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Short communication

Determination of cephalosporins in solid binary mixtures by polarized IR- and Raman spectroscopy

Bojidarka B. Koleva[a,*], Tsonko M. Kolev[b,c], Michael Spiteller[d]

[a] University of Sofia "St. Kl. Ohridsky", Department of Analytical Chemistry, 1 J. Bourchier Boulevard, Sofia 1164, Bulgaria
[b] Institute of Organic Chemistry, BAS, Acad. G. Bonchev Street, Building 9, Sofia 1113, Bulgaria
[c] Plovdiv University, Department of Organic Chemistry, 24 Tzar Assen Street, Plovdiv 4000, Bulgaria
[d] Institute of Environmental Research, University of Dortmund, Otto-Hahn-Strasse 6, Dortmund 44221, Germany

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ABSTRACT

Quantitative IR- and Raman spectroscopic determinations of four cephalosporin antibiotics in six solid binary mixtures have been conducted. This is a new approach for spectroscopic determination of these antibiotics, since the corresponding quantitative analysis in solution only has been reported so far. The correlation coefficient r^2 was found to be in the confidence intervals within 99.32–99.88% and 99.90–95.54% for the systems under study by using the absorption ratios of the characteristic bands at 800 cm^{-1} and 721 cm^{-1} present in the IR- and Raman spectra of the antibiotic compounds cephalexin, cephalotin, cephaloglycin and cephamandole, respectively. Solid-state linear dichroic infrared (IR-LD) spectral analysis of the solid mixtures was carried out in order to obtain experimental IR-spectroscopic assignment of the compounds studied. Independent high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) analysis was performed for the validation of the vibrational spectroscopic data. The application of this instrumental analytical tool for the analysis of 10 tablets of the commercial products Cefamandole and Cefalotin (Actavis) was also studied.

1. Introduction

Cephalosporins, such as penicillins are antibiotics with antibactericidal activity [1] which are known to disrupt the synthesis of the peptidoglycan layer of bacterial cell walls. Their wide pharmaceutical application causes an ever-growing interest for fast, simple and reliable analytical methods for analytical determinations in binary mixtures. Cephalosporins liquid binary mixtures have already been studied by Morelly [2–5] by using first- and second-derivative spectrophotometry. Binary mixtures of cephalothin and clavulanic acid were analyzed by means of first derivative spectrophotometry [6]. However, cephalosposin antibiotics as trade products usually are powder mixtures, prepared for injection purposes and, in such a case, powder X-ray diffraction appears to be a powerful and routine method for their identification. This X-ray technique provides best selectivity; however, it is relatively expensive and requires tedious preliminary sample treatment.

The aim of this work was to elaborate a method for quantification of cephalexin, (**I**), cephalotin (**II**), cephaloglycin (**III**) and cephamandole (**IV**) (supplementary material, Scheme 1S), using IR- and Raman spectroscopy. The last two methods are relatively cheap, fast and easy for technical operation as well as interpretation of data obtained, and do not require preliminary sample dissolution methods. In the present work, we are presenting the quantitative determination of six binary mixtures containing cephalosporin in a solid-state, a method which has not been reported in the literature so far. The IR-LD spectral analysis of oriented colloids in nematic host was used for the experimental IR-band assignment and selection of appropriate bands for the quantitative determination [7–9]. This analytical tool is known to provide additional information about the supramolecular structure of the object under the ambient conditions. Polymorph forms of pharmaceutical products have also been studied [10–12]. The spectroscopic data were compared independently with HPLC tandem mass spectroscopy method for the quantitative evaluation of the systems studied. The correlations obtained were used for the quantitative analytical determination of three trade products provided as powder samples of Cefamandole and Cefalotin.

2. Experimental

The antibiotics studied were Merck & Co. products. The powder of the commercial products Cefamandole and Cefalotin were purchased from Actavis (Bulgaria). The IR spectra were measured

* Corresponding author. Tel.: +359 2 8161208; fax: +359 2 9625438.
E-mail address: BKoleva@chem.uni-sofia.bg (B.B. Koleva).

