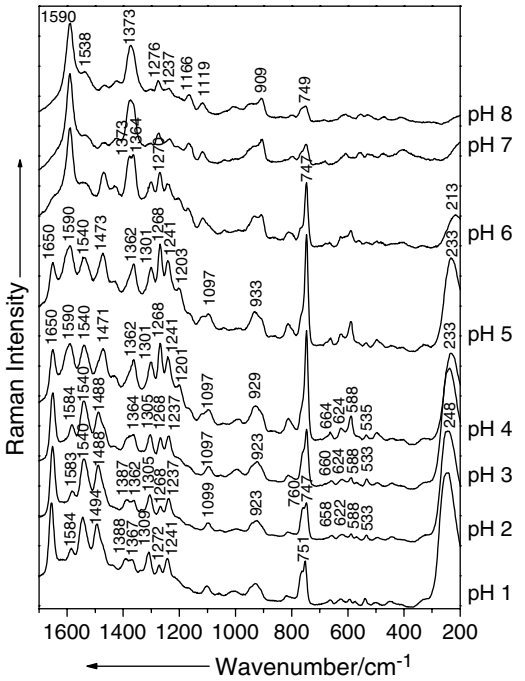

Figure 2. SERS spectra of thiamine (10^{-5} mol l^{-1}) on a gold sol at different pH values. Excitation, 632.8 nm; laser power, 50 mW.

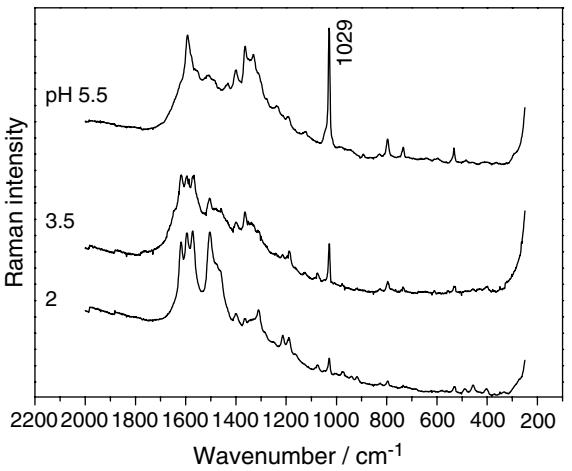

Figure 3. SERS spectra of nicotinamide on a silver colloid at different pH values. Excitation, 488 nm; laser power, 200 mW.

nicotinamide[62,63] were characterized using Raman and SERS techniques. For example, in the case of nicotinamide, the pH-dependent SERS spectra (Fig. 3) revealed two different molecular species adsorbed at the silver surface: a neutral species, at basic pH values, and the protonated form, preponderantly adsorbed in acidic media. Consequently, the change in orientation with respect to the silver colloidal surface was established (Fig. 4). The participation of the ring N atom in the interaction with the surface for the neutral species was concluded from closer examination of the relative

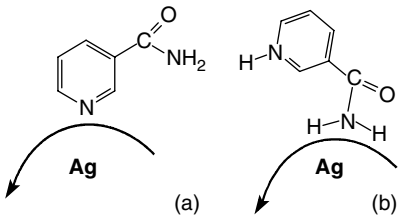

Figure 4. Different orientations of the (a) neutral and (b) protonated species of nicotinamide at a silver surface.

intensity of the SERS bands. The ring breathing mode at 1029 cm^{-1} (Fig. 3) is drastically decreased with protonation upon adsorption. Another interesting SERS study compared the enhancement efficiency of several colloidal or film substrates, in order to guide optimal conditions to detect nicotinamide.[63]

For the SERS adsorption characterization of NAD^+ and $NADP^+$ at silver and gold electrodes,[64,65] the characteristic bands of nicotinamide, adenine and ribose were monitored. In the nucleotide structure, the two termini, adenine (vitamin B_4 or DNA base) and nicotinamide, were found to interact with the surface in a specific manner in the adsorption process. By following the breathing modes of nicotinamide at 1029 cm^{-1} and adenine at 735 cm^{-1}, respectively, it was easier to recognise NAD^+.

Further, a quantitative analysis of nicotinamide in vitamin tablets was proposed using SERS on a silver-coated alumina substrate.[66] This study demonstrated the possibility of quantifying nicotinamide at the ppm level in commercial vitamin B_3 complex preparations.

