Available online at www.sciencedirect.com
SCIENCE DIRECT
Talanta 69 (2006) 822–828
Talanta
www.elsevier.com/locate/talanta

Validation of reducing-difference procedure for the interpretation of non-polarized infrared spectra of *n*-component solid mixtures

Bojidarka B. Ivanova *, Dimiter L. Tsalev, Michail G. Arnaudov

University of Sofia, Faculty of Chemistry, 1 J. Bourchier Blvd., 1164 Sofia, Bulgaria

Received 25 July 2005; received in revised form 21 October 2005; accepted 4 November 2005
Available online 13 December 2005

Abstract

Validation of reducing-difference procedure based on subtracting of non-polarized infrared (IR) spectra of *n*-component solid mixtures is presented. The accuracy and precision are established. The limits of detection are 3.0, 2.5, 1.5 and 1.0 wt.% for 5-, 4-, 3-and 2-component mixtures, respectively. Smoothing procedures for IR spectral analysis, based on Savitzky–Golay or Fourier methods are applied as well. The mean values and relative standard deviations for peak position (ν_i) and integral absorbance (A_i) obtained by this data processing approach have been examined using Student's *t*-test.

Keywords: Reducing-difference procedure; Fourier transform infrared spectroscopy (FT-IR); *n*-Component systems; Limit of detections; Smoothing methods; Amino acids; Peptides

1. Introduction

Infrared spectroscopy (IR) is a fundamental and well established analytical technique, whose development with a view to improving possibilities for quantitative and qualitative determination is worthwhile. Moreover, substantial interest in development of robust, inexpensive and rapid procedures for quantitative and qualitative determination of major components exists in some industrial, pharmaceutical or forensic fields and customs authorities.

The so-called reducing-difference procedure for interpretation of non-polarized IR spectra of binary, solid or liquid systems has been described in [1–4], allowing the separation and determination of different components, associates [1,3] and tautomeric forms in mixtures [4]. The question about feasibility of interpretation for *n*-component systems and optimal experimental conditions for obtaining valid quantitative results is well grounded. This calls for optimization of critical experimental variables such as the number of IR scans, deconvolution and curve-fitting parameters [5]. In reducing-difference IR-spectroscopic approach as well as in any other IR subtracting procedures, could be encountered other problems. The signal-to-noise ratio decreases with every subsequent subtraction, however the comparison between the spectral curves and the identification of unknown compounds versus standards may become ambiguous. The optimization of smoothing IR spectral curve is mandatory in such cases.

Therefore, the present work is devolved to optimization and validation of the reducing-difference procedure for non-polarized IR spectra interpretation for *n*-component solid systems. The effect of smoothing parameters, based on Savitzky–Golay [6–10] and Fourier [7,8] methods has been carefully evaluated. The limits of detection (LODs) for *n*-component systems have been determined, using as model systems the solid-state mixtures of amino acids and peptides. Their choice has been governed by biological activity of these components and also by their suitable IR spectroscopic characteristics, comprising of numerous and intensive absorption maxima in the whole 4000–400 cm^{-1} IR spectral region [11–13]. On the other hand, DL-isoleucine (Ile) has been used as a model compound for validation of the technique for orientation as suspension in nematic liquid crystal in linear-dichroic infrared spectroscopy [5].

* Corresponding author. Tel.: +359 2 8161 208; fax: +359 2 9625 438.
E-mail address: ahbi@chem.uni-sofia.bg (B.B. Ivanova).

0039-9140/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2005.11.026

Table 1
Concentrations (wt.%) of the components in the solid state mixtures studied

Sample no.	n	wt.%				
		L-phe-L-phe	L-tyr	L-phe	gly-met-gly	Ile
(1)		31.33	31.33	31.33	3.00	3.00
(2)	5	31.67	31.67	31.67	2.50	2.50
(3)		32.33	31.33	31.33	1.50	1.50
(4)		32.67	32.67	32.66	1.00	1.00
(5)		32.33	32.33	32.33	–	3.00
(6)	4	32.50	32.50	32.50	–	2.50
(7)		32.83	32.83	32.83		1.50
(8)		33.00	33.00	33.00	–	1.00
(9)		48.75	48.75	–	–	2.50
(10)	3	49.25	49.25	–	–	1.50
(11)		49.50	49.50	–	–	1.00
(12)		49.75	49.75	–	–	0.50
(13)		–	98.50	–	–	1.50
(14)	2	–	99.00	–	–	1.00
(15)		–	99.50	–	–	0.50
(16)		–	–	–	–	100.00
(17)	1	–	–	–	100.00	–

2. Experimental

2.1. Materials, measurements and methods

L-tyrosine (L-tyr), L-phenylalanine (L-phe), L-phenylalanyl-L-phenylalanine (L-phe-L-phe), glycyl-L-methionyl-glycine (gly-met-gly) and Ile have been purchashed from Bachem (Darmstadt, Germany).

