Overdetermined linear systems in multicomponent spectrophotometry

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OVERDETERMINED LINEAR SYSTEMS
IN
MULTICOMPONENT SPECTROPHOTOMETRY

ISRAEL SAMUEL HERSCHBERG

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN
DE TECHNISCHE WETENSCHAPPEN AAN DE TECHNISCHE
HOGESCHOOL TE EINDHOVEN OP GEZAG VAN DE
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DIT PROEFSCHRIFT IS GOEDGEKEURD DOOR DE PROMOTOREN

PROF. DR. IR. A.I.M. KEULEMANS

EN

PROF. DR. J.F. BENDERS

Aan Sjifra, Benno en Dan,
in vertrouwen,
aan Sonja,
met liefde,
aan mijn ouders,
uit dankbaarheid.

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TABLE OF SYMBOLS

Unless the context indicates otherwise, the following meaning is to be assigned to the symbols listed below.

(All arrays will be underlined; vectors are understood to be column vectors. Notations such as $(\underline{A}^T\underline{A})_{ij}$ denote the element in the i-th row and j-th column of the matrix $\underline{A}^T\underline{A}$.)

a	absorbance
$\underline{a}_o$	see $\underline{a}$
$\underline{a}$	(synonymous with $\underline{a}_o$) an observable vector of k elements, the i-th element of which, a_i or a_{oi}, represents the observed absorbance of the unknown at the i-th wavelength, and hence is a stochastic quantity
$\underline{A}$	an observable matrix of k rows and n columns, the i,j-th element of which, a_{ij}, represents the observed absorbance of the j-th reference at the i-th wavelength, and hence is a stochastic quantity
$\underline{b}_o$	see $\underline{b}$
$\underline{b}$	a vector of k elements; the non-observable $\underline{b}$ represents the "true" value of $\underline{a}$; it is synonymous with $\underline{b}_o$
$\underline{B}$	a matrix of k rows and n columns; the non-observable $\underline{B}$ represents the "true" value of $\underline{A}$
c	concentration
d	optical path length
E(...)	the expected value of ...
i	an index running from 1 to k
$\underline{I}$	a unit matrix
j	an index running from 0 or 1 to n
k	the number of rows of $\underline{A}$, i.e. the number of wavelengths of observation of absorbances
l	litre
n	the number of columns of $\underline{A}$, i.e. the number of unknown concentrations to be estimated
n^*	equals n for Model I, equals n + 1 for Model II, see Section 3.5
nm	nanometre, 10^{-9} metre

P(...)	the probability that ... obtains
$\underline{r}$	the residual vector $\underline{a} - \underline{A\hat{x}}$
s	optical slit width
$\hat{s}$	the root-mean-square value of an element of $\underline{r}$
T	transmittance
$\underline{V}^{\tau}$	the left-inverse of $\underline{A}$, $\underline{V}^{\tau} \equiv (\underline{A}^{\tau}\underline{A})^{-1}\underline{A}^{\tau}$
$\underline{x}$	the vector of relative concentrations of the unknown
α	probability
$\Delta\nu_{\frac{1}{2}}$	band half-width
ε	the error in an observation of $\underline{A}$ and/or $\underline{a}$; also, the molar absorptivity
λ	wavelength, also, an eigenvalue
λ_s	the smallest eigenvalue of $(\underline{A},\underline{a})^{\tau}(\underline{A},\underline{a})$
μ	micrometre; also, the smallest eigenvalue of $\underline{A}^{\tau}\underline{A}$
ν	frequency
$\underline{\xi}$	the vector $\binom{\underline{x}}{-1}$
σ(...)	the standard deviation of ...
$\underline{\Sigma}$	a covariance matrix
τ	(as a superscript) denotes transposition of arrays
$\hat{}$	(caret) placed over a symbol denotes an estimate
$\sim$	(tilde) placed over a symbol denotes that the array has been referred to its column means
$\doteq$	is approximately equal to
$\equiv$	is by definition equal to

CHAPTER I

INTRODUCTION

1.0 A synopsis

The investigation reported in the present thesis is directed toward improving the precision of spectrophotometry, especially as applied to multicomponent analyses in the region accessible to quartz prism instruments. The simple linear model usually assumed for the relation between absorbance and concentration quite naturally leads to the use of overdetermined systems of linear equations, which offers increased precision and even some possibility of verifying the accuracy of the analytical results.

Some refinements of the usual observational technique are imposed by the desire to maintain the linearity of the model; that no elaborate recording equipment was available for the investigation proved beneficial to the accuracy and the precision, though no doubt it sometimes severely tried the observers' patience.

It must be regarded as fortunate that access to an electronic computer became available to the author's Laboratory some time before the present work was started. To say that the use of a computer facilitated the project would be a gross understatement; the lack of such a facility would effectively have prevented progress beyond an application to a very few components, whereas systems of up to at least eight components can in effect be handled with chemically meaningful results.

Though the use of a computer thus solved the routine numerical problems and proved valuable for gaining an insight into departures from linearity, the difficulty of establishing the validity of the simple, classical least-squares procedure adopted still had to be faced. As it happened, the legitimacy of this simple method could be proved for the point estimation problem; the correctness of the use of some interval estimators could be made plausible for the rather special case aimed at in the spectrophotometric application. In this validation study, the help of a simulation on a computer proved indispensable.

