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ORIGINAL ARTICLE

Polarized spectroscopic elucidation of *N*-acetyl-L-cysteine, L-cysteine, L-cystine, L-ascorbic acid and a tool for their determination in solid mixtures

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Abstract Method of linear polarized vibrational (both IR- and Raman) spectroscopy of oriented colloids in nematic host is applied on *N*-acetyl-L-cysteine, L-cysteine, L-cystine and L-ascorbic acid with a view to obtain experimental bands assignment and local structural elucidation in solid-state. Structural results are compared with available crystallographic data for all of the systems studied. Scopes and limitations of the polarized method are shown. Discussion on the correlation between polarized spectroscopic data and the space group type as well as the number of the molecules in the unit cell (Z) is performed. Compounds with monoclinic space group $P2_1$, containing $Z = 1$ (*N*-acetyl-L-cysteine) and 2 (L-cysteine and L-ascorbic acid) are elucidated. One of the rare for organic molecules, hexagonal $P6_122$ space group and $Z = 6$ (L-cystine) is also elucidated. Experimental assignment of the characteristics frequencies is obtained, explaining the typical for the crystals Fermi-resonance, Fermy–Davydov and Davydov splitting effects. For first time in the literature we are reported the orientation of the solid-mixture in nematic host, using the trade product ACC (Hexal, Germany), containing mainly *N*-acetyl-L-cysteine and L-ascorbic acid. Quantitative IR-spectroscopic approach for determination of solid mixtures is presented as well. The intensity ratio between 1,716 cm^{-1} (characteristic for *N*-acetyl-L-cysteine) and 990 cm^{-1}, (attributed *N*-acethyl-cysteine and vitamin C) is used. Linear regression analysis between content and the peak ratio data for ten solid-binary mixtures, leads to straight-line plot $y = 1.08_2$ $(\pm 0.04_9) + (-0.11_4 \pm 0.01_1)x$, where $x = 1/X_i$. Factor r of 0.9641 and a reliability of 98.85% are obtained. The analysis of ACC 200 (Hexal, Germany) show that the IR measurements leads to standard deviation of 0.010 and 0.011 at P about 0.0500 for the systems and a confidence of $>98.77_1\%$.

Keywords *N*-Acetyl-L-cysteine · L-Cysteine · L-Cystine and L-ascorbic acid · IR-LD spectroscopy · Raman spectroscopy · Determination and analysis of solid mixtures

Introduction

Considerable commercial interest of the pharmaceutical industry in *N*-acetyl-L-cysteine as derivative of the sulfur-containing amino acid, L-cysteine (Lappas et al. 2005; Alipour et al. 2007) is base on its biological properties. Pharmaceutical industry offers *N*-acetyl-L-cysteine as powder usually with vitamin C (L-ascorbic acid). In this respect the determination of *N*-acetyl-L-cysteine in solids is an ever-growing interest. The development of fast, simple and reliable analytical methods for the determination is reasonable. One of the powerful and routine method for determination of solids in pharmaceutical industry is powder X-ray diffraction, moreover it is unique due to the combination of absolute specify with high degree of accuracy. However, the method is relatively expensive, difficult for operation and/or requiring a preliminary samples treatment. Conventional infrared

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(IR-) and Raman spectroscopy are also wide used in pharmaceutical industry, however the choice of suitable for analysis often is difficult on account of the typical for crystals effects of Fermi-resonance, Fermi–Davydov, Davydov or Evans' hole (Davydov 1962, 1968; Winston 1951; McClure 1954; Lisitsa et al. 1972, 1974; Evans 1960). Linear-polarized IR-spectroscopy of oriented colloids in nematic host around many of these problems. The method is unique for experimental assignment of the characteristic bands and local structural elucidation independently of the crystalline or amorphouse character of the samples (Ivanova et al. 2004, 2006, 2007; Koleva et al. 2008a). The method has been successful applied for analysis of amino acid derivatives and small peptides in series of research and review articles and book chapter (Kolev et al. 2007, 2008a, b, c, d, e; Koleva et al. 2008b). Investigation of the internal motion of biomolecules and its biological functions is one of the most important topics in molecular biology. The present study was devoted to use polarized vibrational spectroscopy to study biological interesting objects with pharmaceutical application. Therefore, the aim of presented study is polarized spectroscopic and structural elucidation in solid-state of *N*-acetyl-L-cysteine, free amino acid L-cysteine, using IR-LD and Raman spectroscopy of oriented colloid suspensions in nematic host. A development of quantitative method for determination of these compounds in solid mixtures also shown. In the pharmaceutical industry above stated compounds are offered as powders with vitamin C, thus requiring a detail characterization of L-ascorbic acid as well. The possibility for dimerisation of all HS-containing amino acids (Ivanova et al. 2004), forming S–S disulphide bridges under different conditions proposed an elucidation of corresponding derivatives of *N*-acetyl-L-cysteine and L-cysteine. The independent methods of HPLC tandem mass spectrometry (ESI MS/MS) are also applied. Studying much marvelous function in biomacromolecules from dynamic point of view (Chou 1988, 1989) is a most challenging project today, and the technique described in this paper might greatly empower the ability of scientists in this area. The Biological Functions of Low-Frequency Phonons' (Chou and Chen 1977) was published, many follow-up studies and evidences have indicated that studies of low-frequency (or terahertz frequency) collective motions in proteins and DNA hold a very high potential to reveal the profound dynamic mechanisms of many marvelous biological functions in biological systems (Chou 1983a, b, 1984a, b, c, 1985a, b, 1986, 1987, 1988, 1989; Chou and Chen 1977; Chou et al. 1981, 1994, 1989; Chou and Kiang 1985; Chou and Maggiora 1988; Gordon 2007, 2008; Martel 1992; Sinkala 2006; Sobell et al. 1983; Zhou 1989).