Seventeen solid mixtures (Table 1) have been prepared by means of mechanical mixing of individual compounds in hydraulic ball-crusher (Vibrator according to M. von Adrenne, Germany), for 5 min. All the measurements have been preformed with three individually prepared samples and three scans with 150 repetitions for each sample.

The IR spectra have been recorded on a Bomem–Michelson 100 FT-IR-spectrometer within the 4000–400 cm^{-1} range at 2 cm^{-1} resolution. The conventional (non-polarized) solid IR spectra have been obtained using KBr disk technique, where 200 mg of KBr were mixed with 1 mg of solid sample in hydraulic ball-crusher for 5 min. Prepared solid mixtures were pressed under 9.8 MPa in hydraulic press (Decimal DP36, Germany) during 3 min. The IR spectra were obtained from 150 scans. The position (ν_i) and corresponding integral absorbancies (A_i) for each i-peak were determined by deconvolution and subsequent curve-fitting procedure at 50%:50% ratio of Lorentzian to Gaussian peak functions, χ^2 factors between 0.00047–0.00039 and 2000 iterations [5]. The means of two treatments (even if based on different numbers of replicates) were compared by Student's t-test [14–16]. The experimental IR spectral curves have been acquired and processed by means of GRAMS/AI 7.01 IR spectroscopy (Thermo Galactic, USA) and STATISTICA for Windows 5.0 (StatSoft, Inc., Tulsa, OK, USA) program packages.

2.2. Reducing-difference analysis of non-polarized IR-spectra

As mentioned above and described in [1,2] for binary systems, the method consists in *subtraction* of two IR spectral curves, corresponding to samples, containing diverse concentrations of given structural units. The measurements of both samples must be made under the same conditions and sample preparation techniques (e.g. liquids, solutions in selected solvents, Nujol mull or KBr-technique). The subtraction procedure is performed until a definite band is eliminated in the obtained *reducing-difference IR spectrum*. The following equations are used:

$$A_{11} - \kappa_1 A_{12} = \varepsilon_1 b_1 c_1 - \kappa_1 \varepsilon_1 b_2 c_2 = 0 \quad (1)$$

or

$$k_1 = \frac{c_1 b_1}{c_2 b_2} \quad (2)$$

A_{11} and A_{12} in Eq. (1) are the integral absorbancies of manipulated absorption maxima in both spectra, characterized with molar absorptivity ($\varepsilon_1(\nu_1)$), concentration ($c_{1,2}$) and pathlength ($b_{1,2}$). The Eq. (1) should be valid for other IR spectral band (ν_2) characteristic for the same structure [1,2]:

$$A_{21} - \kappa_2 A_{22} = \varepsilon_2 b_1 c_1 - \kappa_2 \varepsilon_2 b_2 c_2 = 0 \quad (3)$$

and

$$\kappa_2 = \frac{c_1 b_1}{c_2 b_2} \quad (4)$$

The equalization of Eqs. (2) and (4) results in $k_1 = k_2$, i.e. the elimination of the IR spectral characteristics of given structure leads to disappearance of the obtained reducing spectra of all the bands assigned to a given compound.

For i-component system and j IR-characteristic maxima, Eq. (1) is transformed to

$$A_{ij} - \kappa_i A_{i(j+1)} = \varepsilon_i b_j c_j - \kappa_i \varepsilon_i b_{(j+1)} c_{(j+1)} = 0 \quad (5)$$

where $i, j = 1$–n.

And finally resulting in

$$k_1 = \kappa_2 = k_3 = \cdots = k_i = \frac{c_j b_j}{c_{(j+1)} b_{(j+1)}} \quad (6)$$

2.3. IR spectra subtraction

By analogy to the case of binary mixtures [1,2], for a n-component system the subtraction [17–19] of the IR spectra is obtained from the relation:

$$\mathrm{RS} = \mathrm{SS} - (\mathrm{SbS}F) \quad (7)$$

In Eq. (7), RS is the result spectrum, SS the sample spectrum, SbS the subtracted spectrum and F is the subtraction factor. By varying the F factor and looking at the obtained *reducing curve*, can be observed the disappearance of a given absorption peak. However, this procedure entails the problem with shifting the data point spacing sets. It is obvious that the proper subtraction