1.1 Summary

Chapter I states the objective of the study, sketches its development, provides a summary of its contents and reviews some relevant literature. In addition, an opinion on the possibility of full automatization is given.

Chapter II gives a detailed description of the experimental procedure and deals with the drift in time of observed absorbances; it is concluded that it is necessary to obtain reference absorbances as nearly simultaneously as possible with the absorbances of the unknown mixture(s). The theoretical desirability of using unusually high values of the absorbance is discussed and the feasibility of the use of $a \leq 1.6$ (without material loss of the precision of an observation) is shown. The rationale of overdetermined systems is given; among the advantages of this approach, the increased precision, a limited assurance of accuracy, the elimination of clerical or observation errors, the detection of slit width bias and extraneous components and the relative insensitivity to differences in cell properties are touched upon. The availability of a measure of the precision of an analysis, derived directly from the data of that analysis, is also mentioned as a major advantage.

In Chapter III, a mathematical model is defined; it is shown that, on the assumption of errors that are small relative to the values observed, the classical least-squares point estimator $\hat{\underline{x}}$ has some reasonable properties. A preference for the use of $\hat{\underline{x}}$ is stated and the relation of the theoretically preferable estimator, $\hat{\underline{z}}$, to $\hat{\underline{x}}$ is discussed. Upper bounds for some relevant functions of $\hat{\underline{x}} - \hat{\underline{z}}$ are obtained and used to demonstrate that $\hat{\underline{x}} = \hat{\underline{z}}$ holds to a sufficient degree of approximation on the assumption of small errors; this also yields an efficient algorithm for $\hat{\underline{z}}$. A variant of the model is introduced and two calculation methods for this variant are shown to be equivalent.

Chapter IV is concerned with the problem of interval estimation. The covariance matrix of $\hat{\underline{x}}$ is derived and its use for some simple tests and/or confidence intervals is pointed out. The difficulties of more sophisticated interval estimation, which arise from the errors affecting $\underline{A}$, the matrix of coefficients, are discussed. From the analogy to the classical case, three hypotheses on the distribution of certain statistics are formulated, assuming a normal distribution of errors. A test, by a Monte Carlo method, of these hypotheses leads to the conclusion that the distributions concerned are sufficiently similar to χ^2- and F-distributions for the cases of interest. Hence, tests and confidence regions similar to those available for multivariate classical regression are deduced to be valid for these cases; their use is discussed.

Chapter V is devoted to a closer analysis of the assumed linear relation between absorbance and concentration. Observation of absorbances at non-vanishing slit widths is known, in principle, to introduce a non-linear dependence. The effect is studied on a model consisting of a Lorentz absorbance and a triangular slit function, which conforms reasonably well to practice. By exploration of this model, maximum permissible ratios $s/\Delta\nu_{\frac{1}{2}}$ are found for single-component systems; the slit width influence on multicomponent systems is discussed: in such cases as show considerable slit width error, diagnosis of this type of non-linearity is shown to be possible by the presence of a characteristic and persistent pattern in the residuals of the least-squares solution.

Chapter VI explores the use of known mixtures of components as references. Some theory is presented; the device is applied to bring the notoriously non-linear near infra-red region within the scope of the method. Analyses are shown to be successful for some rather strongly non-linear two-component systems. A three-component near infra-red system is exhibited in which the technique fails to give chemically satisfactory results. However, this is shown to be due to the poor distinguishability of the spectra, not to the use of mixtures as references, since this device does not, in principle, impair the precision.

In the final chapter, VII, the method is applied to a few practical problems. In the first example (cortisone and prednisone acetates), the pronounced spectral similarity provides the difficulty. As a second example, it is shown that multicomponent analysis of the C_9 aromatic fraction of a mineral turpentine is feasible for up to at least eight components; the results obtained are consonant with those of gas-liquid chromatography and are estimated to be of better precision for major constituents. Some other practical applications are referred to.

1.2 Some relevant literature

When the present study was begun in 1958, rather little had been published on the application of linear algebra or automatic computing equipment to problems of analytical chemistry.

Opler 1950 described the use of punched-card machinery for the multicomponent IR analysis of up to 10 components, which he, however, fails to identify; from his description, one can infer that the 10 × 10 matrix of molar absorptivities was pre-inverted by some method and then applied, row by row, to the column vector of observed absorbances.

A project of considerable magnitude was undertaken around 1958: our sources are an editorial (Müller 1958) in Analytical Chemistry and two editorials (Anonymous 1958, 1959) in Chemical and Engineering News. An instrument was developed by the International Telephone and Telegraph Corp. to scan IR spectra and to convert them to digital form on punched tape, up to 3000 sampling points being envisaged. The calculation of the constituents of a multicomponent mixture is then entrusted to an electronic computer equipped with a library of reference spectra. For qualitatively known mixtures "a set of biorthogonal functions" is previously computed. (We suspect this set to be identical to the rows of the left-inverse $\underline{V}^T$, Sec. 5.6) The set is then applied to the mixture spectrum. The first model,developed by Taplin and Rogoff, was delivered to the Sloan-Kettering Institute in the autumn of 1958; a commercial prototype was reported to be offered in the summer of 1959, but has not, to the author's knowledge, been heard of since.

Anderson and Moser 1958 programmed an IBM 650 computer for the routine conversion of emission spectrographic film line transmittances to concentrations, using non-linear calibration curves.