Experimental part

Materials and methods

The *N*-acetyl-L-cysteine, L-cysteine, L-cystine, L-ascorbic acids were purchased from Sigma-Aldrich and Merck, respectively. ACC 200 was Hexal product (Germany).

The IR-spectra were measured on a Thermo Nicolet FTIR Spectrometer 6,700 (4,000–400, 0.5 cm^{-1} resolution, 150 scans) equipped with a Perkin Elmer wire-grid polarizer. Non-polarized solid-state IR spectra were recorded using the KBr disk technique. The oriented samples were obtained as a suspension in a nematic liquid crystal (MLC 6815, Merck). This new method has been presented for first time in (Ivanova et al. 2004, 2006, 2007; Koleva et al. 2008a). Its validation for accuracy, precision and the influence of the liquid crystal medium on peak positions and integral absorbances of the guest molecule bands has been presented (Ivanova et al. 2004, 2006, 2007; Koleva et al. 2008a). Optimization of experimental conditions and an experimental design for quantitative evaluation of the impact of four input factors has been presented (Ivanova et al. 2004, 2006, 2007; Koleva et al. 2008a). The number of scans, the rubbing-out of KBr-pellets, the amount of studied compounds included in the liquid crystal medium and the ratios of Lorentzian to Gaussian peak functions in the curve fitting procedure on the spectroscopic signal at five different frequencies has been studied. It has been found that the procedure for the position (ν_i) and integral absorbancies (A_i) determination for each i-peak have been carried out by deconvolution and curve-fitting procedures at 50:50% ratio of Lorentzian to Gaussian peak functions, χ^2 factors within 0.00033–0.00023 (in our cases) and 3,000 iterations. The means of two treatments were compared by Student t test. The experimental IR-spectral patterns have been acquired and processed by GRAMS/AI 7.01 IR spectroscopy (Thermo Galactic, USA) and STATISTICA for Windows 5.0 (StatSoft, Inc., Tulsa, OK, USA) program packages. The applicability of the last approach for experimental IR-spectroscopic band assignment as well as an obtaining of stereo-structural information has been demonstrating in series of amino acid derivatives, and small peptides in a series of papers (Kolev et al. 2007, 2008a, b, c, d, e).

Raman spectra in solid-state are recorded on HORIBA Jobin-Yvon Raman Spectrometer.

The analyses of the samples by HPLC-ESI MS/MS were performed with a Thermo Finnigan surveyor LC-Pump. Compounds were separated on a Luna C18 column (150 × 2 mm, 4 μm particle size) from Phenomenex (Torrance, CA, USA). The mobile phase consisted of water +0.1% HCOOH (A) and acetonitrile +0.1% HCOOH (B) using a gradient program presented

Table 1 HPLC ESI MS–MS conditions

N	*t* (min)	*A* (%)	*B* (%)	Rate (μl min^{-1})
0	0.00	100	0	200
1	3.00	100	0	200
2	8.00	65	35	200
3	9.00	0	100	200
4	14.00	0	100	200
5	14.50	100	0	200
6	20.00	100	0	200

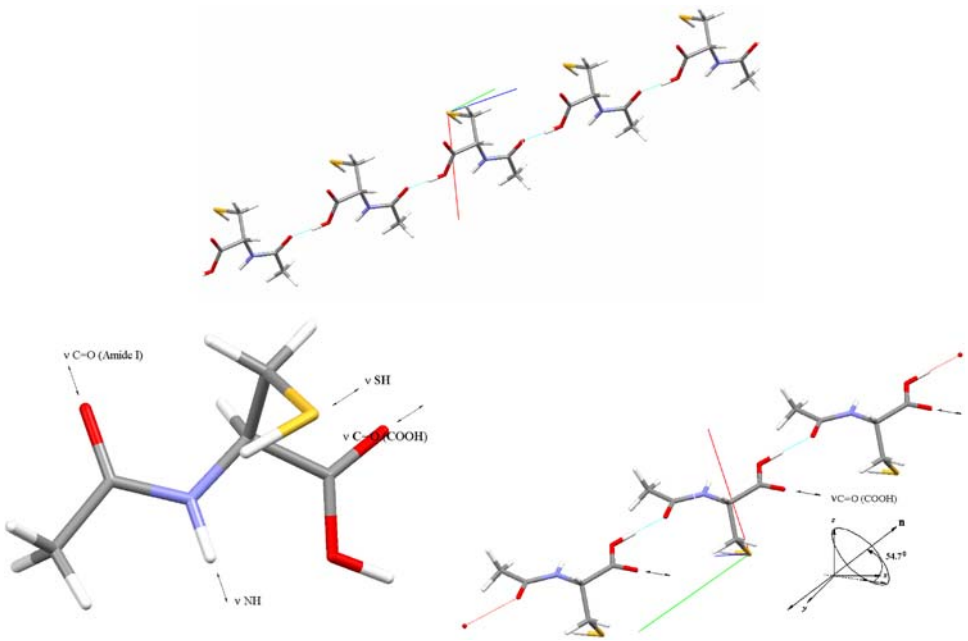
in Table 1. The compound was detected via UV and a TSQ 7000 (Thermo Electron Corporation, Dreieich, Germany) mass spectrometer.

