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Food additives characterization by infrared, Raman, and surface-enhanced Raman spectroscopies

Edyta Podstawka,[1]* Małgorzata Światłowska,[2] Ewa Borowiec[2] and Leonard M. Proniewicz[3]

[1] Regional Laboratory of Physicochemical Analysis and Structural Research, Jagiellonian University, 3 Ingardena Str., 30-060 Krakow, Poland
[2] Higher Vocational School, 8 Mickiewicza Str., 33-100 Tarnow, Poland
[3] Faculty of Chemistry, Chemical Physics Division, Jagiellonian University, 3 Ingardena Str., 30-060 Krakow, Poland

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Fourier-transform infrared (FT-IR), Raman (RS), and surface-enhanced Raman scattering (SERS) spectra of β-hydroxy-β-methylobutanoic acid (HMB), L-carnitine, and *N*-methylglycocyamine (creatine) have been measured. The SERS spectra have been taken from species adsorbed on a colloidal silver surface. The respective FT-IR and RS band assignments (solid-state samples) based on the literature data have been proposed. The strongest absorptions in the FT-IR spectrum of creatine are observed at 1398, 1615, and 1699 cm^{-1}, which are due to ν_s(COOH) + ν(CN) + δ(CN), $\rho_s(NH_2)$, and ν(C=O) modes, respectively, whereas those of L-carnitine (at 1396/1586 cm^{-1} and 1480 cm^{-1}) and HMB (at 1405/1555/1585 cm^{-1} and 1437–1473 cm^{-1}) are associated with carboxyl and methyl/methylene group vibrations, respectively. On the other hand, the strongest bands in the RS spectrum of HMB observed at 748/1442/1462 cm^{-1} and 1408 cm^{-1} are due to methyl/methylene deformations and carboxyl group vibrations, respectively. The strongest Raman band of creatine at 831 cm^{-1} ($\rho_w(R-NH_2)$) is accompanied by two weaker bands at 1054 and 1397 cm^{-1} due to ν(CN) + $\nu(R-NH_2)$ and ν_s(COOH) + ν(CN) + δ(CN) modes, respectively. In the case of L-carnitine, its RS spectrum is dominated by bands at 772 and 1461 cm^{-1} assigned to $\rho_r(CH_2)$ and $\delta(CH_3)$, respectively.

The analysis of the SERS spectra shows that HMB interacts with the silver surface mainly through the $-COO^-$, hydroxyl, and $-CH_2-$ groups, whereas L-carnitine binds to the surface via $-COO^-$ and $-N^+(CH_3)_3$ which is rarely enhanced at pH = 8.3. On the other hand, it seems that creatine binds weakly to the silver surface mainly by $-NH_2$, and C=O from the $-COO^-$ group. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: Fourier-transform infrared spectroscopy; FT-IR; Raman spectroscopy; RS; surface-enhanced Raman scattering; silver colloid; food additives; β-hydroxy-β-methylobutanoic acid; HMB; L-carnitine; *N*-methylglycocyamine; creatine

INTRODUCTION

During the last several years, different products that deliver necessary energy food compounds to the human body have appeared on the food market, which assist in body mass building and regeneration of body strength after intense effort or fast burning of fatty acids. These products include vitamin/mineral nutrients, proteins, energy- and isotonic drinks, and many other beverages. The most popular examples of these food additives are β-hydroxy-β-methylobutanoic acid (HMB), L-carnitine, and *N*-methylglycocyamine (creatine). (Fig. 1 presents the structures of these compounds.)[1–4] However, we are slightly confused because according to us the proper name of HMB should be α-hydroxy-α-methylobutanoic acid and not β-hydroxy-β-methylobutanoic acid as it is commonly named. Not to add to the confusion, we will use only the abbreviation, i.e. HMB.

The extensive biological implications of HMB, L-carnitine, and creatine on proper physiological functions of the human body and their increased demand on the food market are the reasons for our interest in these compounds. Thus, for the purpose of understanding their action at the molecular level, one has to determine their molecular structure, geometry of the adsorbed species deposited onto the silver colloidal surface, and the mechanism of their interaction at the solid/solution interface.

HMB is a derivative of L-leucine.[5] In the biological world, it may be found in small amounts in food products originating from plants and animals. It is present in lucerne, corn, or grapefruit.[6,7] It is a potent and safe derivative used

*Correspondence to: Edyta Podstawka, Regional Laboratory of Physicochemical Analysis and Structural Research, Jagiellonian University, 3 Ingardena Str., 30-060 Krakow, Poland.
E-mail: podstawk@chemia.uj.edu.pl