In the field of mass spectrometry, we find,in a book by Barnard (1953:214 sqq.) a pilot study, on the ACE computer, of the application of least-square methods to quantitative analysis. Pre-inversion of the relevant reference matrices is advocated; the chief difficulties are the fact that the matrices were ill-conditioned and, more importantly, could not be assumed to be constant in time.

Mihm 1958 decribes a system for the remote calculation of mixture composition from mass-spectrometric data. The data obtained are punched into cards and then transmitted by Teletype to a computer centre, where they are matched with a paper tape containing the relevant pre-computed inverse of the 'calibration' matrix, described as "semipermanent" and needing "infrequent changes'. The results, normalized to sum to 100% on an air- and water-free basis, are transmitted back to the factory.

McAdams 1958 gives a great deal of information about a set of computer programs designed to set up calibration matrices and to invert them; the sophisticated routines allow for a great many corrections to be applied; the matrix finally inverted is always square, with orders of up to 26 occurring for the mass-spectrometric application.

Hopp and Wertzler 1958 advocate a particular method of solving the linear equations resulting from mass-spectrometric multicomponent analyses with square matrix. Their method, equivalent to Gaussian elimination, prevents the propagation of errors due to (physically meaningless) negative elements of the solution vector, which are set to zero.

Of considerably greater relevance to the present thesis is an important paper by Sternberg, Stillo and Schwendeman 1960. In their study of a five-component system (see Fig. 1.1), which would be considered moderately difficult by our standards, the procedure is as follows. The system is simplified to one of four components by assuming the fifth to be the original ergosterol minus the four irradiation products found. The four components are determined by least squares from various sets of 8 and 12 absorbances in the ultra-violet, a set of 12 equidistantly chosen wavelengths giving the best results. Their work relies on published values of the specific absorptivities obtained by others, in a different solvent from that used for the analysis of the mixture; the mixture spectra were recorded on a Beckman DK-2 spec-

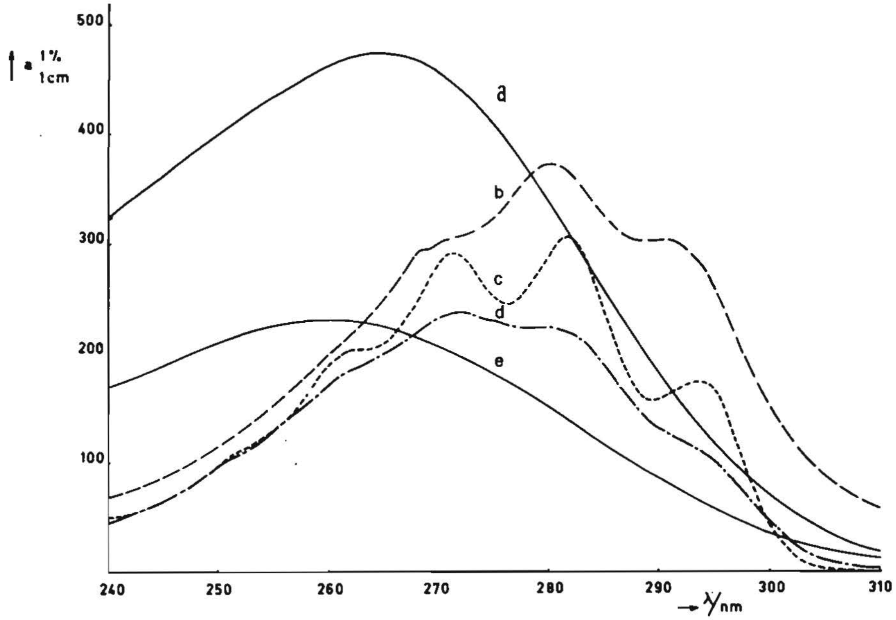

Fig. 1.1 The absorption spectra in the ergosterol irradiation system (after Sternberg, Stillo and Schwendeman 1960):

(a): calciferol$_2$, (b): tachysterol$_2$ ($\frac{1}{2}a^{1\%}_{1\,cm}$),
(c): ergosterol, (d): lumisterol$_2$, (e): precalciferol$_2$.

trophotometer. The 4 x 4 matrices $\underline{A}^{\tau}\underline{A}$ were inverted on the MISTIC computer and $(\underline{A}^{\tau}\underline{A})^{-1}\underline{A}^{\tau}$ calculated by hand. For their best matrix, results as low as -9.1 % (for precalciferol$_2$) occasionally were found for a known concentration of zero. The observed standard deviations (from 11 analyses) range from 1.2 % of total concentration for the best to 5.8 % for the worst component.

Barnett and Bartoli 1960 present a method for determining, by least squares, the coefficients of what is essentially an inverse square matrix, $\underline{A}^{-1}$, of order n, such that concentrations can be found from $\underline{A}^{-1}\underline{a}_o$, where $\underline{a}_o$ is an observed vector of mixture absorbances. They use $m > n$ mixtures to establish the coefficients and, from the residuals, determine correction terms for non-linearity. For the IR, a number of complex systems, $n \leq 8$, yields very good standard deviations.