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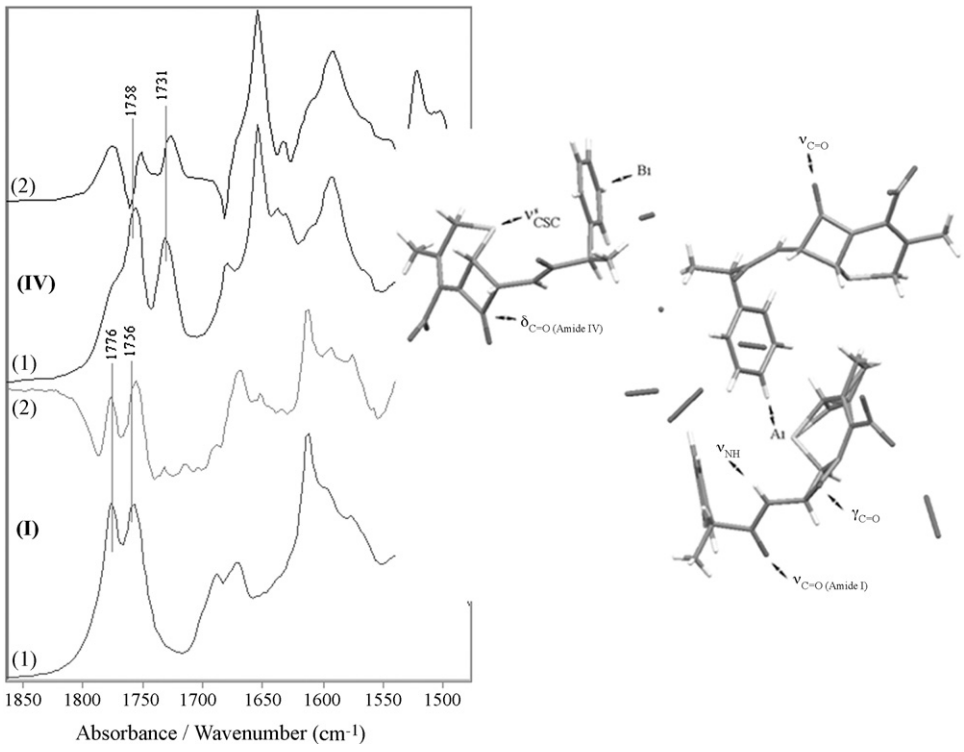

Fig. 1. Non-polarized IR- (1) and reduced IR-LD (2) spectra of (**I**) and (**IV**) after elimination of the bands at 1776 cm^{-1} and 1758 cm^{-1}, respectively. Selected transition moments in (**I**).

on a Thermo Nicolet FTIR spectrometer 7000 (4000–400 cm^{-1}, 0.5 cm^{-1} resolution, 150 scans), equipped with PerkinElmer wire-grid polarizer. Non-polarized solid-state IR spectra were recorded using the KBr disk technique at ambient conditions (T = 298 K, p = 1 atm). The oriented samples were prepared as colloid suspensions in nematic liquid crystals (MLC 6815, Merck). This linear-dichroic infrared (IR-LD) approach was previously presented recently by Ivanova et al. [7–9]. The method was validated for accuracy, precision and the influence of the liquid crystal medium on the peak positions and the integrated absorbencies of the guest molecule bands [10–12]. Raman spectra in solid-state were recorded on Horiba Jobin-Yvon Raman spectrometer. HPLC–MS/MS measurements were made using TSQ 7000 instrument (Thermo Electron Corporation). Two mobile phase compositions were used: (A) 0.1% (v/v) aqueous HCOOH and (B) and 0.1% (v/v) HCOOH in CH_3CN. Electrospray ionization (ESI) mass spectrometry: A triple quadruple mass spectrometer (TSQ 7000 Thermo Electron, Dreieich, Germany) equipped with an ESI 2 source was used and operated under the following conditions: capillary temperature 180 °C; sheath gas 60 psi, corona 4.5 μA and spray voltage 4.5 kV. The sample was dissolved in acetonitrile (1 mg ml^{-1}) and was further injected into the ion source by an autosampler (Surveyor) with a flow rate of pure acetonitrile of 0.2 ml min^{-1}. Data processing was performed by Excalibur 1.4 software. Samples for HPLC–MS/MS were prepared and analyzed by using the procedure described for the quantitative determination [2–5].