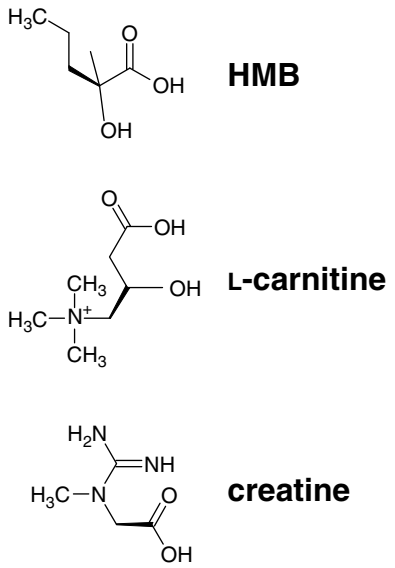

Figure 1. Molecular structures of HMB, L-carnitine, and creatine.

as a supplement by sportsmen for improving their fitness, strength, and body mass. In addition, HMB is the forerunner of cholesterol. The study suggests that supplementation by HMB significantly decreases cell membranes by decreasing the amount of phosphocreatinine kinase in muscle cells after intense physical training.[8] On the other hand, HMB plays a key physiological role in stimulating the immune system and preventing diseases. Supplementation by HMB decreases the risk of heart attack, stroke, and coronary diseases, as well as helps maintain proper blood pressure and reduce cholesterol levels[9,10] This compound is also recommended during convalescence after diseases involving huge loss of nitrogen.

L-Carnitine was discovered in 1905.[11] Its most significant source for human nutrition is meat, although humans are also capable of synthesizing L-carnitine from dietary amino acids.[12] L-Carnitine appears to be a conditionally essential nutrient. It is a cofactor required for transformation of free long-chain fatty acids into acylcarnitines, and for their subsequent transport into the mitochondrial matrix, where they undergo β-oxidation for cellular energy production. Conditions that seem to benefit from exogenous supplementation of L-carnitine include anorexia, chronic fatigue, coronary vascular disease, diphtheria, hypoglycemia, male infertility, muscular myopathies, and Rett syndrome.[13] In addition, preterm infants, dialysis patients, and HIV positive individuals seem to be prone to deficiency of L-carnitine and greatly benefit from its supplementation.

Creatine was discovered in 1832 by the French chemist Chevreula.[14] The chemical name of creatine is *N*-methylglycocyamine. It is a natural component of the human body and is synthesized in the liver, pancreas, and kidney from three amino acids: L-glicyne, L-arginine, and L-methionine.[15] Creatine is usually delivered to the body through food. It is widespread in meat (beef and pork) and fish (cod, herring, salmon, and tuna).[16] It is recognized as the most important and powerful supplement in sports diet and ensures high energy levels in the muscle cells during extensive physical effort.[1]

Fourier-transform infrared (FT-IR) and Raman spectroscopy (RS) are generally very sensitive analytical and quantitative methods commonly used for the examination of the molecular structure of food additives.[17] However, currently there is great interest in creating extremely sensitive and specific biochemical drugs and food supplements that interact with different interfaces.[18] Thus, the use of an extremely sensitive technique, namely, surface-enhanced Raman spectroscopy (SERS), to study the interaction of a molecule with the metal surface on the nanoscale, permits one to solve the structure of the adsorbed species and thus, in some cases, allows understanding the mechanism of binding of different substrates to their receptors.[19,20]

SERS spectroscopy is a very sensitive technique that employs rough substrates at the nanometer scale to enhance the Raman signal produced by the adsorbed and immobilized species.[21] This signal is too weak to be detected for solute concentrations below 10^{-1} M with conventional RS. In SERS spectroscopy, the effective Raman cross section can be increased up to 10^{14}–10^{15} orders of magnitude to allow information to be obtained even from a single molecule adsorbed at the surface of the substrate.[22] Therefore, this technique combines ultrasensitive detection limits with detailed structural information. Not all the vibrational modes are intensified equally in the SERS spectra. In this technique, the orientation[23,24] and the distance[25,26] of functional groups (responsible for the adsorption process of the molecule) with respect to the surface are critical for enhancement of particular Raman bands. Thus, only the molecular fragments on the metal surface or very close to it can contribute to the SERS signal.

The main goal of this work is to structurally characterize the above-mentioned compounds by using vibrational spectroscopy and propose a mechanism of interaction between these molecules and the silver surface. This may help in understanding their biological activity in the physiological conditions at the basic pH. The information obtained is also useful in understanding the adsorption process of amino acids,[27,28] peptides,[28–31] or proteins[32,33] at the aqueous solution/silver (gold) interface.

EXPERIMENTAL

Food additives

Food additives such as HMB, L-carnitine, and *N*-methylglycocyamine (creatine) were obtained from Sigma-Aldrich (Poznan). The purity and chemical structures of the samples

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were proved by means of the ^{1}H-NMR spectra and electrospray mass spectrometry.

Raman spectroscopy

For the RS measurements, about 1 mg of each sample was placed in a glass capillary tube and measured directly (180° geometry). The RS spectra were obtained with a triple grating spectrometer (Jobin Yvon, T 64 000) equipped with a liquid-nitrogen-cooled charge-coupled device (CCD) detector (Jobin Yvon, model CCD3000). A spectral resolution of 4 cm^{-1} was set. The 514.5-nm line of an argon ion laser (Spectra-Physics, model 2025) was used as the excitation source. The laser power at the sample was set to 20 mW.