Zscheile, Murray, Baker and Peddicord 1962, apparently unaware of the existence of theoretical results that accomplish the same purpose, obtain a crude measure of the variance of the unknowns in least-square UV spectrophotometry by a laborious process of simulation. Their three- and four-component systems of RNA constituents are termed "unstable" because constituent spectra exhibit some correlation. An attempt to find less "unstable" sets of wavelengths by restricting the number of points at which the spectrum is sampled quite naturally yielded no results.

In 1961, Grinev, Rau and Svishchev in Russia worked out a program for the Ural computer to perform least-squares calculations with spectrophotometric determination of concentrations as an objective. Their work has been applied by Romanovskii c.s. 1964 to a three-component mixture (IR data); they state an accuracy of 3%, using 100 sampling points in the spectrum at a low rate of scan; a Strela computer was used to process the data.

White, Shapiro and Pratt 1963 apply linear programming to the problem of estimating the composition from an overdetermined system of linear equations, the components being RNA nucleotides. This of course automatically disallows chemically meaningless negative elements of the solution vector. It would appear to be extremely difficult to assess the merit of their method and the statistical behaviour of their solution.

Lee, McMullen, Brown and Stokes 1965 derive the 'error coefficients' (proportional to our $\hat{\sigma}(\hat{x}_j)$) relating the error in calculated concentration to the errors in absorbances for the spectrophotometric least-squares application. They propose a use of the method for mixtures of up to nine oligonucleotides,the aim being automatic nucleotide sequence analysis.

In an introductory textbook of fairly recent origin (Bauman 1961), the use of least squares for infra-red analysis is touched upon. The examples given are restricted to weakly overdetermined systems, e.g. four equations in three unknowns.

In all the above references, the coefficient matrix is assumed to be known exactly, i.e. without error. This, though facilitating the treatment, hardly seems in accordance with reality.

In the related subject of rank analysis of spectral data, where the question of interest is the number of species present in a reaction mixture, the papers by Ainsworth 1961, 1963, Wallace 1960, Wallace and Katz 1964 and Katakis 1965 deserve mention.

Finally, we should note here a few articles in which partial results of the present study have been published: Herschberg and Sixma 1962, Cerfontain, Duin and Vollbracht 1963, Herschberg 1964 and Arends c.s. 1964; we also wish to cite the theses of Vollbracht 1962, Kaandorp 1963 and Wanders 1964, in which the method presented has been applied extensively, chiefly to mixtures of various arylsulfonic acids.

1.4 Prospects and retrospects

During the work described in this thesis, the point-by-point observation of absorbances was of course felt to be somewhat burdensome. The desirability of some method of automatic data gathering was felt keenly, the more so since data reduction had already been relegated to a computer. When the opportunity of obtaining punched paper tape output from a spectrophotometer presented itself, we were therefore eager to experiment with the device, since it promised to reduce the effort of observation to negligible proportions.It was somewhat disappointing to find that the precision obtained from this instrument was decidedly inferior to that afforded by the laborious manual method. The chief cause of this was the non-reproducibility of the wavelengths at which the absorbances were punched. Though the instrument operated on the theoretically preferable double beam principle - the point-by-point readings were taken on single-beam equipment - it proved less reliable in practice: the double-beam construction in fact obliged us to record a complete spectrum (reference or mixture) before another spectrum could be observed. When contrasting this with the manual method, in which all absorbances at a given wavelength could be observed nearly simultaneously, and which, moreover, permitted using higher absorbance values, one finds the superiority of the non-automatic method quite explicable.

In spite of this we believe that, eventually, automatic data gathering will prove capable of giving a precision at least as good as that now available from manual observation. In fact, given a computer which assumes an active rather than a passive attitude to its input, the method as presented could be automatized in much like its present form: reading an absorbance, changing the cell seen by the beam and setting a new wavelength are all simple commands with proper instrumentation. Even the problem of variable precision of an automatically recorded value of the absorbance, which manually is compensated for by a slower read-out, could be overcome by programming. When obtaining a value known to be more affected by noise than is desirable, an active computer could rather simply be made to read out that value as often as is necessary to reduce it, by averaging, to one having the proper weight. Thus all values could be brought to a common and high level of precision. Moreover, such a program, requiring the computer to be active once every few seconds only, would occupy a negligible fraction of the attention of any computer and would not interfere measurably with other computer programs in the course of simultaneous execution.

We therefore hold our method to be eminently capable of full automatization; in fact, the practice of observing at equidistant points in the spectrum seems more suitable to an automatic procedure than any scheme of attempting to choose some "best" set of wavelengths.

Looking back, the author wishes to recall with profound gratitude the encouragement and guidance given him by the late Professor of Organic Chemistry at Amsterdam, Dr. F.L.J. Sixma, in whose laboratory most of the experimental part was performed; the assistance of Drs. D. de Jager in the experiments described in Chapter VI is also gladly acknowledged.Among the many others from whose help he had profited, the author wishes to mention especially Drs. P.J. van der Haak for stimulating discussions and Dr. A.C.M. Wanders for his enthusiastic help in programming.

It is a pleasant duty to record the material assistance given this work by the Mathematical Centre, Amsterdam, which provided generous access to facilities on its computers and by the 'Stichting Scheikundig Onderzoek Nederland' which granted a contribution toward the requisite instrumentation.

