

The spectrophotometric analysis of mixtures with overlapping absorption bands

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The subject of this work was to estimate the precision and accuracy of the results of the determination of the individual component concentrations in their mixtures obtained by four absorption spectroscopic methods. The spectrum of any substance can be expressed by a linear combination or by a product of Lorentz or Gauss functions; therefore the spectrum approximated by two overlapping Lorentz functions was used as the model system. Small concentrations of unknown components were replaced by a polynomial.

Provided that the Lambert—Beer law and the law of the additivity of components absorbances are valid, the relationship between concentrations of the individual components and absorbance at analytical wavenumbers can be expressed in matrix notation [1]

$$\bar{a} = \bar{K} \times \bar{c}, \quad (1)$$

where \bar{a} — the column matrix of absorbances at wavenumbers $g = 1, 2, \dots, k$,

\bar{K} — the matrix of absorptivities k_{gj} of the components $j = 1, 2, \dots, n$ at wavenumbers $g = 1, 2, \dots, k$ (matrix of the $k \times n$ type),

\bar{c} — the column matrix of concentrations of the components $j = 1, 2, \dots, n$.

In the simplest case, when $k = n$, one wavenumber, usually corresponding to the maximum of an absorption band, is chosen for each component. Equation (1) then represents a system of n linear equations with n unknown concentrations at n different wavelengths.

The absorptivities k_{gj} can be found as follows. Series of solutions of the individual components are prepared, absorbances on their spectra are measured and then the line slopes of the dependences of absorbances on concentration are estimated graphically or calculated by the least-squares method [1, 2]. In a favourable case when the spectra of the components do not overlap too much, the main diagonal elements of the matrix of absorptivities are relatively large in comparison with other elements. The system of equations represented by equation (1) can be solved by some common methods [3].

When the absorption bands of the individual components overlap to a large extent, this classical method is not efficient enough and the determined concentrations of components are imprecise and often inaccurate. Better results can be obtained by means of overdetermined systems of equations together with the least-squares method. An overdetermined system of equations can be chosen by two different ways:

1. The matrix $\overline{\overline{K}}$ is obtained as described above, using the spectra of individual standards, but the absorbances are measured at more wavelengths (wavenumbers) than is the number of components [1, 4–7]. After *Zscheile* and co-workers [8] the best results are achieved if maximum of the accessible wavelengths is used and the same was concluded by *Sternberg et al.* [9]. Such method was applied by many authors especially in the ultraviolet region where absorption bands are relatively broad and the precision of the measurement of absorbances is high. Thus, reproducible measurements are possible there even at the sides of absorption bands.

2. *Barnett and Bartoli* [10] used the spectra of mixtures of the standards for calibration instead of the individual standards, the number of calibration mixtures being greater than the number of the components. The number of analytical wavelengths is equal to the number of components. *Niebergall and Mattocks* [11] applied a similar procedure in the ultraviolet region. Absorbances were measured at the maximum of the band of each component. Such procedure removes problems occurring especially in the infrared region. A number of the specific absorption bands for each component are usually available there but the Lambert–Beer law is often not valid exactly and measurements of absorbances at sharply sloped parts of absorption bands are not precise.

Vasilyev [12] extended the validity of *Barnett's* and *Bartoli's* relationships for any number of wavenumbers and expressed them in matrix notation. In other works [13–15] the generalization of the method of the analysis of multicomponent mixtures has been theoretically worked out and practical application in u.v. and i.r. regions pointed out. The method joins the advantages of the overdetermined system of equations in the number of calibration mixtures with those of overdetermination in analytical wavenumbers.

The aim of the present work is to compare the accuracy and precision of the above-cited methods in relation to the extent of overlapping of absorption bands and to check the possibility of determination of the individual components from absorbances measured at the sides of absorption bands in the infrared region.