FT-IR spectroscopy

A standard procedure with 1 mg of investigated compounds dispersed in 200 mg of KBr was used for the FT-IR measurement. The spectra were recorded at room temperature as the average of 128 scans using a Brucker infrared spectrometer, model EQUINOX 55, equipped with a Nernst rod as the excitation source and a DTGS detector in the 400–4000 cm^{-1} range with the spectral resolution of 4 cm^{-1}.

SERS spectroscopy

$AgNO_3$ and $NaBH_4$ were purchased from Sigma-Aldrich Co. (Poznan, Poland) and used without further purification. A solution of the colloidal silver was prepared three times according to the standard procedure.[27] Briefly, 8.5 mg of $AgNO_3$ dissolved in 50 ml deionized water at 4 °C was added dropwise to 150 ml of 1 mM solution of $NaBH_4$ immersed in an icebath and stirred vigorously. After the addition of $AgNO_3$ was completed, the resulting pale-yellow solution was stirred and maintained at 4 °C for approximately 1 h. The excitation spectra of three batches of the Ag sol prepared in this manner showed an absorbance maximum at 396 nm.

Aqueous sample solutions were prepared by dissolving the samples in deionized water. The concentration of the samples before mixing with the colloid was set to 10^{-4} M. The freshly prepared aqueous sample solution was added to the silver sol (the final sample concentration in the silver colloid was $\sim 10^{-5}$ M) and brought to pH = 8.3. In addition, a 10^{-5} M KCl solution was added to enhance the SERS signals from the samples.

The SERS spectra of the compounds investigated in this work were collected twice for each batch of the three silver colloids using the same equipment as for the Raman measurement. In addition, the SERS spectra were recorded at the same time after sample addition. The obtained spectra were almost identical except for small differences (up to ~5%) in some band intensities. No spectral changes that could be associated with a destroyed sample or a desorption process were observed during these measurements.

RESULTS AND DISCUSSION

Figures 2–4 present the FT-IR (trace A), RS (trace B), and SERS (trace C) spectra of HMB, L-carnitine, and creatine, respectively, in the wavenumber range of 400–1850 cm^{-1}. So far, no vibrational studies of the HMB and L-carnitine structures have been carried out. On the basis of the characteristic

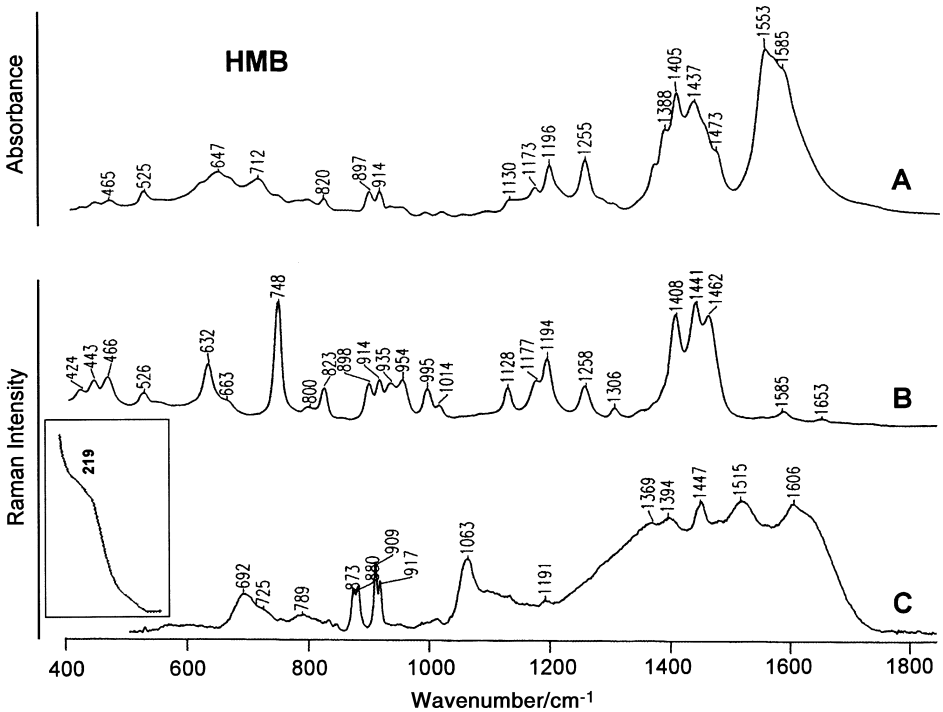

Figure 2. The FT-IR (A), RS (B), and SERS (C) spectra of HMB in the spectral range of 400–1850 cm^{-1}.