3. Results and discussion

The quantitative analysis requires an adequate identification of the characteristic IR-spectral bands of all of the cephalosporin antibiotics employed (supplementary material, Scheme 1S). The corresponding IR-spectroscopic patterns were complicated as a result of strong overlapping effects. The IR-LD characterizations discussed below overcame these difficulties to a significant degree. Independently, in all cases the experimental IR curves were processed preliminarily by deconvolution and curve fitting procedure, employing a validated approach [7–9]. The analysis below was aimed at selecting the appropriate bands for quantitative analysis. Single band, characteristic for each of the compounds that did not overlap with the bands of the matrix compound as well as a second peak, typical for each of the compounds in the corresponding binary mixtures were sought for all of the six systems under study.

The solid-state IR spectra of the samples in KBr pellets as well as the non-polarized ones prepared as nematic liquid crystal suspension of the four compounds indicated identical spectral patterns, thus avoiding the effect of the orienting mesophase on both the intensity and peak positions of the compound studied. The difference IR-LD spectra obtained (supplementary material, Fig. 1S) indicated a significant degree of macro-orientation [7–9] of the suspended particles, which resulted in the precise interpretation of the spectroscopic data and vibrational assignments.

The spectroscopic assignment of the phenyl fragment vibrations was performed in accordance with the Wislon's notation [13].

3.1. Linear-polarized IR- and Raman spectroscopic data

In the case of (**I**), the polarized IR-spectroscopic data (supplementary material, Fig. 1S) were correlated with the known crystallographic ones [14]. As far as in the solid-state (**I**) is stabilized as zwiterrionic structure [14], the protonation causes the formation of COOH group. The observed broad absorption maximum within the whole 3500–2400 cm^{-1} region was assigned to stretching asymmetric ($\nu^{as}_{NH_3^+}$) and symmetric ($\nu^{s}_{NH_3^+}$) vibration modes of the NH_3^+-group. These data correlated well with other analogous data for peptide systems [15]. The sub maxima at 3619 and 3550 cm^{-1} corresponded to ν_{OH} of H_2O and OH group of COOH

fragment. The band at 3369 cm^{-1} was assigned to the ν_{NH} stretching mode of amide (O=C–NH) group. The 1800–1475 cm^{-1} spectroscopic region was characterized with a series of bands such as the pair of bands at 1776 cm^{-1}/1756 cm^{-1} ($\nu_{C=O}$ stretching mode in β-lactame fragment); 1698 and 1687 cm^{-1} ($\delta^{as}_{NH_3^+}$ and $\delta^{as'}_{NH_3^+}$); the strong maximum at 1670 cm^{-1} amide I ($\nu_{C=O}$) mode); 1596 cm^{-1} (**8a**, Ph), 1575 cm^{-1} (**8b**, Ph), 1538 cm^{-1} (δ_{NH}, Amide II), 1496 cm^{-1} (**19a**, Ph); 759 cm^{-1} and 698 cm^{-1} of **11**-γ_{CH} and **4**-γ_{Ar} out-of-plane mode of monosubstituted benzene ring, respectively. The band at 721 cm^{-1} could be assigned to ν^{S}_{CSC}, which is usually characterized by a band within 680 ± 45 cm^{-1} region. The experimental evidence for the assignments described above was confirmed by the following IR-LD analysis, compared to the crystallographic data (Fig. 1).

What was the origin of these maxima? The splitting effect could result from the crystal field splitting effect or combination modes. The precise interpretation was obtained by the theoretical data (supplementary material) provided. As far as the protonated form was characterized by pairs of bands at 1806/1785 cm^{-1} belonging to symmetric ($\nu^{s}_{C=Ocomb}$) and asymmetric ($\nu^{as}_{C=Ocomb}$) stretching modes of O=C(OH)–CNC=O, the observed bands were combination modes. The ($\nu^{as}_{C=Ocomb}$) had low intensity in the corresponding Raman spectra. The cephalosporin nucleus was characterized by the common band at 800 cm^{-1} of Amide IV ($\delta_{C=O}$) in β-lactams (theoretical value of 799 cm^{-1}) and could be used in the IR- and Raman spectra for quantitative analysis. The characteristic bands of (**II**)–(**IV**) in Table 1S were assigned in a similar manner. The low symmetry of the cephalosporin antibiotic molecules leads to the observation of the discussed maxima both in the IR- and Raman spectra as a relatively strong band. Some discussion about the influence of the different substituents on the IR-spectroscopic patterns in (**I**)-(**IV**) was also carried out (supplementary material).