Experimental

Precision and accuracy of the results of determination of the component concentrations in the mixtures obtained by the methods chosen were studied on a model system and verified on the binary mixture of chlorobenzene with *o*-dichlorobenzene. Two overlapping absorption bands were used as the model system which enabled to check the influence of experimental errors on the determination of concentrations in a multicomponent mixture. Each band was expressed by the Lorentz function [16, 17]

$$A_{\tilde{\nu}} = \frac{A_0}{1 + \frac{4(\tilde{\nu} - \tilde{\nu}_0)^2}{A\tilde{\nu}_{1/2}^2}}, \quad (2)$$

where $A_{\tilde{\nu}}$ — absorbance at frequency $\tilde{\nu}$,
 A_0 — absorbance at frequency $\tilde{\nu}_0$,
 $\tilde{\nu}_0$ — frequency at the maximum of the band,
 $A\tilde{\nu}_{1/2}$ — half-band width.

The influence of background [18, 19] causing deviations from the Lambert–Beer law was approximated by the function

$$A_{\bar{\nu}}(\text{obs}) = \alpha(\bar{\nu} - \bar{\nu}_{02})^2 - \beta(\bar{\nu} - \bar{\nu}_{02}) + \gamma, \quad (3)$$

where $\bar{\nu}_{02}$ is the frequency at the maximum of the second band. The coefficients α , β , γ were estimated from the spectra of chlorobenzene and *o*-dichlorobenzene. Another inaccuracy was brought in the model system by rounding off the values of $A_{\bar{\nu}}$ to three decimal places (which corresponded to the common precision of measured absorbances).

Numerical values representing the concentrations of the components in the calibration solutions of standards, in the calibration mixtures and samples, as well as other parameters were chosen to be similar to the parameters of the binary system chlorobenzene—*o*-dichlorobenzene, used in the infrared region (Table 1).

Table 1

Comparison of the model and experimental parameters of the binary system

Parameter	Lorentz functions		Chloro- benzene $j = 1$	<i>o</i> -Dichloro- benzene $j = 2$
	$j = 1$	$j = 2$		
c_{max} [% w/v]	0.5000	0.5000	0.4920	0.5030
A_0	0.750	0.550	0.714	0.551
$\Delta\bar{\nu}_{1/2}$ [cm ⁻¹]	4.0	4.5	5.7	5.3
$\bar{\nu}_0$ [cm ⁻¹]	2.0	10.0	737.6	746.2
Region of spectrum [cm ⁻¹]	0—12.0		735.4—748.4	
Distances of maxima			/	
$\Delta\bar{\nu}_0 = \bar{\nu}_{01} - \bar{\nu}_{02}$ [cm ⁻¹]	8.0		8.72	
Distances of analytical wavenumbers				
$\Delta\bar{\nu} = \bar{\nu}_g - \bar{\nu}_{g-1}$ [cm ⁻¹]	2.0		2.18	

Model samples

The absorbances of model samples were calculated by the same way as the absorbances of components of the binary model system. The matrices \bar{B} of ten chosen samples ($m = 10$; $k = 2$ and $k = 7$) were calculated to compare different methods. Total concentration of samples did not exceed the interval of 0.4952—0.5025% w/v while the concentration ratio of the two components was always 0.2585 : 0.2415.

Binary system chlorobenzene—*o*-dichlorobenzene

The selected methods of the spectrophotometric analysis of multicomponent mixtures were applied to determine the mixture of chlorobenzene with *o*-dichlorobenzene in the infrared region (720—760 cm⁻¹). Measurements were performed on a double-beam UNICAM SP-100 infrared spectrophotometer (NaCl prism) using 1-mm cells with NaCl windows.

Standard solutions and calibration mixtures were prepared from the standards of the individual components; redistilled carbon disulfide was used as a solvent. The concentrations of the standard solutions of chlorobenzene and *o*-dichlorobenzene varied from 0.05 to 0.5% w/v. The concentration of chlorobenzene in calibration mixtures decreased from 0.5111% w/v in the 1st mixture to 0% in the 21st one. The concentration of *o*-dichlorobenzene was chosen so as to make the total sum of concentrations ($c_{11} + c_{12}$) equal to ca. 0.5000% w/v. The values of absorbances used for calculation of the calibration data were corrected for the solvent absorbances.