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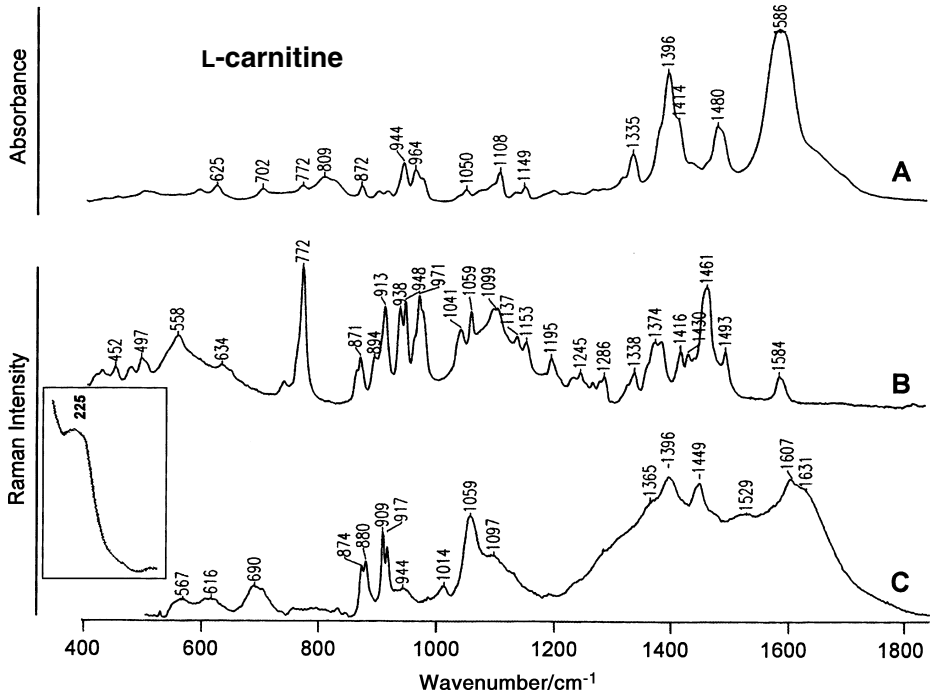

Figure 3. The FT-IR (A), RS (B), and SERS (C) spectra of L-carnitine in the spectral range of 400–1850 cm^{-1}.

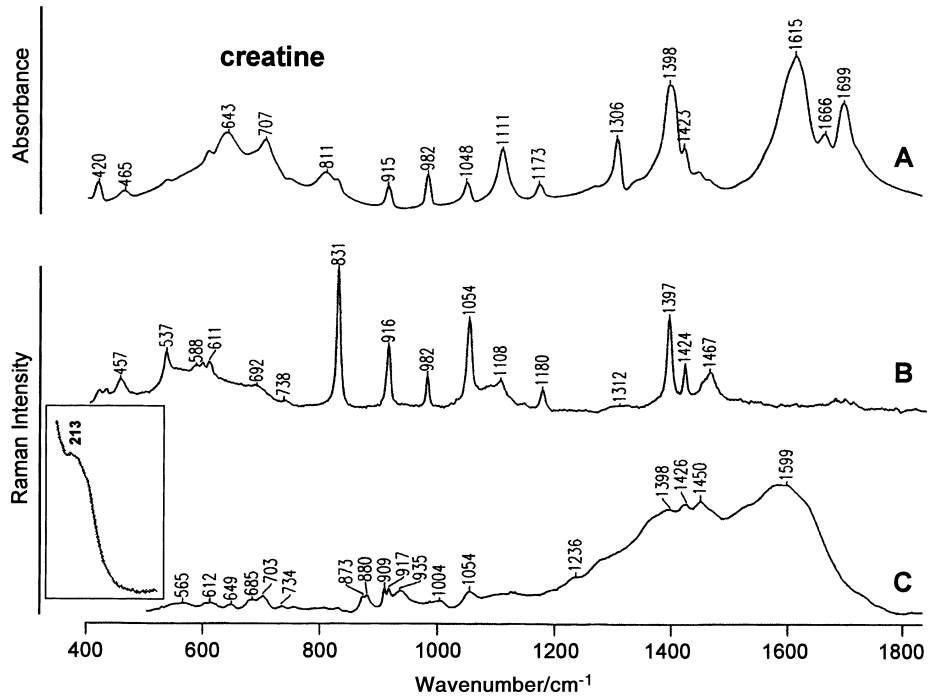

Figure 4. The FT-IR (A), RS (B), and SERS (C) spectra of creatine in the spectral range of 400–1850 cm^{-1}.

wavenumbers of the group atoms' vibrations, we briefly discuss the characteristic FT-IR, RS, and SERS bands of HMB and L-carnitine, which are crucial for the characterization of these compounds.[34,35] On the other hand, on the basis of the spectroscopic studies of creatine[36] and its complexes with palladium(II and III)[37] and phenylmercury(II),[38] we have made band assignments for this compound. Here, we only briefly discuss some FT-IR, RS, and SERS bands that are characteristic of this compound. The wavenumbers together with our proposed band assignments for HMB, L-carnitine, and creatine are summarized in Tables 1–3, respectively.