3.2. Quantitative determinations

For a two-component mixture, the total absorbance A^t at a given frequency is the sum of the absorbances of the two components, *i* and *j*, at the specified frequency: $A^t = A^i + A^j = a^i \times b \times c_i + a^j \times b \times c_j$. Molar absorbtivities a^i and a^j were determined from the absorption measurements of mixtures, containing known amounts of the compounds *i* and *j* at two different frequencies, ν_1 and ν_2. If the ratio of the total absorbance of given band (A^t) to the absorbance of a second band (A^i), typical only for one component of mixture is known, then the corresponding equation can be expressed as follows [16]:

$$\frac{A^t_{\nu 1}}{A^i_{\nu 2}} = \frac{a^i \times_{\nu 1} - a^j \times_{\nu 1}}{a^i \times_{\nu 2}} + \frac{a^j \times_{\nu 1}}{a^i \times_{\nu 2}} \times \frac{1}{X_i}$$

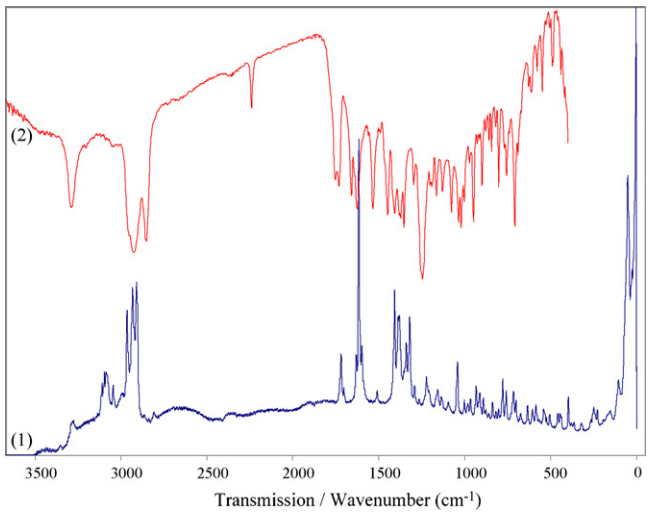

Fig. 2. Raman (1) and IR-(2) spectra of (**II**) in solid state.

The frequencies used were as follows: System (**I**)/(**II**): ν_1 = 800 cm^{-1} and ν_2 = 721 cm^{-1}; System (**I**)/(**III**): ν_1 = 800 cm^{-1} and ν_2 = 721 cm^{-1}; System (**I**)/(**IV**): ν_1 = 800 cm^{-1} and ν_2 = 721 cm^{-1}, System (**II**)/(**III**): ν_1 = 800 cm^{-1} and ν_2 = 738 cm^{-1}, System (**II**)/(**IV**): ν_1 = 800 cm^{-1} and ν_2 = 723 cm^{-1} and System (**III**)/(**IV**): ν_1 = 800 cm^{-1} and ν_2 = 723 cm^{-1}, respectively.

In addition, the (**I**)/(**II**) system was determined by using the values ν_1 = 800 cm^{-1} and ν_2 = 1756 cm^{-1}, in the corresponding IR-spectra (Fig. 2).

Similar equations were used for the Raman spectroscopy measurements [16]. For adequate comparison of the reliability of IR and Raman data, the same bands were utilized for the quantitative analysis. In the corresponding Raman spectra, the character of the bands employed was the same but their intensity was different.

The ratios of the intensity of the IR characteristic peaks mentioned above and typical for both the compounds in the corresponding solid mixtures were evaluated for quantification purposes by applying the mathematical model described above. Repeating IR- and Raman analyses of the samples (three replicates) for each molar fraction and each system was applied (supplementary material, Fig. 4S). The results of the mean peak intensity ratios are shown in Fig. 3. Linear regression analysis between the contents and the peak ratio data gave linear plots which are depicted in supplementary material, Tables 2S and 3S. The corresponding correlation coefficients *r* and r^2 are given in supplementary material, Table 2S as well. In all cases, $p < 0.0001$ values indicated significant correlation. The r^2 values gave confidence within 99.32–99.88% and 99.90–95.54% for the systems studied, using the absorption