*Methods of calibration and calculations of the sample concentrations**Method I*

The calibration data were calculated from the known concentrations of the solutions of the individual standards and from the absorbances measured at the maxima of the corresponding bands (wavenumbers 2 and 6). The arithmetic series of seven concentrations taken from the interval of 0.07–0.5% w/v was used. k_{g1} and k_{g2} were calculated by minimalization of deviations from the Lambert–Beer law *via* the least-squares method [1, 2, 15]; the resulting matrix \bar{K} was inverted to \bar{K}^{-1} .

The unknown concentrations of components in the samples were calculated by multiplication of the transposed matrix \bar{K}^{-1} by the matrix of the sample absorbances \bar{B} which were measured at wavenumbers 2 and 6 of the spectra of samples. The formula is

$$\bar{X} = (\bar{K}^{-1})^T \times \bar{B}, \quad (4)$$

where \bar{X} — the matrix of concentrations of the components $j = 1, 2, \dots, n$ in the samples $i = 1, 2, \dots, m$ (type $n \times m$),

\bar{B} — the matrix of absorbances of the samples $i = 1, 2, \dots, m$ at wavenumbers $g = 1, 2, \dots, k$ (type $k \times m$).

Method II

The matrix of absorptivities \bar{K} was evaluated in the same way as in method I but absorbances were measured also at the sides of the absorption bands ($k = 7$). The resulting matrix \bar{K} is a rectangular one, its inverse matrix is not defined. The required concentrations of samples can be calculated by minimalization of the squares of absorbance deviations with respect to concentrations [1, 15, 20]

$$\bar{X} = \bar{Q} \times \bar{B}, \quad (5)$$

where \bar{Q} is the matrix (type $n \times k$) for calculation of concentrations

$$\bar{Q} = (\bar{K}^T \times \bar{K})^{-1} \times \bar{K}^T \quad (5a)$$

Method III

The calibration data were obtained here by measurements of $m = 21$ mixtures of the known composition. Concentrations of the first component decreased regularly from 0.5000% w/v in the 1st mixture to 0% w/v in the 21st one while concentrations of the second component increased at the same time from 0% to 0.5000% w/v. The total concentration in any mixture was always 0.5000% w/v. The absorbances were measured at two wavenumbers corresponding to the maxima of absorption bands of components. The values obtained were arranged to form the matrix of concentrations \bar{C} and the matrix of absorbances \bar{A} . Multiplying these matrices after equation (6) we can obtain directly the matrix for calculation of concentrations [15]

$$\bar{Q} = \bar{C} \times \bar{C}^T \times (\bar{A} \times \bar{C}^T)^{-1}, \quad (6)$$

where \bar{C} — the matrix of concentrations of the components $j = 1, 2, \dots, n$ in the mixtures $i = 1, 2, \dots, m$ (type $n \times m$),

\bar{A} — the matrix of absorbances of the mixtures $i = 1, 2, \dots, m$ at wavenumbers $g = 1, 2, \dots, k$ (type $k \times m$).

The concentrations of components in the samples were calculated after equation (5).

Method IV

The calibration data were calculated from absorbances obtained as in method III supplemented by absorbances measured at the sides of absorption bands ($k = 7$) of calibration mixtures composed as in method III. The matrix \bar{Q} was calculated after the following formula, derived for the case $k > n$ [15]

$$\bar{Q} = \bar{C} \times \bar{C}^T \times (\bar{C} \times \bar{A}^T \times \bar{A} \times \bar{C}^T)^{-1} \times \bar{C} \times \bar{A}^T \quad (6a)$$

The concentrations of components in the samples were again calculated after equation (5).

Method V

The matrix \bar{Q} was calculated in the same way as in method IV but instead of the matrices \bar{C} and \bar{A} ($m = 21$) their submatrices ($m = 7$, mixtures 8–14) were used. For the calculation of the concentrations of components equation (5) was used.