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Table 1. Wavenumbers (cm^{-1}) and proposed band assignments for FT-IR, RS, and SERS spectra of HMB

HMB			
Assignment	FT-IR (cm^{-1})	RS (cm^{-1})	SERS (cm^{-1})
ν(C═O)		1653	**1630**
			1606
ν_{as}(COOH)/ν_{as}(COO^-)	1585	1585	
	1553		
			1515
$\delta(CH_3)$	1473	1462	
$\delta(CH_2)$	1437	1441	**1447**
ν_s(COOH) and ν(CC)/ν_{as}(COO^-)	1405	1408	**1394**
$\delta(C_\alpha H)$	1388		**1369**
$\delta_{in-pl.}$(CH)		1306	
ρ_w(COH)	1255	1258	
$\nu_{out\text{-}of\text{-}ph.}$(CCO)	1196	1194	**1191**
	1173	1177	
ν(CC)	1130	1128	
			1063
ν(CC)		1014	
		995	
		954	
		935	
ν(C–COOH)/ν(C–COO^-)	914	914	**917**
			909
$\rho_w(CH_2)$	897	898	**880**
$\nu(C_3O)$	820	823	**873**
		800	
$\rho_r(CH_2)$		748	**789**
$\rho_t(CH_3)$	712		**725**
δ(CH)			**692**
ρ_w(COOH)		663	
Skeletal and δ_b(COH)	647	632	
Skeletal	525	526	
	465	466	
		443	
		424	

Table 2. Wavenumbers (cm^{-1}) and proposed band assignments for FT-IR, RS, and SERS spectra of L-carnitine

L-carnitine			
Assignment	FT-IR (cm^{-1})	RS (cm^{-1})	SERS (cm^{-1})
ν(C═O)			**1631**
			1607
ν_{as}(COOH)/ν_{as}(COO^-)	1586	1584	**1529**
$\delta(CH_2)$	1480	1493	
$\delta(CH_3)$		1461	
$\delta(CH_2)$		1430	**1449**
δ(CH)	1414	1416	
ν_s(COOH) and ν(CC)/ν_{as}(COO^-)	1396	1385	**1396**
ν(CN) and/or δ(CN)		1374	**1365**
$\delta(CH_2)$	1335	1338	
$\rho_{t(in-ph.)}(CH_2)$		1286	
ρ_w(COH)		1245	
$\nu_{out\text{-}of\text{-}ph.}$(CCO)	1149	1195	
		1153	
		1137	
ν(CN) and/or ν(CC)	1108	1099	**1097**
	1050	1059	**1059**
		1041	**1014**
ν(C–$N^+(CH_3)_3$)	964	971	
ν_{as}(C–$N^+(CH_3)_3$)	944	948	**944**
ν(CC)		938	
ν(C–COOH)/ν(C–COO^-)		913	**917**
			909
$\rho_w(CH_2)$	872	894	**880**
		871	**874**
$\rho_r(CH_2)$		772	
ρ_w(CN)	809		
ν_s(C–$N^+(CH_3)_3$)	772		
δ(CH)	702		**690**
Skeletal and δ_b(COH)	625	634	**616**
Skeletal		558	**567**
		497	
		452	

HMB

In the FT-IR (Fig. 2(A)) and RS (Fig. 2(B)) spectra of HMB, the enhanced bands are mainly due to the carbonyl, hydroxyl, methyl, and methylene group vibrations. The strong IR and weak RS band at 1585 cm^{-1} is due to the–COOH asymmetric stretching vibration, ν_{as}(COOH). At slightly lower wavenumbers (~1405 cm^{-1}), in both spectra, a band of rather strong intensity of the symmetric stretching vibration of the carboxyl group is observed coupled with the stretching vibration of the C–C bond, ν_s(COOH) + ν(CC). On the other hand, the rather weak 914 cm^{-1} band is due to the oscillation of the C–C bond adjacent to the carboxyl group, ν(C–COOH). In addition, only in the Raman spectra of HMB, two additional bands of the–COOH group are observed at the 1653 and 663 cm^{-1} (very weak), which are due to the C═O stretching ν(C═O), and –COOH wagging vibrations ρ_w(COOH), respectively.

In the wavenumber ranges of 1480–1300 and 900–690 cm^{-1}, in the FT-IR and RS spectra, bands due to the methyl and methylene group vibrations are enhanced. The intense bands at 1462–1473 and 1437–1441 cm^{-1} are due to the $-CH_3$ and $-CH_2-$ deformations, respectively, while the ~897 cm^{-1} band is due to the $-CH_2-$ wagging vibration, $\rho_w(CH_2)$. The 748 cm^{-1} Raman and 712 cm^{-1} IR bands are assigned to the $-CH_2$ rocking, $\rho_r(CH_2)$, and $-CH_3$ twisting vibrations $\rho_t(CH_3)$, respectively. In addition, in the RS spectrum

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Table 3. Wavenumbers (cm^{-1}) and proposed band assignments for FT-IR, RS, and SERS spectra of creatine