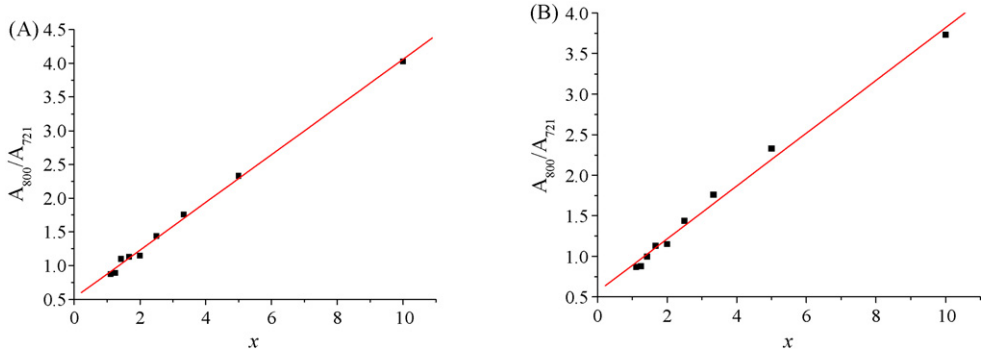

Fig. 3. IR-spectroscopic (A) and Raman (B) dependences of absorption peak ratios A_{800}/A_{721} vs. *x* ($x = 1/X_i$, where X_i is mole fraction of (**I**) in the mixtures with (**IV**).

ratios of the bands at 800 cm^{-1} and 720 cm^{-1} in both the IR- and Raman spectra, respectively. For the system (**I**)/(**II**) a straight-line plot $y = -19.020\,(\pm 1.05) + 15.84\,(\pm 0.25) \times x$, confidence interval of 0.11–0.88, $r = 0.9991$ and $r^2 = 0.9982$ were obtained. The corresponding analysis, using the Raman spectra gave r-value of less than 0.9700, due to the low intensity of the band at 1756 cm^{-1}. The comparison of the IR- and Raman data led to the conclusion that, for the systems studied, the IR-data produced minor improvement with respect to the Raman ones.

The validity of these equations was confirmed by the spectra of pure compounds, from which the ratios a^i_{800}/a^i_{721} were found to be within 0.552–0.211, respectively. Taking into account that the absorptions measured in the Raman spectrum depends on the intensity of the laser as irradiation source and on the factor K_ν, including the frequency-depending terms $A_\nu = I_0 \times K_\nu \times X$ [16], the K^i_{800}/K^i_{721} ratios were also calculated. The values obtained within $0.55 \pm 1.1 \times 10^{-2}$–$0.22 \pm 1.0 \times 10^{-2}$ were very close to the experimental ones. The limit of detection (LOD) was evaluated to be of, approximately, 0.012 molar fraction for the IR and Raman methods, respectively. The better results obtained for the IR-spectra, as compared to the Raman ones could be explained by the fact that, in the IR spectra, the characteristic bands used for evaluation were characterized by higher integral absorbencies.

The correlations between the results for samples with different amounts of (**I**)–(**IV**) fpor all of the systems studied, obtained by spectroscopic and HPLC ESI MS/MS techniques demonstrated good agreement with correlation coefficients >0.9998.

The application of this mathematical model for the analysis of the commercial products was demonstrated with ten different powder samples of the commercial antibiotics Cefamandole and Cefalotin (Actavis, Bulgaria), containing 1.108 g and 2.216 g cepamandol, respectively. The analysed samples contained 1.055 g cephalotin. Three replicates were made for each sample. The IR measurements gave a standard deviation of 0.011, 0.010 and 0.013 at p-value of, approximately, 0.0500 for the corresponding systems. For the Raman data, values of 0.013, 0.011 and 0.012 at p of, approximately, 0.0550 were obtained. The confidence value of >99.98% was obtained by using the vibrational model of quantitative analysis presented in this paper.

4. Conclusion

Quantitative determination of four antibiotic compounds such as cephalexin, cephalotin, cephaloglycin and cephamandole in six solid binary mixtures is performed by IR- and Raman spectroscopy. The correlation coefficient r^2-values within the 99.32–99.88% and 99.90–95.54% confidence are obtained by using the absorption rations of the bands at 800 and 720 cm^{-1}. The absorption bands used in the quantitative analysis are selected by a detailed linear-polarized IR- and Raman spectroscopic analysis. The mathematical model and the correlations are also applied to ten tablets of the pharmaceutical products Cefamandole and Cefalotin (Actavis, Bulgaria), giving a confidence value of >99.98% by employing the vibrational model of evaluation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2008.04.026.

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