Vitamin B_6 (pyridoxine)

This displayed a different SERS behaviour, in spite of skeletal structural similarities with nicotinamide.[67] The characteristic N-adsorption of the pyridine derivatives species on the surface was absent in the case of the B_6 ring after deprotonation. A flat orientation of the ring with respect to the colloidal surface was proposed.

Vitamin C (ascorbic acid)

FT-IR and FT-Raman techniques were used as complementary tools to quantify vitamin C in foods and pharmaceutical products.[68] In this study, near-IR (NIR), Fourier transform (FT) NIR, FT IR-attenuated total reflectance (FTIR-ATR), diffuse reflectance (DRIFTS), FT IR–photoacoustic (FTIR-PAS) and FT-Raman spectroscopy were used in conjunction with partial least-squares (PLS) regression to quantify vitamin C in powders, mixtures and solutions. The results indicated that the methods adopted have high prediction correlation with an overall prediction error of 0.2–3.0%. The time required to complete an experiment ranged from 5 s (NIR) to 3 min (FT-Raman).

Water-insoluble vitamins

Vitamin A consists of three biologically active molecules, retinol, retinal (retinaldehyde) and retinoic acid. Each of these compounds is derived from the plant precursor molecule β-carotene (a member of a family of molecules known as carotenoids). β-Carotene, which consists of two molecules of retinal linked at their aldehyde ends, is also referred to as the provitamin form of vitamin A.

Quantitative analysis of vitamin A degradation by Raman spectroscopy was recently reported.[69] An extraction method was then used to obtain the SERS spectra of water-insoluble drugs such as vitamin A, aspirin, salicylic acid and acetaminophen.[70] Based on the strong affinity of the drug molecules to the silver particle surfaces, it was concluded that the method can be extended to identify and analyse many other water-insoluble compounds by SERS. The high sensitivity of SERS and the linear calibration curve make it feasible in trace quantitative analysis; the limit of detection is comparable to or better than those of calorimetric and spectrophotometric methods.

SERRS spectra have been reported for the β-carotene and lycopene carotenoids present in low-density lipoproteins (LDLs).[71] The silver surface was modified by the formation of a self-assembled monolayer (SAM) of carboxylate-terminated linear alkanethiols in order to simulate the LDL binding region of the cellular LDL receptor. Thiols of different chain lengths were used to produce SAMs of varying thicknesses. It was shown that carotenoids are not released from the LDL particle on adsorption on the bare and thiol modified silver surfaces. The SERRS studies indicated that β-carotene and lycopene were present in the shell of the LDL particle. The authors concluded that the two carotenoids are located in different places on the LDL particle from the dependence of SERRS on the distance from the silver surface.

Vitamin B_{12} (cyanocobalamin)

This has been subject of resonance Raman and SERRS studies[72–74] because of its important biological effects. For instance, it was shown that the Co—C bond remains essentially unaffected when vitamin B_{12} binds to methylmalonyl-coenzyme A mutase.[74]

Vitamins K_1 and K_3

Resonance Raman and FTIR spectra of the radical anions of 2-methyl-1,4-naphthoquinone and 2-methyl-3-phytyl-1,4-naphthoquinone, also known as vitamin K_3 and vitamin K_1, respectively, have been reported.[75] The study was also extended to 1,4-naphthoquinone for comparison. The vibrational assignments were carried out on the basis of comparison with earlier time-resolved resonance Raman studies on photochemically generated radical anions of 1,4-naphthoquinone and 2-methyl-1,4-naphthoquinone. For the moment, no SERS data on vitamin K are available.

Alkaloids

These compounds are all nitrogen heterocycles, which mainly occur in plants as salts of common carboxylic acids such as citric, lactic, oxalic, acetic, malic and tartaric acids and also fumaric, benzoic, aconitic and veratric acids. Their amine character produces an alkaline solution in water, hence the origin of their name: alkaloids.

Nicotine

Of the monocyclic alkaloids, nicotine was briefly characterized.[66] Its SERS spectrum on a silver–alumina substrate reveals a sharp band at 1032 cm^{-1} (trigonal ring breathing) and several broad bands similar to those for nicotinamide, making it difficult to distinguish between the two compounds in the given SERS system. This feature is supported by the interaction of nicotine with the surface through the N six-membered ring, while the characteristic five-membered ring of the compound stays far from the surface.