Creatine			
Assignment	FT-IR (cm^{-1})	RS (cm^{-1})	SERS (cm^{-1})
ν(C═O)	1699		
ν(C═N)	1666		
$\rho_s(NH_2)$	1615		**1599**
$\delta(CH_3)$		1467	**1450**
$\delta(CH_2)$	1423	1424	**1426**
ν_s(COOH), ν(CN), and/or δ(CN)	1398	1397	**1398**
$\delta(CH_3)$, $\delta(NH_2)$, and/or $\rho_t(NH_2)$	1306	1312	
ν(R-NH_2)			**1236**
δ(N–CH_3) and/or ν_{as}(CNC)	1173	1180	
ν_{as}(N–CH_3)	1111	1108	
ν_{as}(CN) and/or ν(R–NH_2)	1048	1054	**1054**
δ(CH) and/or $\rho_w(CH_2)$	982	982	**1004**
			935
ν(C–COOH)/ν(C–COO^-)	915	916	**917**
			909
$\rho_w(CH_2)$			**880**
			873
ρ_w(R–NH_2)	811	831	
$\rho_t(CH_3)$		738	**734**
$\rho_w(NH_2)$	707	692	**703**
			685
Skeletal	643	611	**649**
	465	588	**612**
	420	537	**565**
		457	

(Fig. 2(B)) the 1306 cm^{-1} band is observed and assigned to the in-plane deformation vibration of the C–H moiety.

It is worth pointing out that two medium intensity bands of the –COH wagging vibration, ρ_w(COH), and deformation bending vibration, δ_b(COH), are observed in both FT-IR and RS spectra. The former band appears at around 1255 cm^{-1}, while the latter one is at 647–632 cm^{-1} and is coupled to the skeletal vibrations. Other bands that involve the–OH group vibrations are enhanced in the spectral ranges of 1194–1173 cm^{-1} (the C–C–O out-of-phase stretching vibrations, $\nu_{out-of-ph.}$(CCO)) and 800–823 cm^{-1} (the C_3O stretching vibrations, $\nu(C_3O)$).

In the SERS spectrum of HMB (Fig. 2(C)) adsorbed on the colloidal silver surface, bands due to the deprotonated carboxylate, hydroxyl, and $-CH_2$ group vibrations are mainly enhanced. The 1394 cm^{-1} band of medium intensity is due to the $-COO^-$ group symmetric stretching vibration, $\nu_s(COO^-)$. Its appearance suggests that this group interacts with the silver surface. The bands at 909–917 cm^{-1} assigned to $\nu(C–COO^-)$ support the above conclusion. Moreover, the medium intensity of these bands suggests that the C–C bond adjacent to the deprotonated carboxylate group probably adopts a vertical orientation with respect to the silver surface. This phenomenon proves that the HMB molecule binds to the silver surface through the hydroxyl group as well. The 1191 and 873 cm^{-1} bands confirm the postulated mode of interaction. The former band is due to the $\nu_{out-of-ph.}$(CCO) mode, whereas the latter is due to $\nu(C_3O)$. The HMB SERS spectrum shows also the medium intensity bands of the $-CH_2$ and $-CH_3$ vibrations. The 1447, 880, 725, and 692 cm^{-1} bands due to the $\delta(CH_2)$, $\rho_w(CH_2)$, $\rho_t(CH_3)$, and δ(CH) modes suggest that these moieties assist in the HMB binding to the silver surface.

L-Carnitine

As in the case of HMB, in the FT-IR (Fig. 3(A)) and RS (Fig. 3(B)) spectra of L-carnitine, bands due mainly to the carboxyl and –COH group vibrations are enhanced. These vibrations include bands at ~1586, 1396–1385, 1245, 1137–1195, 913, and 625–634 cm^{-1} due to the ν_{as}(COOH), ν_s(COOH) + ν(CC), ρ_w(COH), $\nu_{out-of-ph.}$(CCO), ν(C–COOH), and δ_b(COH) modes (Table 2), respectively.

In addition, in the L-carnitine spectra, bands due to vibrations of the CN and C–$N^+(CH_3)_3$ fragments are enhanced. The C–$N^+(CH_3)_3$ moiety is characterized by the three bands at around 970, 944, and 772 cm^{-1} (see Table 2 for detailed band wavenumbers), which are due to the stretching, ν(C–$N^+(CH_3)_3$), asymmetric stretching, ν_{as}(C–$N^+(CH_3)_3$), and symmetric stretching vibrations, ν_s(C–$N^+(CH_3)_3$), respectively. These bands exhibit higher intensity in the Raman spectrum than in the corresponding FT-IR spectrum of L-carnitine. On the other hand, the C–N bond gives rise to the 1374 cm^{-1} Raman (ν(CN) and/or δ(CN)) and 809 cm^{-1} IR (ρ_w(CN)) bands, as well as to several IR and Raman bands (ν(CN) + ν(CC)) observed in the range of 1041–1108 cm^{-1}.

Additionally, in both vibrational spectra of L-carnitine, the bands due to the methyl and methylene group vibrations are enhanced. Their detailed wavenumbers together with their allocation are summarized in Table 2 and are not discussed here in detail.