of one spectrum from another, requires a strict correspondence of the data points to the same x values. The *data point spacing* problem needs interpolation, which is done automatically by the software. The software also handles the problem of *the subjectivity* of iterative algorithm selection. This algorithm calculates the subtraction factor by minimizing the complexity of the residual spectrum (*Auto Subtraction Algorithm*).Smoothing procedureThe most typical smoothing methods for IR spectral curves are Savitzky-Golay [5–8] and Fourier [6,7]. Fourier smoothing is based on data inversion by Fast Fourier Transform (FFT) to the time domain, where a trapeziodal filter is applied to the high frequency region. Finally, an inverse FFT is applied to give the smoothed result. The degree of smoothing determines the cut-off point of the filter. A trapeziodal filter is applied to the high frequency region, then inverse FFT to give the smoothed result. The degree or percentage (%) of smoothing determines the cut-off point for the filter. Savitzky–Golay smoothing is based on the convolution approach which performs a least-squares fit to a specified part of data points or the procedure is controlled by the level of polynomial and number of points parameters. The degree of polynomial and the number of *points* are controlled during the procedure by specifies the order of the polynomial to fit over the specified number of points. Thus the larger number of points specified and lower order of polynomial heavier the smoothing. Only odd numbers are used for number of smoothing points and even values are rounded. The number of smoothing points must be one more than the "degree of polynomial." The algorithm used for generating the Savitzky–Golay convolution coefficients has been described in [6–9]. It is worth mentioning that the choice of smoothing parameters is somewhat subjective—data-dependent and arbitrary. The $(n-1)/2$ data points, where n is the number of points used in the convolution function, are truncated from the beginning and end of the procedure.

3. Results and discussion

3.1. Limits of detection and smoothing procedure validation

The LODs of the reducing-difference procedure for non-polarized IR spectra are examined for n-component systems starting with optimization of the standard smoothing conditions for IR curves interpretation. For that reason the complicated IR spectrum of five component mixture (No. 1 in Table 1) is stepwise reduced by subtracting the IR spectra of other components. In this case the *reduced IR spectrum* should correspond to pure Ile. A series of IR-characteristic peaks are used in the following steps: (i) from the IR spectrum of sample 1 (Fig. 1.1) is subtracted the IR curve of pure gly-met-gly (Fig. 2.1) until disappearance of the peak at 3295 cm^{-1} to yield a final reduced IR spectrum shown in Fig. 1.2. (ii) From the latter curve, L-tyr IR spectrum (Fig. 2.2) is subtracted the maximum at 3205 cm^{-1}. Its total elimination results in curve in Fig. 1.3. (iii) A subsequent subtraction (Fig. 1.3 minus Fig. 2.3 curves), where Fig. 2.3 corresponds to L-phe, results in IR curve in Fig. 1.4. In this case the subtraction is aimed at disappearance of the 1624 cm^{-1} peak, typical for L-phe. However, the complex character of the spectral curve (Fig. 1.3) calls for monitoring of a second peak, also typical for simple amino acid. For L-phe, the 844 cm^{-1} band is suitable (Fig. 2.3). (iv) Finally, from the spectrum in Fig. 1.4 is subtracted the IR curve for individual L-phe-L-phe (Fig. 2.4) until total elimination of the peak at 1495 cm^{-1} (Fig. 1.5) which corresponds to pure Ile in solid state is reached (cf. Figs. 1.5 and 2.5). The comparison between Figs. 1.5 and 2.5 demonstrates a qualitative correlation. It is noteworthy that the order of subtraction of different components in the mixture leads to identical final results.

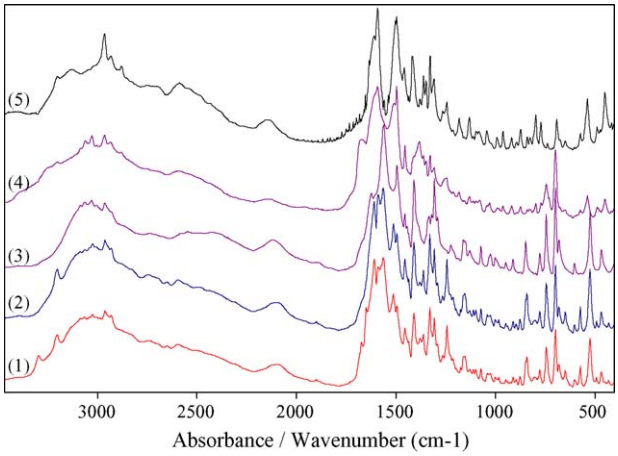

Fig. 1. Solid-state IR spectra of five component system within 3500–400 cm^{-1} (No. 1, Table 1) (1) and subtracted IR curves after stepwise reduction of the components: gly-met-gly (2), L-tyr (3), L-phe (4) and L-phe-L-phe (5).