CHAPTER II

THE EXPERIMENTAL PROCEDURE AND ITS RATIONALE

2.0 Introductory

After a description of the procedure adopted (Section 2.1), the present chapter discusses successively:

- the need to obtain absorbances of the reference compounds as nearly simultaneously as possible with the absorbances of the mixture(s) instead of relying on previously determined absorptivities of the components (Section 2.2);
- the theoretical desirability of observing high values of the absorbances (Section 2.3);
- the feasibility of observing absorbances in the range $0 \leq a \leq 1.6$ (Section 2.4);
- the rationale of gathering observations at k wavelengths, where k considerably exceeds n, the number of components to be determined (Section 2.5);
- some additional advantages accruing from the use of overdetermined systems, i.e. those for which $k \gg n$ (Section 2.6),

these being the features in which our practice departs from conventional procedure.

2.1 The experimental procedure

2.1.1 Preparation and observation

Preparations for an analysis consist of obtaining n solutions of known composition to serve as references. All substances are weighed in accurately, using such amounts that evaporation losses can be excluded; the amounts of solvent used are determined by weighing too. Normally, these reference solutions will be solutions of pure compounds; occasionally, known mixtures of such solutions may be employed as references (Ch. VI). The concentrations of both reference and mixture solutions are preferably adjusted to have maximum absorbances in the range 1.4 to 1.6. Care is taken to use a single batch of solvent for a complete experiment; the solvents usually employed are ethanol (after a spectrophotometric check on the absence of benzene) and *iso*-octane ("special for spectroscopy"). When using test mixtures for the unknown(s), these are made up by careful weighing in of the reference solutions; the composition of such test mixtures is believed to be known to within 1 part in 10,000 of the corresponding reference solutions.

After filling all cells with the solvent employed, the absorbances of the cells are determined at various points in the λ-region of interest, arbitrarily putting the absorbance of cell no. 1 equal to zero; all cells with the exception of no. 1 (which is left undisturbed) are then emptied; each is then filled (after careful rinsing) with a reference solution or a mixture solution. In all, to permit the simultaneous determination of m unknown samples, each to be analysed for n components, $m + n + 1$ cells are required. The wavelength setting is then adjusted to the first wavelength chosen, cell no. 1 is set to $T = 100\ \%$, $a = 0$ and the absorbances of the remaining $n + m$ cells are then read off successively at that wavelength setting, the time-constant (damping factor) of the instrument being so adjusted that the absorbances can comfortably be estimated to within 0.001 of absorbance. In the range $0.96 \leq a \leq 1.6$, use is made of additional electronic amplification by a factor 10 of the signal; with a suitably chosen damping factor, the additional noise introduced by the use of this feature does not prevent a comfortable estimate of a to within 0.001, though, of course, the time taken for a reliable read-out to be obtained is appreciably longer at $a \doteq 1.5$ than at $a \doteq 0.5$, say.

After all observations at a given wavelength have been made, it is customary to read out a for cell no. 1 again; ideally, this should be 0.000; in fact, $|a|$ is very rarely in excess of 0.003 (the instrument permitting $a \geq -0.040$ to be read out) and usually well below that. Occasionally (say every half-hour or so) the position of the zero-transmittance point ($T = 0$, $a = \infty$) is checked and, if necessary, adjusted.

Only after completing the full series of observations at any wavelength - which may take from about 1 minute for $m + n = 3$ up to about 10 minutes for $m + n = 23$ - the wavelength setting is adjusted to a subsequent value of λ; the cycle is then repeated, starting at the adjustment of the slit width and/or amplification to $a = 0$ for cell no. 1.

Usually, observations are made at some 20 to 40 wavelengths, which are nearly always equidistant in λ.

2.1.2 Some observational details

All observations reported in the present work, unless otherwise noted, have been made manually with a Zeiss PMQ II single beam spectrophotometer under the operating conditions as recommended by the manufacturer. The instrument normally has a sliding cell holder accommodating four cells; in the course of the present work, extension of the number of cells in the holder became advisable. Accordingly, Mr. J. Groot successively designed and constructed an eight-cell sliding holder (shown in Herschberg and Sixma 1962, Fig. 1) and a twenty-four-cell holder on the caroussel principle, described and shown in Cerfontain and Groot 1964. The cells employed at the Amsterdam University Laboratory for Organic Chemistry are commercially available 'Ultrasil' fused silica stoppered cells of $d = 1.000$ cm nominal path length; the correctness of the stated d was verified by Bemetel - T.N.O. (Amsterdam) to within 0.0005 cm.

Optically, differences in the absorbances of these cells themselves (when filled with the solvent to be employed) are usually less than 0.01. However, the practice invariably is to determine these cell absorbances immediately prior to an experiment; when they show significant variation with wavelength, they are explicitly allowed for in the subsequent treatment of the absorbances of the mixture(s) and references; when they are constant in the spectral region of interest, no explicit correction need to be made: by data reduction with inclusion of a 'resultant cell constant' the effect of any constant amounts added to or subtracted from the elements of $\underline{a}$ or any column of $\underline{A}$ is nullified (Section 2.6.3).

On account of the increased risk of an undue proportion of scattered light, observations below a certain wavelength should not be included; this lower limit in λ has been set at about 240 nm on the basis of some quite rough experiments. Fortunately, the condition $\lambda > 240$ nm proves not to be hampering to the performance of the analysis, since most of the interesting spectral detail in the applications is present at wavelengths well above this cut-off limit.