In the SERS spectrum of L-carnitine (Fig. 3(C)) adsorbed on the colloidal silver surface, several bands due to the deprotonated carboxylate, –C–$N^+(CH_3)_3$, and $-CH_3$ moieties vibrations are enhanced. The 1396 cm^{-1} band of medium intensity is due to the $\nu_s(COO^-)$ mode. Its enhancement indicates that the deprotonated carboxylate group directly interacts with the colloidal silver surface. The 909–917 cm^{-1} bands which are due to $\nu(C–COO^-)$ strongly support this statement. The intensities of these bands suggest that the C–C bond adjacent to the $-COO^-$ group has to adopt tilted or close-to-perpendicular orientation on the silver surface. The appearance of the 944 and 1014–1097 cm^{-1} weak bands due to the ν_{as}(C–$N^+(CH_3)_3$) and ν(CN) modes points out that the C–$N^+(CH_3)_3$ fragment is also in close proximity to the silver surface.

Creatine

The FT-IR (Fig. 4(A)) and Raman (Fig. 4(B)) spectra of creatine are dominated by the bands due to the $-NH_2$ group and C–N bond vibrations. We propose that the bands at around 692–707, 811–831, 1048–1054, ~1111, ~1173, 1306, and 1615 cm^{-1} (see Table 3 for their detailed wavenumbers) are due to the $-NH_2$ wagging, $\rho_w(NH_2)$, C–NH_2 wagging, $\rho_w(C-NH_2)$, CN asymmetric stretching and C–NH_2 stretching, $\nu_{as}(CN) + \nu(C-NH_2)$, N–$CH_3$ asymmetric stretching, $\nu_{as}(N-CH_3)$, N–CH_3 deformation and CNC asymmetric stretching $\delta(N-CH_3) + \nu_{as}(CNC)$, $-NH_2$ deformation and twisting, $\delta(NH_2) + \rho_t(NH_2)$, and $-NH_2$ scissoring vibrations, $\rho_s(NH_2)$, respectively. In addition, the bands due to the C=N bond stretching, $\nu(C{=}N)$, and CCN deformation oscillations, $\delta(CCN)$, at 1666 and 420 cm^{-1}, respectively, are observed only in the FT-IR spectrum (Fig. 4(A)).

Mieteva *et al.*[37] proposed that the band of strong intensity observed at 1398 cm^{-1} is due to the $\nu(CN) + \delta(NH_2)$ mode. We suggest that, coincidently, the carboxyl group symmetric stretching vibrations, $\nu_s(COOH)$, appear at this wavenumber. The other two bands due to oscillations of the carboxyl fragment are seen at 915 and 1699 cm^{-1} and are readily assigned to the $\nu(C-COOH)$ and $\nu(C{=}O)$ modes, respectively.

Numerous bands of the $-CH_3$ and $-CH_2$ groups are enhanced in the FT-IR and RS spectra. Their wavenumbers and assignments are given in Table 3 and will not be discussed in this work.

The SERS spectrum of adsorbed creatine on the colloidal silver surface (Fig. 4(C)) shows bands due to the amine, deprotonated carboxylate, methyl, and methylene group vibrations. The bands in the spectral region of 1300–1650 cm^{-1} overlap, forming a very complex and broad envelope. The 1398 cm^{-1} band is due to $\nu_s(COO^-)$, which indicates that the deprotonated carboxylate group binds to the silver surface. This observation is supported by the weak bands at 909 and 917 cm^{-1} assigned to the $\nu(C-COO^-)$ mode. The weak intensities of these bands suggest that the C–C bond adjacent to the $-COO^-$ group probably adopts a horizontal position with regard to the silver surface. The enhancement of the 703–685, 1054, 1236, and 1599 cm^{-1} bands of weak intensity indicates that the amine group is also in close proximity to the silver surface. For detailed band assignments of these bands, see Table 3. In addition, in the SERS spectrum of creatine, bands due to the different $-CH_3$ (734 cm^{-1}, $\rho_t(CH_3)$; 1450 cm^{-1}, $\delta(CH_3)$) and $-CH_2$ group vibrations (880–873 and 1004 cm^{-1}, $\rho_w(CH_2)$; 1426 cm^{-1}, $\delta(CH_2)$) are observed. The appearance of these bands points out that carnitine interacts with the silver surface via these two groups.

CONCLUSIONS

In this work, HMB, L-carnitine, and *N*-methylglycocyamine (creatine) have been investigated using FT-IR, Raman, and SERS methods. We have briefly discussed the characteristic RS and IR bands that are crucial for understanding the vibrational structures of these compounds as well as necessary for interpreting the SERS spectra. Such typical strategy allows us to propose a mechanism of interaction of the investigated molecules with the silver colloidal surface, namely, it allows us to suggest the geometry adopted by the title compounds upon adsorption.