However, the exact qualitative or quantitative IR-spectra identifications of unknown compound in mixtures are difficult, since the pathlengths in all solid-state techniques are uncertain. For that reason the analysis requires a comparison between the pairs of IR spectral curves (standard, pure compound and reduced IR spectra) as regards the peak position and integral absorbance ratios of series of IR-characteristic peaks. In DL-isoleu are chosen the multiple absorption maxima in the 1700–1500 cm^{-1} region, where the deconvolution and subsequent curve-fitting yields three absorption peaks (Fig. 3A). The reduced (components $n=5$) and pure IR spectrum of

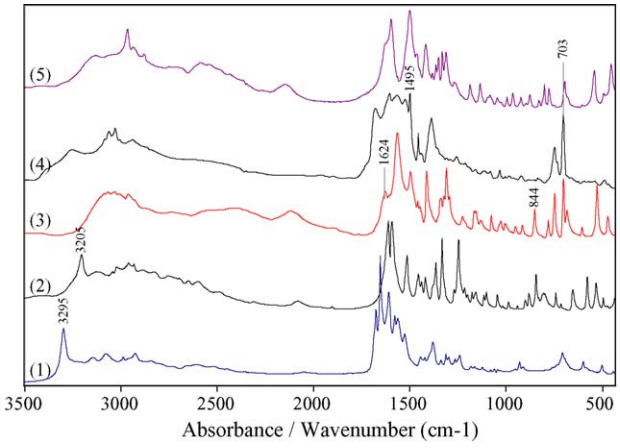

Fig. 2. Solid-state IR spectra of gly-met-gly (1), L-tyr (2), L-phe (3), L-phe-L-phe (4) and Ile (5) within 3500–400 cm^{-1}.

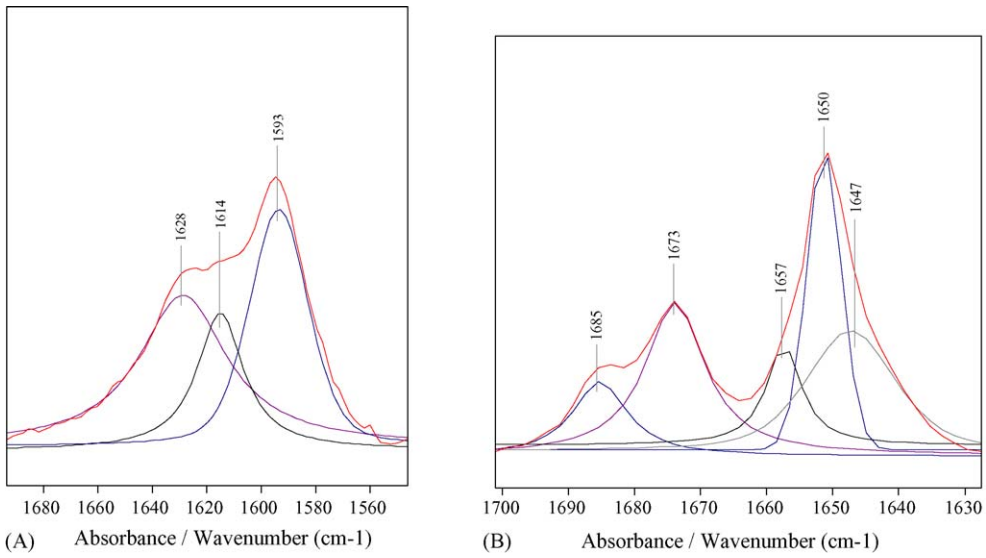

Fig. 3. 1700–1500 cm^{-1} and 1700–1600 cm^{-1} regions of curve-fitted solid-state IR spectra (KBr pellets) of Ile (A) and gly-met-gly (B), respectively.

Ile (Figs. 1.5 and 2.5) are interpreted using the Fourier and Savitzky–Golay smoothing procedures (Fig. 4A and B) with variable smoothing degrees within 0–10 (with step 1) and 5–25 (with step 2) in the first and second methods, respectively. The results for ν_i and A_i (Table 2) are examined using Student's *t*-test and the obtained data are presented in Table 3. The *p* values varied between 0.17 and 0.32 using Fourier smoothing (degrees 1–5) and equals to 0.36, when the IR-spectral curve is smoothed by Savitzky–Golay method (degree 5). The calculated *p*- and *t*-data (Table 3) are compared with tabulated data [14–16] at 99% confidence level, so as to verify the absence of statistically significant differences [16]. This is visualized in Fig. 5A, where the standard deviations (S.D.s) of unprocessed- and Fourier-smoothed (degree 5) IR-spectra of DL-isoleu are presented. These results are well correlated with other known data from quantitative IR analysis [14–16]. In other cases (samples 1–5 in Table 1 and higher degrees of smoothing (7–25 by Savitzky–Golay and 6–9 by Fourier), the critical *p*- and *t*-values are obtained, thus leading to rejection of the hypothesis about the possibility for adequate comparison of resulted curves [14,16]. Some examples are given in Table 3, where the comparison between the original-, Savitzky–Golay (degrees 7, 9 and 25) and Fourier (degree 9) smoothed IR spectral curves of Ile give *p* values around the critical one 0.05 [14–16]. Despite the slightly higher *p* values of 0.076 and 0.072 for degrees 7 and 9 by Savitzky–Golay (Table 3), these degrees cannot be recommended for quantitative assays. The inadequate S.D.s in Fig. 5B for original- and Fourier-smoothed data (degree 9) also tend to supports the above cautiousness.