Results and discussion

Spectroscopic elucidation

According the crystallographic data of *N*-acetyl-L-cysteine, the compound crystallized in the monoclinic space group $P2_1$ (Takusagawa et al. 1981) and the unit cell contains $Z = 1$. This fact leads to advantages to demonstrate the possibilities of the reducing-difference procedure for polarized IR-LD spectra interpretation on the system with only one molecule in the unit cell (Scheme 1). The molecules are joined in the infinite chain by means of the strong intermolecular OH···O interaction (2.540 Å) between the OH group from the COOH fragment and O=C(amide) one (Scheme 1). The NH group participates in the short contact of type NH···S(H) with a distance of 3.669 Å. The amide and COOH planes are near to co-planar disposed in the molecule of *N*-acetyl-L-cysteine closing an angle of 16.1(8)°. The amide fragment is with *trans*-configuration and dihedral angle of 177.9(9)° (Takusagawa et al. 1981). The transition moments of the ν_{NH} is near to collinear with those of $\nu_{C=O}$ (Amide I) in the frame of the crystal. The $\nu_{C=O}$ (Amide I) and $\nu_{C=O}$ (COOH) of the carboxylic group close an angle of 87.8(5)°, while the angle between ν_{SH} and $\nu_{C=O}$ (COOH)–3.2(5)°, respectively (Scheme 1).

The non-polarized IR- and difference IR-LD spectra of *N*-acetyl-L-cysteine (Fig. 1) is characterized with significant degree of macro-orientation of suspended particles in nematic host (Ivanova et al. 2007; Koleva et al. 2008a), allowing the detail and precise assignment of corresponding IR-bands. The observed broad absorption complex band within 3,100–1,800 cm^{-1} belongs to ν_{OH} stretching vibration of the intermolecular interaction OH-group of the COOH, typical for carboxylic acid. The observed multicomponent band is associated with the Fermi-resonance effect, typical for dimers of the carboxylic acids (McClure 1954; Lisitsa et al. 1972, 1974; Evans 1960). The corresponding sub maxima are eliminated at equal dichroic ratio (Fig. 1) that is direct evidence about their origin. The obtained sub maximum at very low frequency like this at 1,918 cm^{-1} is typical for systems with stronger intermolecular hydrogen bonding, like for example squaric acid derivatives (Kolev et al. 2008b, d). In our case the observed OH···O interaction with bond length of 2.540 Å, proves this assumption. The intensive band at 3,374 cm^{-1} belongs to ν_{NH} stretching vibration of the amide fragment, while the maximum at 1,716 $cm^{-1}$to $\nu_{C=O}$ stretching vibration of



Scheme 1 Unit cell and hydrogen bonding in the crystal structure of *N*-acetyl-L-cysteine (Takusagawa et al. 1981); Selected transition moments in the molecule of *N*-acetyl-L-cysteine

COOH group. The observed strong reduction of last band in the difference IR-LD spectrum of *N*-acetyl-L-cysteine (Fig. 1) indicates that the average transition moment of corresponding C=O vibration in the frame of the unit cell is oriented towards the orientation direction (***n***) of the liquid crystal closing an angle of 54.7° (Scheme 2). To Amide I ($\nu_{C=O}$) stretching vibration corresponds the IR-band at 1,693 cm^{-1}.

A direct evidence about this assignment follow by the obtained simultaneously elimination of the bands of ν_{NH} and Amide I at equal dichroic ratio, which is possible only in a trans configuration of the amide HN–C=O fragment (see above stated crystallographic data). The intensive band about 1,575 cm^{-1} belongs to δ_{NH} (Amide II) bending vibration. This maximum is strong reduced with the elimination of the $\nu_{C=O}$ (COOH) band (Fig. 1) which is also in accordance with the fact that both transition moments close an angle of 4.2(4)° (Scheme 1). Same procedure leads to elimination of the maximum at 541 cm^{-1}, thus indicating its character to $\delta_{C=O}$ (Amide IV) vibration (typical values 625 ± 25 cm^{-1}). The band at 1,411 cm^{-1} corresponds to δOH···O bending vibration, while the sharp band at 1,301 $cm^{-1}$to ν_{C-O} (typical values 1,245 ± 75 cm^{-1}). To Amide III (ν_{C-N}) belongs the band at 1,274 cm^{-1}. The other characteristic IR-bands are assigned in a following way: 796 cm^{-1} ($\delta_{C=O}$), 786 cm^{-1} (γ_{NH}, Amide V), 576 cm^{-1} ($\gamma_{C=O}$), 566 cm^{-1} ($\gamma_{C=O}$, Amide VI) and 493 cm^{-1} ($\rho_{C(=O)O}$) (typical IR-spectroscopic regions 705 ± 75, 790 ± 70, 580 ± 100, 540 ± 80 and 465 ± 80 cm^{-1}, respectively). As can be see the relatively wide and overlapping absorption regions of all of these bands leads to a difficult to their precise assignment by the conventional IR-techniques. However, by the polarization IR-LD method a detail analysis is possible, while all of these bands have different orientation of the corresponding transition moments. As for example the elimination of the bands at 786, 576 and 566 cm^{-1} at equal dichroic ratio proves the stated assignment, because of their transition