Remarks

Other two model systems and model samples were studied to compare the efficiency of methods I–V in dependence on the extent of overlapping of the absorption bands. The distances of maxima of two Lorentz bands were 4.0 cm^{-1} ($\bar{\nu}_{01} = 4 \text{ cm}^{-1}$, $\bar{\nu}_{02} = 8 \text{ cm}^{-1}$) and 12.0 cm^{-1} ($\bar{\nu}_{01} = 0 \text{ cm}^{-1}$, $\bar{\nu}_{02} = 12 \text{ cm}^{-1}$), respectively. The other parameters were the same as it was with a maxima distance of 8.0 cm^{-1} .

A desk calculator ELKA 6521 was used for the calculations of methods I and II and a digital computer GIER (Regnecentralen) for the methods III, IV, and V. The computer programme was written in ALGOL; version GIER-ALGOL 4 [21].

Results and discussion

The accuracy of results of the above-listed methods was compared by means of the relative errors of concentration determinations $\Delta_{(\%)jt}$, calculated from the formula

$$\Delta_{(\%)jt} = \frac{x_{jt} - c_{jt}}{c_{jt}} 100\%, \quad (7)$$

where c_{jt} — the known concentration of the component $j = 1, 2, \dots, n$ in the sample $i = 1, 2, \dots, m$,

x_{jt} — the determined concentration of the component $j = 1, 2, \dots, n$ in the sample $i = 1, 2, \dots, m$.

The criteria of precision of the achieved results were standard deviations of the concentration determinations of components from their known values

$$s_j = \sqrt{\frac{\sum_{i=1}^m (x_{jt} - c_{jt})^2}{m}}, \quad (8)$$

where m is the number of determinations.

Table 2
Concentrations determined by methods I-V
Chlorobenzene - o-dichlorobenzene

i1	α_{i1}					c_{i2}	α_{i2}				
	I	II	III	IV	V		I	II	III	IV	V
1	51.11	50.34	50.20	50.30	51.05	-	-0.07	0.11	-0.54	-0.76	-0.29
2	48.37	47.81	47.88	47.89	48.37	3.15	3.75	3.91	3.34	3.33	3.64
3	45.61	45.52	45.59	45.55	45.56	5.87	6.20	6.26	5.83	5.74	5.92
4	42.36	42.50	42.47	42.37	42.51	8.14	8.44	8.56	8.12	8.09	8.12
5	40.32	39.79	39.90	39.71	39.80	9.78	9.84	9.90	9.56	9.47	9.43
6	36.49	36.72	36.92	36.60	36.57	11.83	11.99	12.29	11.77	11.93	11.78
7	36.78	37.40	37.51	37.22	37.26	15.21	15.80	15.98	15.62	15.66	15.36
8	32.02	32.55	32.53	32.22	31.88	17.99	18.47	18.65	18.35	18.41	18.18
9	30.65	31.46	31.55	31.19	30.86	19.88	20.09	20.36	20.01	20.14	19.93
10	27.34	27.91	28.06	27.58	27.30	23.28	23.28	23.43	23.26	23.29	23.10
11	25.65	26.39	26.47	26.00	25.98	26.30	26.65	26.84	26.68	26.76	26.57
12	22.59	23.42	23.49	23.03	22.67	26.97	26.36	26.60	26.42	26.55	26.38
13	20.64	21.30	21.38	20.84	20.46	30.28	30.15	30.34	30.26	30.35	30.18
14	16.87	17.78	17.96	17.25	16.96	33.40	33.69	33.80	33.88	33.89	33.75
15	14.38	14.87	15.13	14.31	14.46	35.62	35.02	35.12	35.25	35.26	35.23
16	12.44	13.09	13.29	12.48	12.54	38.31	38.32	38.47	38.60	38.66	38.68
17	9.68	10.37	10.61	9.71	9.81	41.28	40.89	41.09	41.23	41.34	41.40
18	7.32	7.99	8.16	7.28	7.31	43.27	42.99	43.10	43.37	43.40	43.51
19	6.64	7.16	7.25	6.43	6.36	45.09	44.68	44.61	45.08	44.95	45.09
20	2.80	3.41	3.52	2.65	2.59	47.37	46.17	46.12	46.62	46.51	46.71
21	--	-0.04	-0.06	-0.88	-1.08	50.56	50.25	50.13	50.78	50.61	50.88