These results lead to the following main conclusions: (i) the best correlation between original (No. 16 in Table 1) and smoothed IR spectral data of Ile are obtained using Fourier method with degrees of smoothing between 1 and 5 and by Savitzky–Golay procedure only with degree 5; (ii) as far as for optimization of smoothing conditions is used only a five-component mixture and adequate results are obtained only for

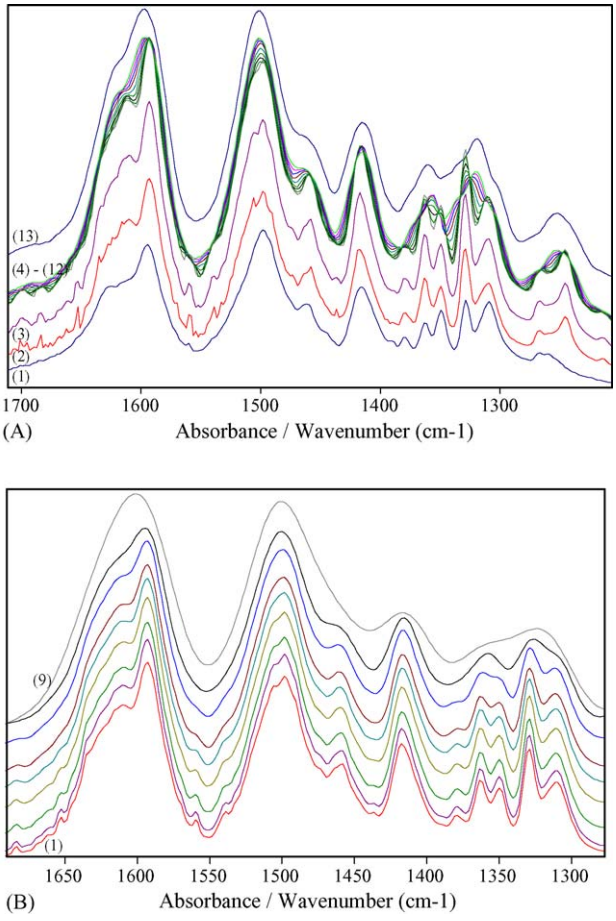

Fig. 4. Solid-state IR spectra of Ile within 1700–1200 cm^{-1} obtained by stepwise reduction from five-component system and interpretated by Savitzky–Golay (A) and Fouried methods (B), varying the smoothing degrees within 5–23 (step 2) and 1–9 (step 1) in the first and second approach, respectively.

Table 2
Peak positions (ν_i) and integral absorbancies (A_i) of non- and smoothed by Savitzky–Golay and Fourier methods, solid-state IR-spectral curves of Ile and gly-met-gly

	ν_1	A_1	ν_2	A_2	ν_3	A_3
Ile						
Non-smoothed IR-spectral curve of pure compound	1627.86	3.935_0	1613.72	0.417_2	1593.56	8.727_3
Smoothed IR-spectral curve ($n=5$)						
Savitzky–Golay						
Degree 5	1627.00	3.929_8	1613.65	0.415_8	1593.18	8.721_4
Degree 7	1625.13	3.770_0	1615.67	0.754_1	1596.56	8.999_7
Degree 9	1620.22	2.178_6	1618.44	2.089_0	1611.56	9.061_7
Degree 25	1622.77	0.256_6	1619.22	3.096_7	1604.88	11.852_1
Fourier						
Degree 1	1627.76	3.933_4	1613.70	0.417_0	1593.64	8.726_6
Degree 2	1627.64	3.912_2	1612.80	0.416_6	1593.23	8.721_1
Degree 3	1627.23	3.904_4	1612.67	0.401_4	1593.11	8.716_0
Degree 4	1627.25	3.900_2	1612.55	0.398_8	1593.15	8.706_0
Degree 5	1628.67	3.045_4	1611.98	0.400_2	1594.35	8.890_1
Degree 9	1630.21	0.800_0	1615.78	0.699_9	1594.55	9.800_7
gly-met-gly						
Non-smoothed IR-spectral curves of pure compound	1685.33	2.862_1	1673.31	5.697_3	1650.88	4.057_4
Smoothed IR-spectral curve ($n=5$)						
Savitzky-Golay						
Degree 5	1685.12	2.877_2	1672.89	5.598_8	1651.05	4.047_0
Fourier						
Degree-1	1685.25	2.869_3	1673.22	5.694_4	1650.91	4.053_8

3 wt.%, this value should be accepted as limit of detection for the discussed system. The second conclusion is supported by the following results. Examination of the second 3 wt.% component gly-met-gly (Table 1) versus a series of absorption peaks, determined also by deconvolution and curve-fitting procedure (Fig. 3B) is carried out. The corresponding data are given in Tables 2 and 3, where a comparison with standard gly-met-gly IR spectral data (No. 17 in Table 1) has been made. The calculated integral absorbance ratios and *t*-test data (Tables 4 and 5) brings to conclusions similar to those for Ile-likewise the S.D.s for $n=5$ (Fig. 6).