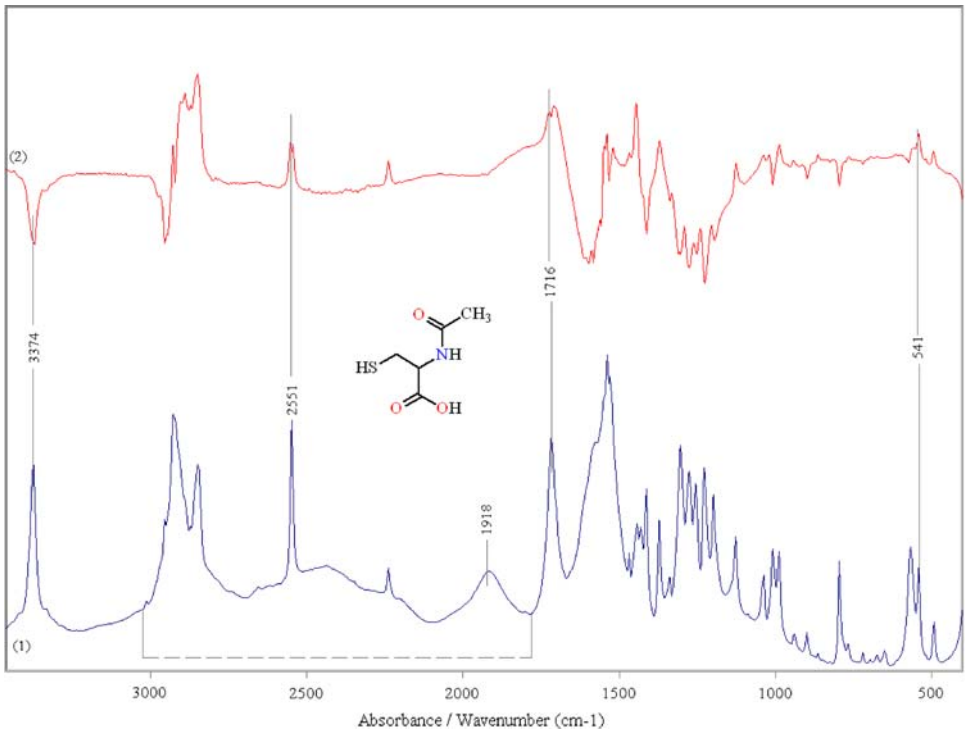
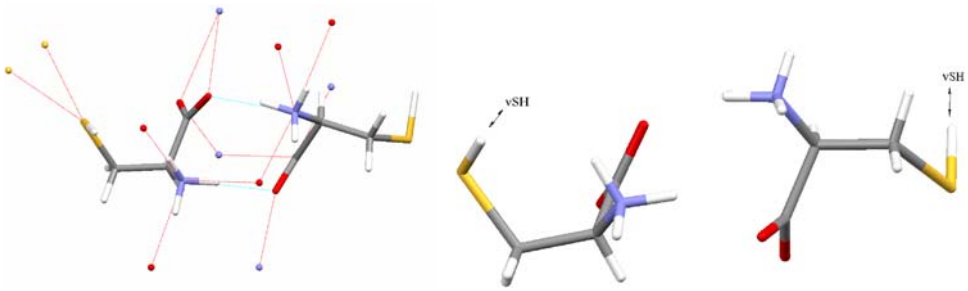


Fig. 1 Non-polarized IR-(*1*) and difference IR-LD (*2*) spectra of *N*-acetyl-L-cysteine



Scheme 2 Unit cell and hydrogen bonding in the crystal structure of L-cysteine (Görbitz and Dalhus 1996); transition moments of the ν_{SH} stretching vibration

moments are near to co-linear oriented in the frame of the near to co-planar disposition of the amide and COOH fragments in the molecule of *N*-acetyl-L-cysteine (Scheme 1). The total disappearance of the band of $\nu_{C=O}$ stretching vibration leads to a strong reduction of the maximum of ν_{SH}, which is also in accordance with the obtained geometry of the molecule, where both transition moments are near to co-linear oriented and close and angle of 3.1(2)° (Scheme 1).

The precise elucidation of the first compound requires a comparison with the data of the neutral amino acid L-cysteine. The crystallographic data (Görbitz and Dalhus 1996) show that the compound crystallized in the monoclinic $P2_1$ space group and the unit cell contains $Z = 2$ or two molecules, oriented as shown in Scheme 2. The molecules are joined in 3D network by series of intermolecular interactions: SH···S (3.589 Å) and NH_3^+···O (2.960, 2.894, 2.897, 3.027, 2.703 Å) (Scheme 2). On the basis of this facts and comparing with the previous IR- and Raman analysis of zwitterion L-cysteine (Pawlukojć et al. 2005), the solid-state IR-spectrum can be assigned in following way. It must be underlined that the interpretation and experimental assignment of the IR- and Raman bands in our study is focused on the vibrational characteristics, which are not discussed (Pawlukojć et al. 2005). The polarized IR-method and comparison with the crystallographic data is appeared to be unique for the experimental proving of the vibrational bands to corresponding modes (Fig. 2). The broad absorption band within whole 3,320–1,800 cm^{-1} belong to $\nu^{as}_{NH_3^+}$ and $\nu^{s}_{NH_3^+}$ stretching vibrations. The highest frequency sub maximum at 3,170 cm^{-1} belonging namely to $\nu^{as}_{NH_3^+}$. The observation of the IR-band at 2,069 cm^{-1} corresponds to $\delta^{a}_{NH_3^+} + \tau_{NH_3^+}$ combination mode. The intensive band at 2,551 cm^{-1} corresponds to ν_{SH} stretching vibration of the SH-group. In contrast to *N*-acetyl-L-cysteine, where the ν_{SH} stretching vibration is eliminated totally, because of $Z = 1$, in the case of L-cysteine the presence of two different oriented molecules in the unit cell leads to observation of a spitted band, with sub maxima having different polarization (Fig. 2) (Davydov 1962, 1968; Winston 1951; McClure 1954; Lisitsa et al. 1972, 1974; Evans 1960). This data indicate directly that the observed splitting is a result of Davydov splitting effect (Davydov 1962, 1968). A difference of 11 cm^{-1} with the data in (Pawlukojć et al. 2005) is obtained. The bands at 1,589 and 1,397 cm^{-1} correspond to ν^{as}_{COO-} and ν^{s}_{COO-} stretching vibrations of COO-fragment, while those at 1,654, 1,610 and 1,530 cm^{-1} to bending δ^{as}_{NH3+}, $\delta^{as'}_{NH_3^+}$ and $\delta^{s}_{NH_3^+}$ vibrations of NH_3^+-group. The definition of two symmetric bending vibrations of NH_3^+, which are degenerated in gas phase, is possible to be defining in solid-state because of the participation of the NH_3^+ group in different type of interactions leads to remove the degeneration. To ν_{C-N} stretching mode belongs the band at 1,349 cm^{-1}. The other characteristic bands can be assigned to the following way: 1,193 cm^{-1} ($\rho_{NH_3^+}$), 1,139 cm^{-1} ($\beta'_{NH_3^+}$),