2.2 Nearly simultaneous observations.

2.2.1 Wavelength reproducibility in multicomponent systems

If a single unknown substance is to be determined it is usual to adopt the following procedure: a number of solutions of known, different concentrations c_i is made up and their absorbances a_i observed at a given wavelength setting λ_1. If the (a,c) relationship is linear and passes through the origin, the slope ε of the calibration line $a = \varepsilon . c$ is taken to be the absorptivity of that substance at λ_1. The concentration x of an unknown sample of that substance, observed to have, at some later time, an absorbance a_x, is then estimated from $\hat{x} = a_x/\varepsilon$.

It may be remarked in passing that, as far as the author has been able to ascertain, neither the linearity nor the non-significance of the intercept, i.e. the hypothesis that the calibration line passes through the origin, has been subjected to statistical tests. This is somewhat surprising for a procedure that purports to test the validity of Beer's law for a given compound at a certain wavelength, the more so since such tests are readily available; it must also be considered regrettable that published values of ε very often lack an indication of the precision achieved.

Estimating $\hat{x}$ from a_x and a previously determined ε evidently implies constancy in time of ε and, as ε is dependent on λ, clearly also is based on the assumption of reproducibility of the wavelength setting λ_1. Since, however, it is known that the wavelength setting is liable to some drift in all instruments, it is usual to choose λ_1 at some stationary value (nearly invariably a maximum) of $\varepsilon(\lambda)$,

$$\left(\frac{d\varepsilon}{d\lambda}\right)_{\lambda = \lambda_1} = 0,$$

since a variation $\Delta\lambda$ in the wavelength setting will then have minimal effect on the observed absorbances.

While there is no doubt that this constitutes good practice, it is unfortunately inapplicable if one wishes to determine two or more substances simultaneously. Even if the shapes of the n spectra $\varepsilon_j(\lambda)$ allow extrema to be found for all n compounds independently,

$$\left(\frac{d\varepsilon_j}{d\lambda}\right)_{\lambda = \lambda_j} = 0 \; ; \; j = 1,\ldots,n,$$

as they often do, the slopes of the other spectra, $\left(\frac{d\varepsilon_i}{d\lambda}\right)_{\lambda = \lambda_j}$ will not generally vanish for $i \neq j$; a maximum in any one curve $\varepsilon_j(\lambda)$ will correspond to points of non-zero slope on the other $n - 1$ curves $\varepsilon_i(\lambda)$; quite generally, there will be $n(n - 1)$ such points of non-zero slope.

It then becomes necessary to give some attention to the variation of the absorptivities with time such as will be caused by instrumental drift in the wavelength setting. The same necessity also arises, independently of the number of components, in the proposed scheme of observing absorbances at a large number of points in the spectrum of the sample to be analyzed.

It will be sufficient to discuss short-time drift of the wavelength scale, say such as may be expected to occur within the course of a single day, since it is comparatively easy to keep the instrument free from gross errors of wavelength calibration. A regular check on the calibration, in conjunction with adjustment whenever necessary, which may be performed daily, will serve to define the wavelength scale sufficiently well to exclude any such gross errors; a convenient method has been indicated, which consists in taking readings of two absorbance values of a solution of known concentration on the steep slopes on either side of the maximum of a narrow absorption band (p-xylene, λ_{max} = 274.5 nm) and readjusting the wavelength scale so as to make these absorbances coincide with the values obtained at the time that the ε_{ij} were determined (Tunnicliff, Brattain and Zumwalt 1949).

2.2.2 Short-time drift of the wavelength setting

In order to obtain an approximate value of the rate of drift of the wavelength scale, $\frac{d\lambda}{dt}$, the apparent wavelength, as read on the scale, of the strong hydrogen emission line λ = 486.133 nm (solar F) was observed as a function of time. The slit width was chosen as 0.13 mm, corresponding to 2.1 nm at this wavelength; at this width the emission line appears as an almost triangular maximum ($\Delta\lambda_{\frac{1}{2}} \doteq 4$ nm), superimposed on a smooth continuous background (Fig. 2.1). If the natural line width is neglected and if

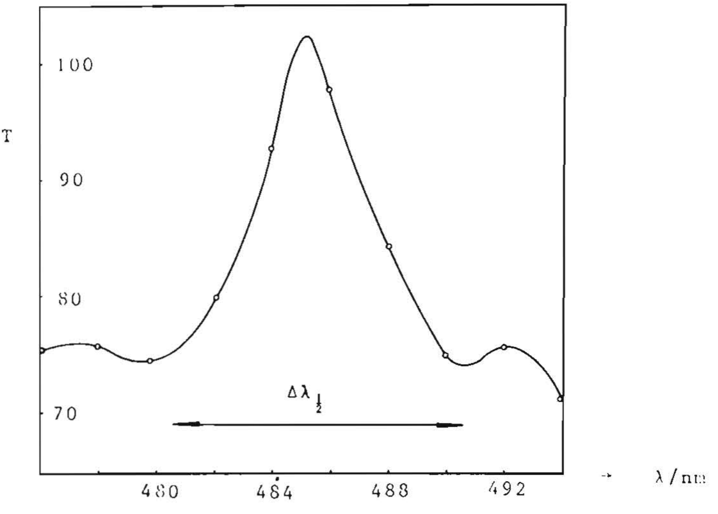

Fig. 2.1 The shape of the hydrogen line F = 486.133 nm when viewed at an optical slit width of 2.1 nm.

the background intensity and the detector sensitivity are assumed constant over the small region (∿ 4 nm) observed, the apparent energy T received at the detector will be determined entirely by the triangular slit transmission function. If the maximum of the slit function, which occurs at the position of the wavelength set, coincides with the position λ_{max} of the emission line, the energy observed will evidently be a maximum, T_{max}. At some other setting, λ_i, the energy arriving at the detector from the emission line will be proportional to $|\lambda_{max} - \lambda_i|$, provided that $|\lambda_{max} - \lambda_i|$ is at most equal to the slit width.