The LODs for different component systems (Table 1) are determined using the described procedure. The obtained *p* values around 0.42 (Tables 4 and 5) have indicated difference between means at 99% level [16]. The listed data correspond to concentrations of Ile at 2.5, 1.5 and 1.0 wt.% for $n=5$, 4, 3 and 2, respectively (samples 6, 10, 14 in Table 1). In all other systems (Table 1), the *p* values are below the critical value of 0.05 [14,16], while in some cases the corresponding values are <0.0044.

Table 3
Student's *t*-test data for the smoothed by Savitzky–Golay and Fourier methods, solid-state IR-spectral curves of Ile and gly-met-gly

	S.D.	*N*	Difference	S.D. difference	*t*	d.f.	*p*
Ile							
Savitzky-Golay							
Degree 5	1.87	6	−1.00	2.45	−1.00	5	0.36
Degree 7	5.88	6	−4.67	5.13	−2.23	5	0.076
Degree 9	3.76	6	−2.67	2.88	−2.28	5	0.072
Degree 25	4.14	6	−5.00	3.90	−3.14	5	0.025
Fourier							
Degree 1	2.37	6	−0.50	1.23	−1.00	5	0.36
Degree 2	2.32	6	−0.33	0.52	−1.58	5	0.17
Degree 3	2.37	6	−0.50	1.23	−1.00	5	0.36
Degree 4	2.16	6	−0.17	0.41	−1.00	5	0.36
Degree 5	2.93	6	−0.17	3.62	−1.13	5	0.31
Degree 9	3.27	6	−10.00	3.52	−6.96	5	0.00094
Gly-met-gly							
Savitzky-Golay							
Degree 5	2.83	6	−0.50	1.23	−1.00	5	0.36
Fourier							
Degree 1	2.48	6	−0.33	0.82	−1.00	5	0.36

S.D.: standard deviation; *N*: parameters number; *t*: parameter; *p*: parameter; d.f.: degree of freedom.

Table 4
Integral absorbencies (A_i) ratios of the peaks with ν_1–ν_3 (Table 2 for the solid-state IR spectra of Ile and gly-met-gly, obtained by reducing procedure in different *n*-component systems

	A_1/A_2	A_1/A_3	A_2/A_3
Ile			
IR-non-smoothed spectrum of pure compound	9.436_4	0.450_7	0.047_8
Smoothed IR-spectra (*n*-component mixture)			
$n=5$	9.435_5	0.4500	0.0443
$n=4$	9.436_0	0.4501	0.0456
$n=3$	9.436_0	0.4504	0.0471
$n=2$	9.4363	0.4506	0.0477
gly-met-gly			
IR-non-smoothed spectrum of pure compound	0.273_4	0.444_2	1.624_5
Smoothed IR-spectrum ($n=5$)	0.271_3	0.443_9	1.610_6

Table 5
Student's *t*-test data about the integral absorbance ratios listed in Table 4 for *n*-component systems

n	S.D.	*N*	Difference	S.D. difference	*t*	d.f.	*p*
Ile							
5	1.53	3	−0.67	1.15	−1.00	2	0.42
4	1.53	3	−0.67	1.15	−1.00	2	0.42
3	1.53	3	−0.33	0.58	−1.00	2	0.42
2	2.08	3	−1.67	2.89	−1.00	2	0.42
gly-met-gly							
5	1.53	3	−0.33	0.58	−1.00	2	0.42

S.D.: standard deviation; *N*: parameters number; *t*: parameter; *p*: parameter; d.f.: degree of freedom.

3.2. Accuracy and precision

The accuracy and precision are obtained for *n*-component systems in the cases of the best (lowest) LODs, namely samples 1, 6, 10, 14 corresponding to $n=5$, 4, 3, 2 component systems (Table 6, Fig. 7) and for standards 16 and 17 for $n=1$ (Table 1). All the spectral curves are Fourier-smoothed at degree 1. The obtained data are presented in Table 6, including the calculated S.D.s. The S.D.s for ν_i are obviously lower than those for their corresponding values for A_i. The result is in agreements with [5] and could be explained by uncertainties involved by curve-fitting stage for complicated absorption maxima [5]. The S.D.s for ν_i ($i=1$–3, Table 2) and A_i (Table 4) for Ile are: $\nu_1 = 1627.80 \pm 0.03_4$, $\nu_2 = 1613.69 \pm 0.01_6$, $\nu_3 = 1593.60 \pm 0.02_0$, $A_1/A_2 = 9.436_1 \pm 0.011_4$, $A_1/A_3 = 0.450_3 \pm 0.015_9$ and $A_2/A_3 = 0.046_5 \pm 0.015_8$, respectively.