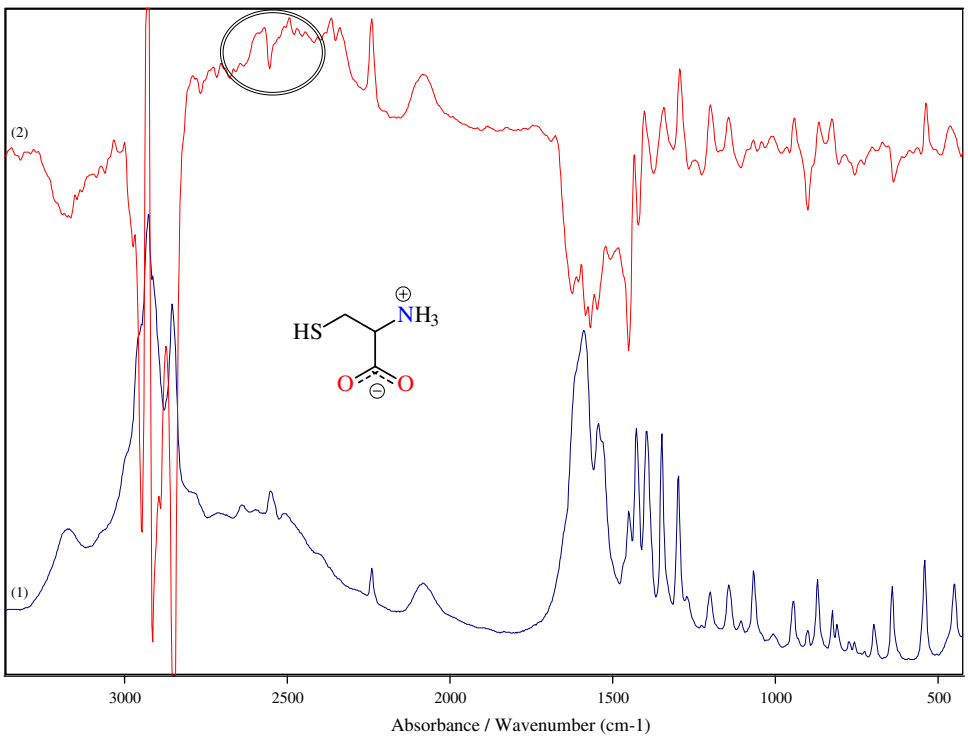
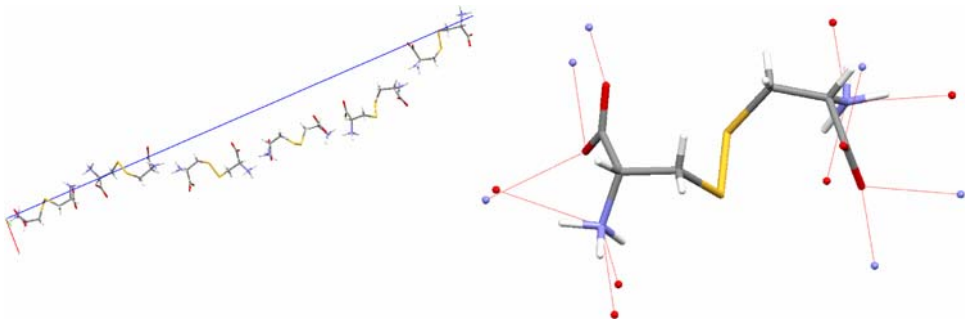


Fig. 2 Non-polarized IR-(*1*) and difference IR-LD (*2*) spectra of L-cysteine



Scheme 3 Unit cell and hydrogen bonding of L-cystine (Steinrauf and Jensen 1956; Chaney et al. 1974; Oughton and Harrison 1957)

1,064 cm^{-1} ($\nu_{CC}/\rho'_{NH_3^+}$), 822 cm^{-1} (wagging COO^-), 804 cm^{-1} (bending COO^-) and 636 cm^{-1} (δ_{COO-}), respectively. Direct evidence about the assignment above stated follow by the observation of the elimination of out-of-plane (o.p.) maxima t equal dichroic ratio.

The possible formation of the S–S disulphide bridge in these systems is on the basic to study the L-cystine, which crystallized in the primitive hexagonal $P6_122$ space group and the unit cell contains six molecules in the unit cell (Scheme 3). The hexagonal space group is rare for organic compounds and this is another reason to apply the polarization IR-LD method. The presence of the different oriented molecules in the unit cell leads to a relatively average orientation of the each of the transition moments in the molecules (Steinrauf and Jensen 1956; Chaney and Steinrauf 1974; Oughton and Harrison 1957) and for this reason the obtained difference IR-LD spectrum (Fig. 3) is characterized with bad orientation of the suspended particles in the nematic host. The molecules are joined into 3D networks by means of the intermolecular interactions of types: $NH_3^+\cdots O$ with bond lengths of 2.866, 2.812 and 2.788 Å, respectively (Scheme 3). One of the main difference between the spectra of L-cysteine and L-cystine as well as between the corresponding *N*-acetyl-L-cysteine and its derivative is the absent of the ν_{SH} band about 2,250 cm^{-1} and observation of a relatively low intensive in the IR-spectra ν_{SS} band about 500 cm^{-1}. In contrast this maximum is relatively strong intensive in corresponding Raman spectra.