Readings were taken at three wavelength settings λ_1, λ_2 and λ_3 such that one of these lies on the other side of the maximum than do the other two. Then, for the right hand branch of the triangular transmission function T(λ) in Fig. 2.2,

$$T - T_1 = \frac{T_2 - T_1}{\lambda_2 - \lambda_1} (\lambda - \lambda_1),$$

and, by symmetry, for the left hand branch

$$T - T_3 = - \frac{T_2 - T_1}{\lambda_2 - \lambda_1} (\lambda - \lambda_3).$$

Hence λ_{max}, the abscissa of the point of intersection, follows from

$$\lambda_{max} = \frac{\lambda_1 + \lambda_3}{2} + \frac{\lambda_2 - \lambda_1}{2} \cdot \frac{T_3 - T_1}{T_2 - T_1} .$$

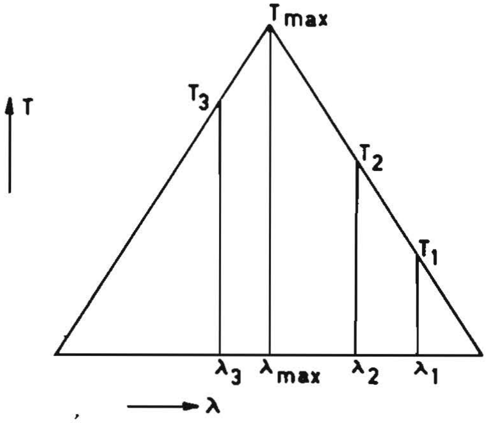

Fig. 2.2 To illustrate the determination of the abscissa of the maximum of an observed transmission function.

In the course of the experiment, the slit setting was left undisturbed; the radiation from the hydrogen light source was thus allowed to fall on the prism continuously, just as would be the case during an actual measuring period. At regular intervals T was read off at the three wavelength settings of 484, 486 and 488 nm; λ_{max} as determined by the above formula was plotted as a function of time, the latter being reckoned from the moment of switching the instrument on. The results are shown in Fig. 2.3 and summarized in the following table.

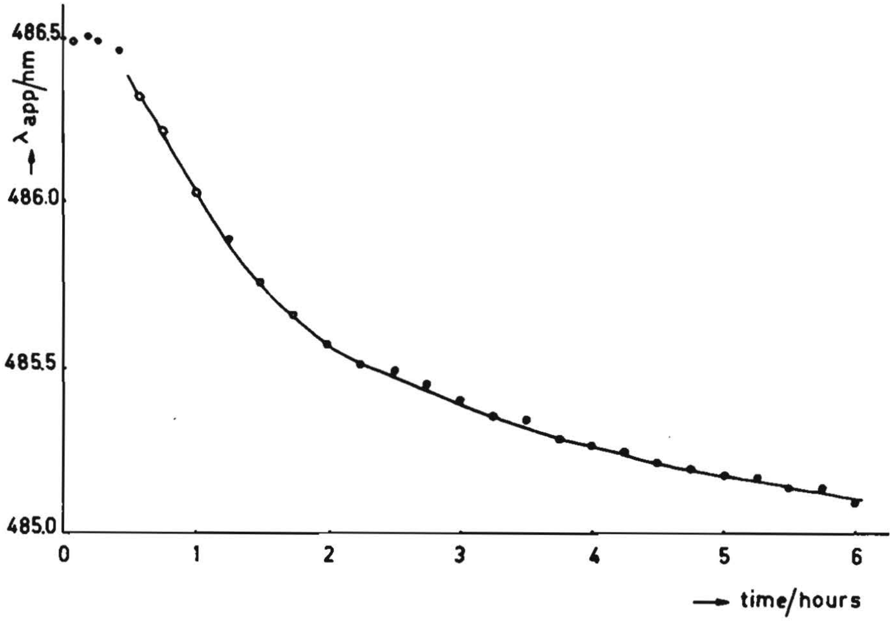

Fig. 2.3 The apparent wavelength λ_{app} of F = 486.133 nm as a function of the time elapsed since the instrument was switched on.

Period after switching on extending from (hours)	to	$-\frac{\Delta\lambda}{\Delta t}$ (nm hr^{-1})
1.0	2.0	0.45
2.0	3.0	0.17
3.0	4.0	0.14
4.0	5.0	0.09
5.0	6.0	0.09

From the fit of a smooth curve the standard deviation of a single determination of λ_{max} may be roughly estimated as 0.02 nm, which, being one hundredth of the distance of the divisions on the λ-scale, seems satisfactory. The shape of the curve indicates that eventually a steady state will be reached for which $\frac{d\lambda}{dt} = 0$; however, this will not occur within an ordinary working day. The cause of the drift of the λ-scale must be sought in the unavoidable heating of the prism by the incident radiation, which causes its refractive index to change. The expansion of the prism material is another possible contributing factor, which may be neglected in our case, since the coefficient of expansion happens to be particularly small for vitreous silica.