Cocaine

Cocaine, a bicyclic alkaloid, was investigated by SERS in a series nine drugs on tetrahydroborate and citrate colloids, silver on ion-exchange filter-paper, silver on cellulose filter-paper and etched silver foil.[46] However SERS spectra could be obtained for a concentration as high as 1000 $\mu g\ ml^{-1}$ only on the tetrahydroborate silver colloid. Except for triamterene, all other investigated compounds 'prefer' a specific surface in order to achieve a high-quality SERS signal. For instance, atenolol and acebutolol were SERS active only in colloids or on silver on ion-exchange filter-paper.[46]

There are a number of alkaloids which are derivatives of quinoline, isoquinoline and their hydrogenated analogues, e.g. papaverine, emetine, quinine, reserpine, strychnine, morphyne, heroin and codeine, most of which have been the subject of vibrational investigations.

Papaverine hydrochloride

A complete experimental and theoretical vibrational study of papaverine hydrochloride (Fig. 5) and its neutral species has been reported.[76] The DFT-computed structural parameters and harmonic vibrational wavenumbers reproduced well the experimental data and helped to characterize the vibrational behaviour of the adsorbed species.

A pH-dependent Raman study was possible at pH values <6.5; at higher values the solubility was dramatically diminished. However, the vibrational analysis of the Raman spectra allowed the two species of papaverine, protonated and neutral, to be distinguished.

The pH-dependent SERS spectra of papaverine (Fig. 6) on citrate-reduced silver colloid reveal two different chemisorbed species on the Ag colloidal surface: protonated and neutral, the protonated one being adsorbed with the phenyl group closer to the surface and the neutral one through the isoquinoline part, more probably through the

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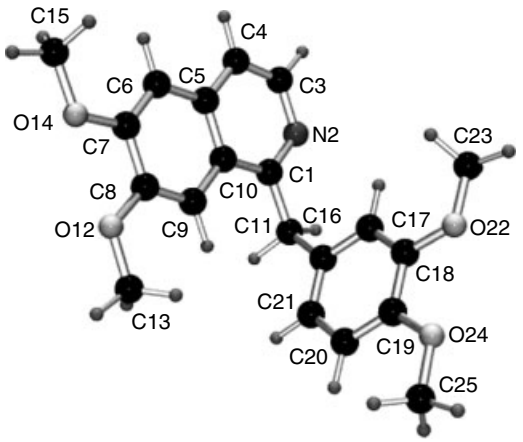

Figure 5. B3LYP/P2-optimized geometry of the neutral form of papaverine.

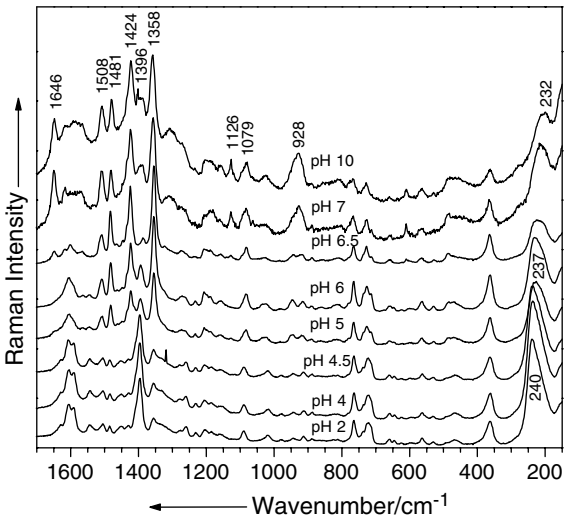

Figure 6. SERS spectra of papaverine on Ag colloid at different pH values. Excitation, 514.5 nm; laser power, 120 mW.

nitrogen atom. In either acidic or basic media, the oxygen adsorption of papaverine species was excluded. SERS spectra could evidence the protonated or neutral species below the micromole level.

Quinolines

SERS spectra in silver sol and Raman spectra in the bulk and in solution of 8-hydroxyquinoline[77] and 2,2′-biquinoline (BQ) molecules have also been reported.[78] For the latter, the existence of both the *cis* and *trans* forms of the BQ molecule in solution and in the bulk is inferred from the normal Raman and FTIR spectra, whereas the SERS study revealed that in the surface-adsorbed state the molecule exists in the *cis* form. Definite evidence of the charge-transfer (CT) mechanism contribution to the SERS enhancement was concluded in both cases. The excitation profile also supported the CT interaction. The enhancement factor of the principal SERS bands showed that the molecule is adsorbed on the silver surface through both the nitrogen atoms with the molecular plane almost perpendicular to the surface.