As far as the L-ascorbic acid is a basic matrix component in ACC 200 pharmaceutical product of *N*-acetyl-L-cysteine, the performed spectroscopic elucidation of this compound is important for the next quantitative determination in *N*-acetyl-L-cysteine in solid-binary mixtures. IR-LD spectroscopic analysis of L-ascorbic acid is also compared with the crystallographic data of vitamin C (Hvoslef 1968). The

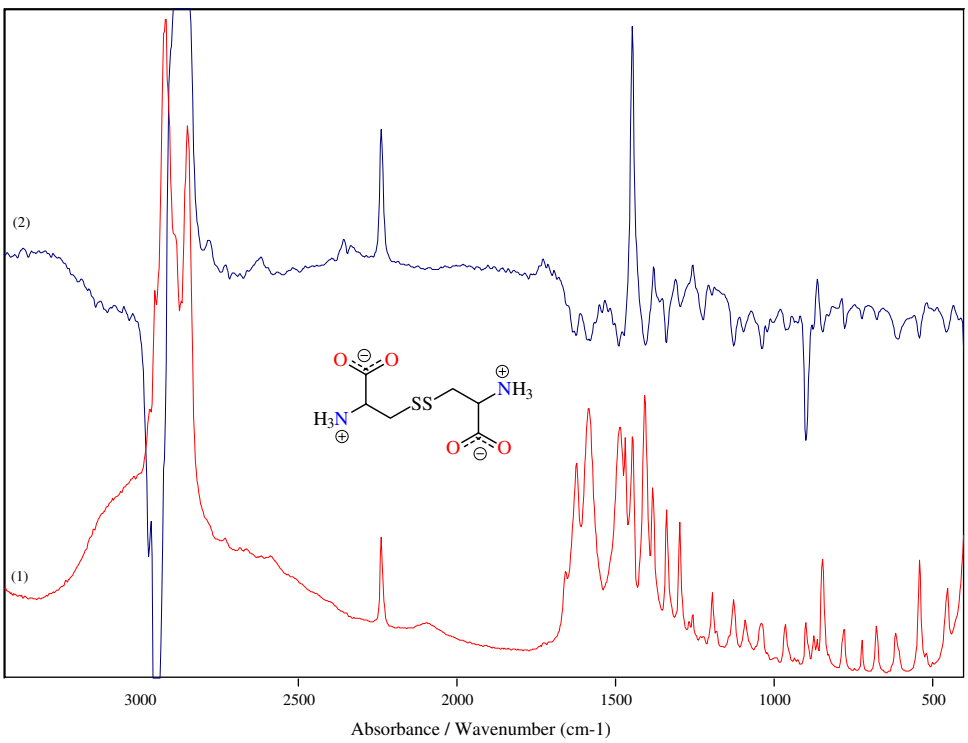


Fig. 3 Non-polarized IR-(*1*) and difference IR-LD (*2*) spectra of L-cystine

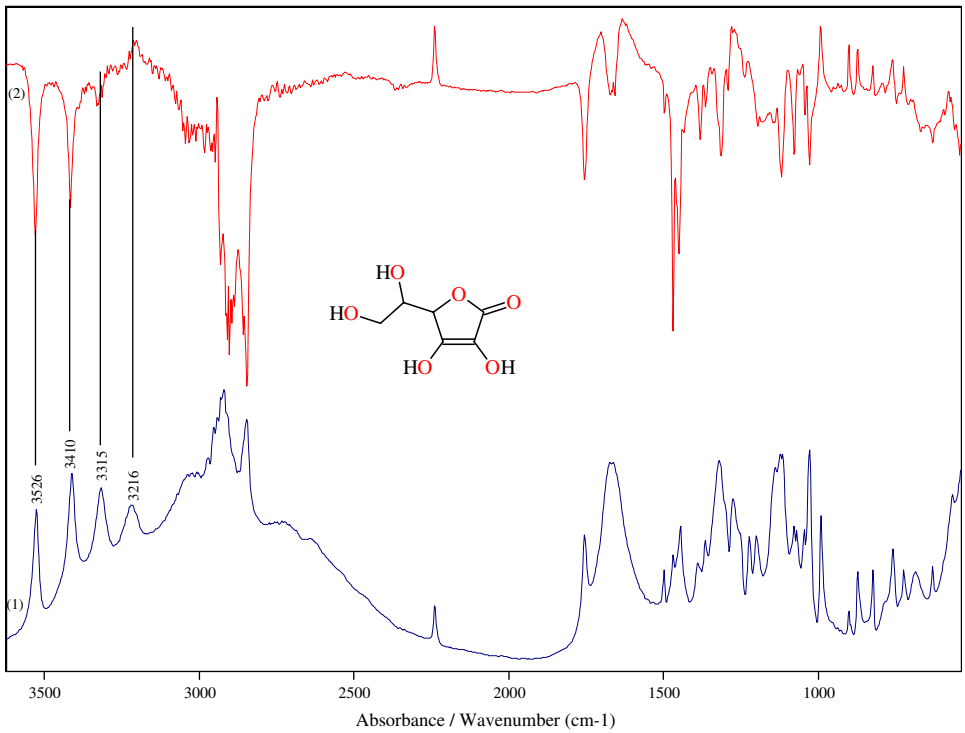


Fig. 4 Non-polarized IR-(*1*) and difference IR-LD (*2*) spectra of L-ascorbic acid

compound crystallized in the monoclinic space group $P2_1$ and the unit cell contains $Z = 2$. Similar to *N*-acetyl-L-cysteine, the compound is characterized with significant degree of orientation of suspended particles leading to adequate interpretation of the IR-bands (Fig. 4). The IR-bands at 3,525, 3,410, 3,315 and 3,216 cm^{-1} correspond to ν_{OH} stretching vibrations of the OH groups in the molecule. The last two bands are eliminated at equal dichroic ratio (see the difference IR-LD spectrum in Fig. 4), indicating a co-linear orientation of corresponding transition moments of OH groups. These data are in accordance with the crystallographic structure of the molecule. The obtained frequencies