Summarizing, the strongest absorptions in the FT-IR spectrum of creatine are observed at 1398, 1615, and 1699 cm^{-1}, which are due to $\nu_s(COOH) + \nu(CN) + \delta(CN)$, $\rho_s(NH_2)$, and $\nu(C{=}O)$ modes, respectively, while that of L-carnitine (1396/1586 cm^{-1} and 1480 cm^{-1}) and HMB (at 1405/1555/1585 cm^{-1} and 1437–1473 cm^{-1}) are associated with the carboxyl and methyl/methylene group vibrations, respectively (see Tables 1–3 for detailed assignments). On the other hand, the strongest bands in the RS spectrum of HMB observed at 748/1442/1462 cm^{-1} and 1408 cm^{-1} are due to methyl/methylene deformations and carboxyl group vibrations, respectively. The strongest Raman band of creatine at 831 cm^{-1} ($\rho_w(R\text{-}NH_2)$) is accompanied by two weaker bands observed at 1054 and 1397 cm^{-1}, which are due to $\nu(CN) + \nu(R-NH_2)$ and $\nu_s(COOH) + \nu(CN) + \delta(CN)$ modes, respectively. In the case of L-carnitine, its Raman spectrum is dominated by bands at 772 and 1461 cm^{-1} assigned to the $\rho_r(CH_2)$ and $\delta(CH_3)$ modes, respectively.

We show clearly that HMB directly interacts with the silver surface through the deprotonated carboxylate, hydroxyl, and methylene groups. We have also given evidence that L-carnitine binds to the surface of the silver nanoparticles through the carboxylate and $-N^+(CH_3)_3$ groups. In the case of creatine, we proposed that this molecule interacts with the silver surface mainly through the amine and deprotonated carboxylate groups. However, the methyl and methylene groups of this molecule are in close proximity to this surface. In addition, the C–C bond adjacent to the $-COO^-$ fragment, in HMB and L-carnitine, probably adopts a perpendicular orientation on the silver surface, while in the case of creatine it adopts a rather horizontal position with regard to the silver surface. All the above conclusions are drawn on the basis of the intensity enhancement, broadening, and wavenumber shifts of the observed bands.

Figure 5 illustrates the suggested manner of binding of HMB, L-carnitine, and creatine on the silver surface.

It should be also noted that all the molecules studied in this work belong to the C_1 point symmetry group. Thus, all the IR-active modes are also Raman active with coincident wavenumbers. The presented spectra, in several cases, displayed bands that differ by a few cm^{-1}. This phenomenon is due to an overlapping of bands, which changes the intensity going from IR to RS, and is usually expected, i.e. stronger bands in IR are usually observed as weaker bands in RS and vice versa. Thus, the appearance of additional bands in the SERS spectra is not connected to the changes in selection rules, but simply because these bands are strongly enhanced by the SER effect. In the IR and Raman

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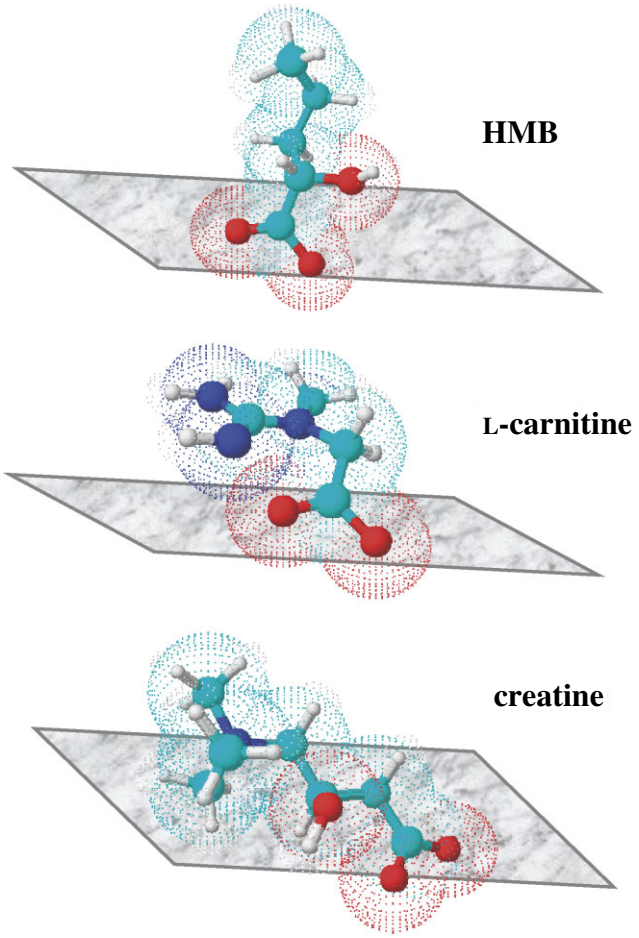

Figure 5. Proposed manner of interactions of HMB, L-carnitine, and creatine with the silver surface.

spectra, they can be seen as very weak bands after expansion of the intensity scale. On the other hand, disappearance of the IR (Raman) bands in the SERS spectra shows clearly that the groups responsible for the proper vibrations observed in the IR (Raman) spectra do not interact with the silver surface.

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