Table 3
Averages of the absolute values of relative errors of the determination
of component concentrations by methods I—V

System	Com- po- nent <i>j</i>	$\bar{\Delta}_{(\%)j}$ [%]				
		I	II	III	IV	V
Chlorobenzene— <i>o</i> -dichlorobenzene	1	4.1444	4.9862	1.3036	1.5388	1.0992
	2	2.5086	3.2443	1.2164	1.4401	1.6623
Model system $\Delta\bar{\nu}_0 = 8 \text{ cm}^{-1}$	1	0.8830	0.5151	0.1740	0.1702	0.1083
	2	0.5886	0.1370	0.1578	0.1287	0.1204
Model system $\Delta\bar{\nu}_0 = 4 \text{ cm}^{-1}$	1	1.7010	0.4881	0.1510	0.1782	0.1084
	2	2.9238	0.1742	0.1203	0.1244	0.1285
Model system $\Delta\bar{\nu}_0 = 12 \text{ cm}^{-1}$	1	0.4184	0.0736	0.0697	0.0580	0.0968
	2	0.5095	0.2982	0.1614	0.1490	0.1448

Table 4
Standard deviations of the concentration determinations of components
by methods I—V

System	Com- po- nent <i>j</i>	$s_j \cdot 10^3$ [% w/v]				
		I	II	III	IV	V
Chlorobenzene— <i>o</i> -dichlorobenzene	1	6.034	7.082	3.833	4.225	1.944
	2	4.422	5.041	3.251	3.684	3.084
Model system $\Delta\bar{\nu}_0 = 8 \text{ cm}^{-1}$	1	2.307	1.364	0.548	0.520	0.346
	2	1.449	0.424	0.458	0.387	0.374
Model system $\Delta\bar{\nu}_0 = 4 \text{ cm}^{-1}$	1	4.395	1.281	0.436	0.510	0.346
	2	7.058	0.480	0.374	0.361	0.361
Model system $\Delta\bar{\nu}_0 = 12 \text{ cm}^{-1}$	1	1.095	0.245	0.224	0.224	0.300
	2	1.296	0.787	0.469	0.412	0.400

Table 2 shows the known concentrations of chlorobenzene (the component 1) and *o*-dichlorobenzene (the component 2) in the mixtures 1–21 and the concentrations determined by methods I—V. The results of method V were calculated by means of the submatrices $i = 1-7, 8-14, 15-21$. The averages of absolute values of relative errors of the concentration determinations $\bar{\Delta}_{(\%)j}$ are shown in Table 3, standard deviations of the concentration determinations s_j are in Table 4.

The accuracy and precision of the determination of the binary mixture chlorobenzene—*o*-dichlorobenzene were appreciably higher in methods III—V where calibration was made by mixtures. However, an independent set of data was used for calibration in methods I and II (calibration by the individual standards) while a regressive calculation of concentrations of the calibration mixtures was, in fact, performed in methods III—V. The increase in wavenumbers resulted in a decrease of the precision and accuracy of determinations, obviously due to the errors of absorbances measured at the sides of the absorption bands.

As follows from the $\bar{\Delta}_{(\%)j}$ and s_j values of the model system (the distance of maxima

8 cm⁻¹), the accuracy and precision of determinations increase in accordance with the sequence of the listed methods. The increase in wavenumbers has a clean-cut influence only on the calibration by individual standards.

The results achieved in both cases by method V proved that the narrowed concentration range of calibration mixtures may improve precision and accuracy, even when a markedly lower number of calibration mixtures was used.