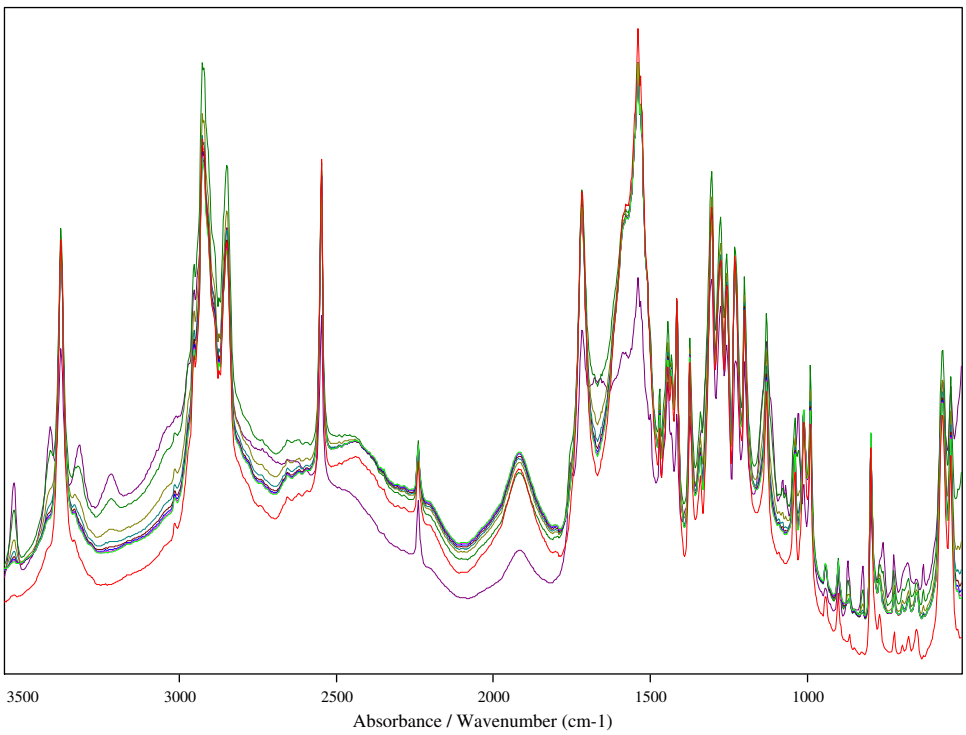


Fig. 5 IR-spectra of the solid-state mixtures of *N*-acetyl-L-cysteine and L-ascorbic acid in different mole fraction of first compound

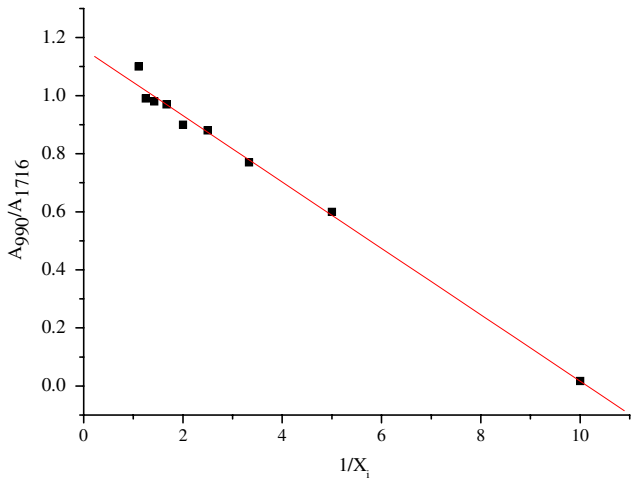


Fig. 6 IR-spectroscopic dependences of absorption peak ratios A_{990}/A_{1716} versus x ($x = 1/X_i$, where X_i is mole fraction of *N*-acetyl-L-cysteine in the binary mixtures with L-ascorbic acid

are similar with those previously reported in (Panicker et al. 2006), however the authors show as a highest frequency IR-band at 3,626 cm^{-1} (Panicker et al. 2006). The band at 1,758 cm^{-1} has been attributed to the C=O stretching of the five member lactone ring system with intense bands at 1,673 and 1,664 cm^{-1} arising from C=C stretching vibrations, coupled with the neighbouring vibrations along the conjugated system. The profile and assignment of the last band is similar to this of the $\nu_{C=C}$ stretching vibration in the squaric acid derivatives, where the stabilization of the (OHC=COH) fragment in the frame of the four-member ring leads to an observation of the high intensive broad band at 1,587 cm^{-1} corresponding namely to $\nu_{C=C}$ stretching vibration. The doublet character of the band about 1,675 cm^{-1} can be associated with the Davydov splitting effect as result of the presence of two molecules in the unit cell. Moreover these maxima are eliminated at different dichroic ratio in inflex point (Arnaudov and Ivanova 2005). The IR-bands at 990 cm^{-1} belongs to CH and OH bending vibration, while the 871 and 821 cm^{-1} to CC stretching vibration. The first vibration is similar to this of *N*-acetyl-L-cysteine.