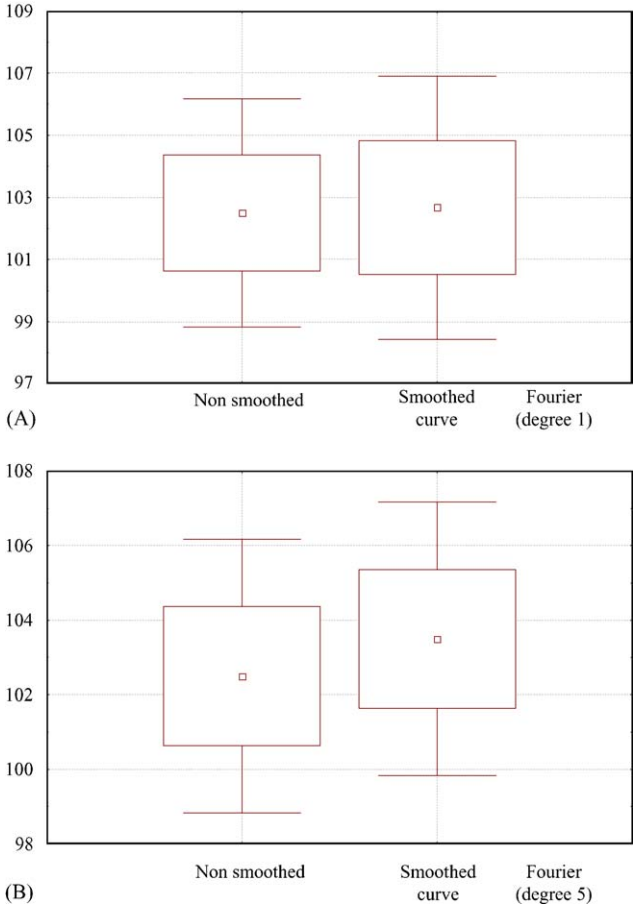

Fig. 5. Mean values and S.D.s for original- and Fourier smoothed curves of Ile, obtained by means of reducing-difference procedure of five component mixture at degree 1 (A) and 5 (B).

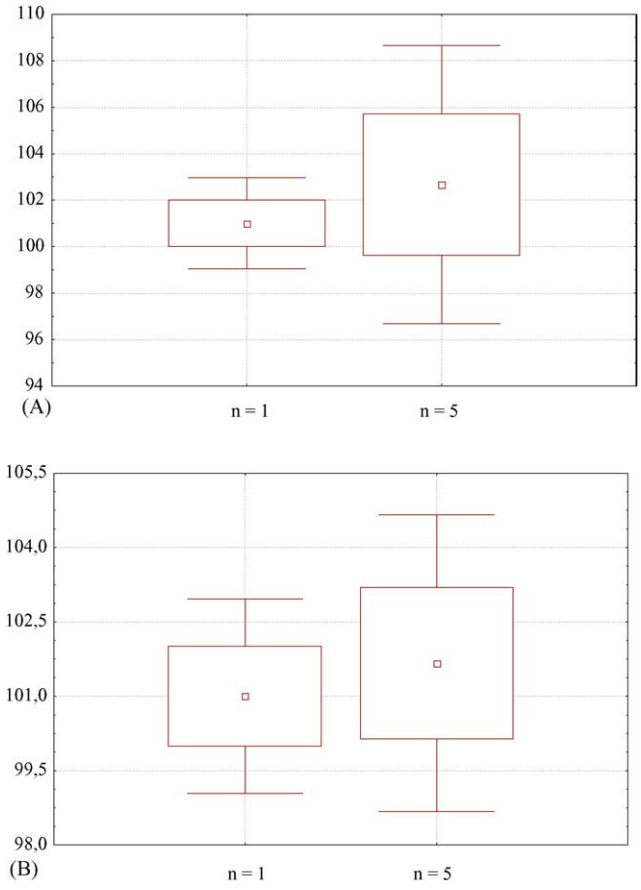

Fig. 6. Mean values and S.D.s for five component systems ($n=5$) for Ile (A) and gly-met-gly (B).