An influence of the overlapping of absorption bands on the precision and accuracy of determinations can be deduced from a comparison of the results for all model systems. When overlapping is larger ($\Delta\bar{\nu}_0 = 4 \text{ cm}^{-1}$), the calibration by mixtures is more advantageous while the increase in wavenumbers influences only the calibration by individual standards. Smaller overlapping ($\Delta\bar{\nu}_0 = 12 \text{ cm}^{-1}$) brings about a slight improvement of precision and accuracy, parallel to the sequence of the above methods. Markedly improved are only the results of method I, which are the least precise and accurate.

On the basis of the achieved results it can be concluded that calibration by mixtures increases substantially the precision and accuracy of the analysis of mixtures with overlapping absorption bands. Furthermore, even the absorbances measured at the sides of overlapping absorption bands can be used in the systems overdetermined in wavenumbers ($k > n$) if they are measured with sufficient precision. This can be reached e.g. by means of a spectrophotometer with a digital recorder of absorbances measured at a great number of wavenumbers. The obtained values are processed by mathematical filtration in a computer [22]. An optimization procedure is applied to choose the optimal analytical wavenumbers and the corresponding absorbances are used for calculation of calibration data or concentrations of components in the samples.

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References

1. Bauman, R. P., *Absorption Spectroscopy*, Chapter 9. J. Wiley, New York, 1967.
2. Maurice, M. J., *Z. Anal. Chem.* **204**, 401 (1964).
3. Rektorys, K., *Přehled užité matematiky*. (The Review of Applied Mathematics.) Státní nakladatelství technické literatury. (State Publishing House of Technical Literature.) Prague, 1963.
4. Bauman, R. P., *Appl. Spectrosc.* **13**, 156 (1959).
5. Herschberg, I. S. and Sixma, F. L. J., *Koninkl. Ned. Akad. Wetenschap. Proc. B*, **65**, 244 (1962).
6. Herschberg, I. S., *Z. Anal. Chem.* **205**, 180 (1964).
7. Doerffel, K., Kugler, P., and Binder, H. J., *Z. Chem.* (Leipzig) **6**, 155 (1966).
8. Zscheile, E. P., Murray, H. C., Baker, G. A., and Peddicord, R. G., *Anal. Chem.* **34**, 1776 (1962).
9. Sternberg, J. C., Stillo, H. S., and Schwendeman, R. H., *Anal. Chem.* **32**, 84 (1960).
10. Barnett, H. A. and Bartoli, A., *Anal. Chem.* **32**, 1153 (1960).
11. Niebergall, P. J. and Mattocks, A. M., *Drug Standards* **28**, 61 (1960).
12. Vasilyev, A. F., *Zavod. Lab.* **31**, 677 (1965).
13. Przybylski, Z., *Chem. Anal.* (Warsaw) **13**, 453 (1968).
14. Kössler, I., *Kvantitativní infračervená spektroskopická analýza*. (Quantitative Infrared Spectroscopic Analysis.) P. 53. Státní nakladatelství technické literatury. (State Publishing House of Technical Literature.) Prague, 1970.

15. Šustek, J., Livař, M., and Schiessl, O., *Chem. Listy* **66**, 168 (1972).
16. Audo, D., Arnaud, P., and Armand, Y., *J. Mol. Struct.* **2**, 287, 409 (1968).
17. Seshadri, K. S. and Jones, R. N., *Spectrochim. Acta* **19**, 1013 (1963).
18. Stone, H., *J. Opt. Soc. Amer.* **52**, 998 (1962).
19. Papoušek, D. and Plíva, J., *Collect. Czech. Chem. Commun.* **30**, 3007 (1965).
20. Przybylski, Z. and Kramarz, J., *Chem. Anal. (Warsaw)* **13**, 249 (1968).
21. Schiessl, O., unpublished *Program SAMZ-L 1968*.
22. Livař, M. and Šustek, J., *Z. Anal. Chem.*, in press.

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