Quantitative determination

The quantitative determination of the *N*-acetyl-*L*-cysteine in the presence of L-ascorbic acid, is based on the known equations for a two-component mixture, where the total absorbance, A^t, of the mixture at a given frequency is the sum of the absorbance of two component compounds, i and j, at the specified frequency: $A^t = A^i + A^j = a^i bc_i + a^j bc_j$. It is necessary to determine a^i and a^j from absorption measurements of mixtures containing known amounts of compounds i and j at two different frequencies, ν_1 and ν_2. This method has been already demonstrated for other pharmaceutical products (Koleva 2006; 2008c, d). If we used the ratio of total absorbance of given band to absorbance of second, typical only for determining for one component in the mixtures well as using the mole fractions (X_i and X_j) as quantity for solids resulted to following

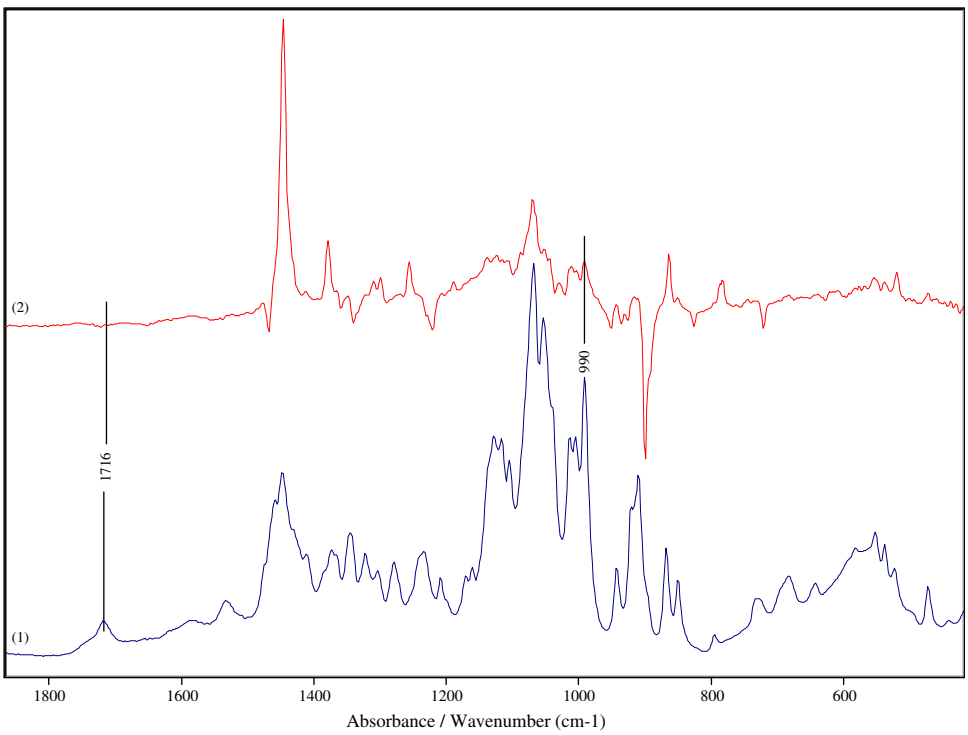


Fig. 7 Non-polarized IR-(*1*) and difference IR-LD (*2*) spectra of ACC 200

equations: $A^t_{\nu_1}/A^i_{\nu_2} = \left(a^i_{\nu_1} bX_i + a^j_{\nu_1} bX_j\right)/\left(a^i_{\nu_1} bX_i\right)$. The $X_i + X_j = 1$, then $A^t_{\nu_1}/A^i_{\nu_2} = \left(a^i_{\nu_1} bX_i + a^j_{\nu_1} b(1 - X_i)\right)/\left(a^i_{\nu_1} bX_i\right)$. The frequencies used are the band at 1,716 cm^{-1}, typical for *N*-acetyl-L-cysteine and 990 cm^{-1}, characteristic for both the compounds (Fig. 5).

Ratios of IR-characteristic peaks of each of determining compound *N*-acetyl-L-cysteine (ν_2, 1,716 cm^{-1}) with second peak at 990 cm^{-1} (ν_1), typically for both compounds in corresponding solid mixtures are used for quantification of the modifications studied using the above state mathematical model. Repeated IR-spectroscopic analysis of three replicated samples for each mole fraction for both systems studied is applied. The results of the mean peak ratio are presented in Fig. 6.

Linear regression analysis between content and the peak ratio data gave straight-line plots: $y = 1.08_2\ (\pm 0.04_9) + (-0.11_4 \pm 0.01_1)x$, where $x = 1/X_i$. The corresponding r factor is 0.9641 and the obtained r^2 value of 0.9885, give a reliability of 98.85%.

The correlations between the results for samples with different amounts of all of the systems studied, obtained by spectroscopic and HPLC ESI MS/MS techniques demonstrate good agreement with correlation coefficients >0.9998. Samples for HPLC ESI MS/MS was done using the procedure described for quantitative determination.

The application of this mathematical model on real commercial product has been demonstrated on ten different powder samples of ACC 200 (Hexal product, Germany), containing 200 mg of *N*-acetyl-L-cysteine and 25 mg L-ascorbic acid, respectively. Independently of that the product is a multicomponent, the used IR-characteristic bands are with lower intensity of the band at 1,716 cm^{-1} (Fig. 7), but the analysis by the three replicates were made for each sample, show that the IR measurements gave a standard deviation of 0.010 and 0.011 at P about 0.0500 for the systems. The confidence of $>98.77_1\%$.

For the commertial product ACC 200 the possibilities of the orientation of multicomponent mixtures are demonstrated for first time. As can be see from the Fig. 7, an underlined degree of macro-orientation of the suspended particles is observed.

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