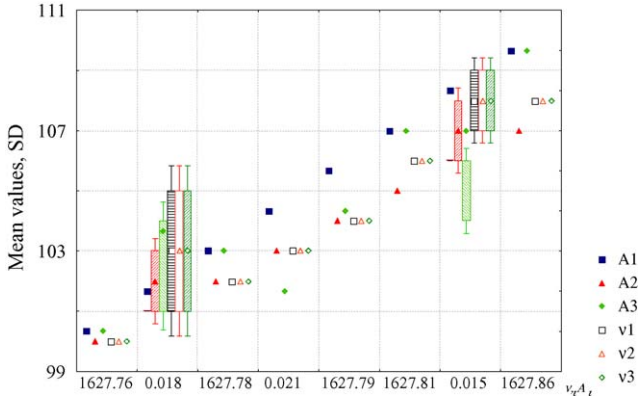

Fig. 7. Mean values and S.D.s of ν_i and A_i for component systems ($n=1$–5) for Ile.

Table 6
Peak position (ν_i) and integral absorbance (A_i) for Ile depending of the component in the mixtures (n)

N	ν_1	A_1	ν_2	A_2	ν_3	A_3
5	$1627.76 \pm 0.01_8$	$3.933_4 \pm 0.010_3$	$1613.70 \pm 0.01_6$	$0.417_0 \pm 0.010_9$	$1593.64 \pm 0.02_0$	$8.726_6 \pm 0.011_7$
4	$1627.78 \pm 0.02_1$	$3.713_1 \pm 0.010_2$	$1613.69 \pm 0.01_9$	$0.512_3 \pm 0.012_1$	$1593.55 \pm 0.02_0$	$11.234_7 \pm 0.012_7$
3	$1627.79 \pm 0.01_8$	$3.135_2 \pm 0.012_1$	$1613.55 \pm 0.01_9$	$0.332_3 \pm 0.011_6$	$1593.66 \pm 0.01_4$	$7.055_2 \pm 0.011_1$
2	$1627.81 \pm 0.01_5$	$2.997_4 \pm 0.013_0$	$1613.77 \pm 0.01_4$	$0.317_6 \pm 0.012_5$	$1593.60 \pm 0.01_5$	$6.658_3 \pm 0.012_2$
1	$1627.86 \pm 0.01_5$	$3.935_0 \pm 0.010_0$	$1613.72 \pm 0.01_7$	$0.417_2 \pm 0.010_5$	$1593.56 \pm 0.01_4$	$8.727_3 \pm 0.011_2$

4. Conclusion

The presented reducing-difference procedure for non-polarized IR spectra interpretation and the obtained results for multicomponent ($n = 2$–5) solid mixtures provide a useful methodology for treatment and identification, with adequate criteria about the presence or absence of a given component and LODs of 3.0, 2.5, 1.5 and 1.0 wt.% for 5-, 4-, 3- and 2-component mixtures, respectively.

References

[1] M.G. Arnaudov, Sh. Dinkov, J. Mol. Struct. 476 (1999) 235.
[2] M.G. Arnaudov, Y. Dimitriev, Phys. Chem. Glasses 42 (2001) 99.
[3] M.G. Arnaudov, B.B. Ivanova, Sh.G. Dinkov, Central Eur. J. Chem. 2 (2004) 589.
[4] B.B. Ivanova, A. Tchapkanov, M.G. Arnaudov, I. Petkov, Central Eur. J. Chem. 4 (2003) 356.
[5] B.B. Ivanova, M.G. Arnaudov, V. Simeonov, 2005, in press.
[6] A. Savitzky, M.J.E. Golay, Anal. Chem. 36 (1964) 1627.
[7] J. Steiner, Y. Termonia, J. Deltour, Anal. Chem. 44 (1972) 1906.
[8] H.M. Madden, Anal. Chem. 50 (1978) 1383.
[9] B.J. Barnes, M.S. Dhanoa, S.J. Lister, Appl. Spectrosc. 43 (1989) 772.
[10] J. Cohen, Statistical Power Analysis for the Behavioral Sciences, 2nd ed., N.J. Lawrence Earlbaum Associates Hillsdale, 1988.
[11] B.B. Ivanova, M.G. Arnaudov, St. Todorov, W.S. Sheldrick, H. Mayer-Figge, Struct. Chem., 2005, in press.
[12] B.B. Ivanova, Spectrochim. Acta Part A, 2005, in press.
[13] B.B. Ivanova, J. Coord. Chem. 58 (2005) 587.
[14] J.L. Myers, A.D. Well, Research Design and Statistical Analysis, Harper Collins, New York, 1991.
[15] M.R. Spiegel, Theory and Problems of Probability and Statistics, McGraw-Hill, New York, 1992, pp. 116–117.
[16] J.W. Park, J.L. Ferracane, Dent. Mater. xi (2005) 7.
[17] P.C. Gillette, J.L. Koenig, Appl. Spectrosc. 38 (1984) 334.
[18] M.A. Friese, S. Banerjee, Appl. Spectrosc. 46 (1992) 246.
[19] S. Banerjee, K. Li, Appl. Spectrosc. 45 (1991) 1047.