Caffeine (1,3,7-trimethyl-2,6-purinedione, Fig. 7)

The structural parameters and the vibrational fundamentals from FT-Raman spectrum have been analysed and discussed with the help of the DFT calculation results in order to improve the previous assignments and for a reliable analysis of SERS spectra.[79] Furthermore, the BPW91 functional gave good results with respect to the calculated harmonic vibrational wavenumbers and no scaling was required. The vibrational behaviour of caffeine on an Ag colloidal surface at different pH values was described (Fig. 8). A flat orientation of mainly chemisorbed caffeine through the π electrons and the lone pair of the N9 atom was considered to occur for normal and basic pH values. At acidic pH values, caffeine was probably adsorbed on the Ag surface through one or both oxygen atoms, more probably through the O11 atom,

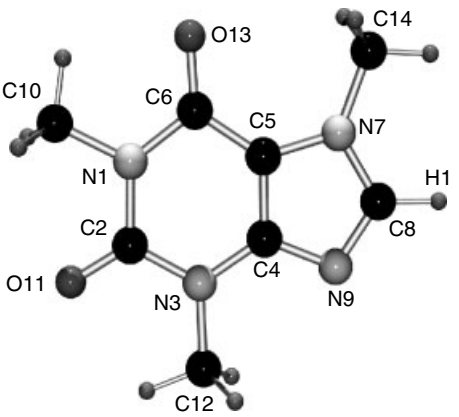

Figure 7. B3LYP/6−311+G(d)-optimized geometry of caffeine.

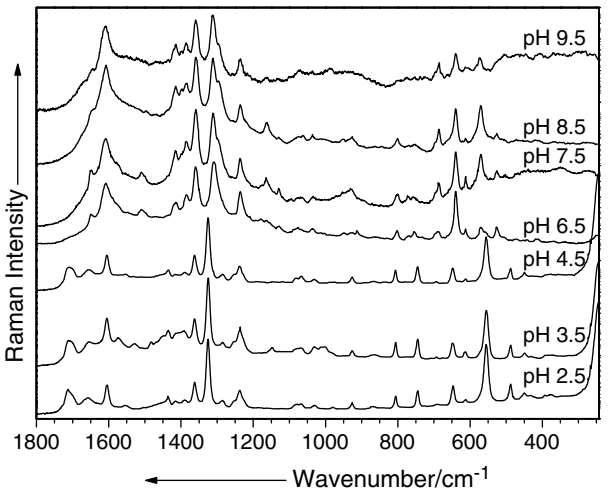

Figure 8. SERS spectra of caffeine on Ag colloid at different pH values. Excitation, 514.5 nm; laser power, 500 mW.

with an end-on orientation. The surface selection rules along with the vibrational assignment of the SERS spectra and the theoretical results have reasonably explained the adsorbate structure.

Miscellaneous

Many other molecular compounds with different pharmacological activity have been analysed using Raman and SERS techniques, including the antihypertensive 1,4-dihydrazinophtalazine sulfate,[80] the antimicrobial agent rivanol,[81] chiral β-blockers,[12] antiretrovirals,[82] narcotics,[83–85] antitumour agents (6-mercaptopurine[86], 9-phenyl- and 9-aminoacridine,[87–88] camptothecin[89] and its water-soluble derivative topotecan[90]) and HIV inhibitors (betulinic acid).[91]

CONCLUSION

The general interest in such studies is justified not only because of the pharmaceutical activity of the compounds concerned, but also because of the potential behaviour in the presence of other compounds, organic or inorganic molecules, metals or metal complexes, membranes or even in the living cells or tissues. On the other hand, SERS adsorption is the key to detecting a given compound at very low concentrations. This fact is a major contributor to the new developments in the nanostructured surface field, providing either a better insight into the mechanisms involved or practical applications. The different Raman and SERS capabilities of providing rich information and detecting minimum amounts of substances down to pico- and femtogram detection limits or monitoring the vibrational behaviour of a given drug have been demonstrated for a variety of molecules not only of pharmaceutical but also of biomedical and environmental interest.

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