From the manufacturer's data for the scale drift as a function of temperature, the observed rate of drift is seen to correspond to about 2.5 to 0.5 degrees per hour. For 1.0 degree/hour the corresponding rate of drift of the wavelength scale is given in the table below:

Wavelength (nm)	$-\frac{d\lambda}{d\theta}$ (nm/degree)
200	0.014
250	0.023
300	0.042
400	0.097
500	0.18
600	0.30
700	0.45
800	0.58
1000	0.73
1200	0.74

Considered as a function of λ, the curve of $|\frac{d\lambda}{d\theta}|$ vs. λ runs approximately parallel to the curve of the reciprocal dispersion, $\frac{d\lambda}{ds}$ vs. λ; this fully agrees with Martens's data (Martens 1904), which also indicate that the temperature coefficient $\frac{dn}{d\theta}$ of the refractive index in air for vitreous silica does not depend very strongly on λ, the total range of variation of $\frac{dn}{d\theta}$ between $\lambda = 257$ nm and $\lambda = 508$ nm being less than 40% of the average value of $\frac{dn}{d\theta}$.

2.2.3 The effect of the wavelength drift

Assuming that 1.0 degree/hour is a reasonable value of the rate of heating of the prism, we may proceed to calculate some representative values of the change in observed absorbance such as may be expected from the corresponding rate of scale drift of the instrument, for which we may put

$$\frac{da}{dt} = \frac{da}{d\lambda} \cdot \frac{d\lambda}{dt} .$$

As a first example, we may take a point on the flanks of the 2-methylnaphthalene absorption peak ($\sim$ 319 nm), for which the maximum molar absorptivity $\varepsilon = a/cd$ is about 510 $\text{mole}^{-1}.\text{l}.\text{cm}^{-1}$ in ethanol. At points 1 nm on either side of this maximum, $|\frac{d\varepsilon}{d\lambda}|$ is graphically determined to be about 170 $\text{mole}^{-1}.\text{l}.\text{cm}^{-1}.\text{nm}^{-1}$. Then for a solution of 3.0×10^{-3} mole l^{-1}, a cell length of 1 cm and, by interpolation, $|\frac{d\lambda}{dt}|_{319} = 0.050$ nm. hour^{-1}, one calculates a drift $|\frac{da}{dt}|$ of $|c.d.\frac{d\varepsilon}{dt}.\frac{d\lambda}{dt}| = 0.026$ units of absorbance per hour. Since a for these points under the stated conditions is about 1.0, it is seen that allowing one hour to elapse between a recalibration and an observation of a may cause an error of about 2.5% in a, if this error is understood to be a departure from the value obtained at the recalibration.

A less extreme example of apparent drift in absorbance might be taken from the case of an alcoholic solution of benzene, $c = 5 \times 10^{-3}$ $\text{mole}.\text{l}^{-1}$ in a 1 cm cell; on the flanks of the band at 254.5 nm the slope is at most equal to 100 $\text{mole}^{-1}.\text{l}.\text{cm}^{-1}.\text{nm}^{-1}$; for the same assumed rate of temperature drift of the prism one finds $|\frac{da}{dt}| = 0.012$ units of absorbance per hour, which, at an absorbance of somewhat less than one, causes an error of > 1%.

The magnitude of the error thus introduced is revealed more clearly if the expected drift rate is compared to the (indirectly determined) standard deviation of a single determination of absorbance, which is about 0.001_2. For the cases quoted the drift rate must therefore be considered highly significant. The same high significance of the drift will be found for practically all bands exhibiting 'fine structure' in the quartz region, say for all bands of up to 5 nm half-width in the ultraviolet or up to several times that half-width in the near infrared (λ > 1000 nm). The drift rate may also be comfortably observed directly by following the apparent absorbance on the flanks of any fine structure band and even on the more moderate slopes of the absorption curve of the chromate ion (at, say, $\lambda = 250$ or 290 nm) for a few hours with an undisturbed wavelength setting.

Fortunately, it is possible to take most absorbance readings at considerably smaller slit widths than were employed for the determination of $\frac{d\lambda}{dt}$ at 486 nm; unless the hydrogen light source shows serious signs of ageing, the mechanical slit width throughout the spectrum may be chosen to be no larger than about 0.02 mm, which presumably causes only about one sixth of the rate of scale drift found above for s = 0.13 mm. Since, with large values of $|\frac{d\varepsilon}{d\lambda}|$, the use of a large s is distinctly inadvisable because of the resulting non-linearity (Ch. V), one will definitely prefer working at small slit widths whenever considerable slopes of absorption bands are likely to be encountered.

If one requires that the error due to scale drift be kept down to say about one half the standard deviation of an absorbance measurement ($\sigma(a) = 0.001_2$), one calculates that for s = 0.02 mm, an assumed rate of heating of the prism of 1 degree per hour at s = 0.13 mm and the values of $|\frac{d\varepsilon}{d\lambda}|$ and concentration quoted above, the observation of the absorbances of the known and unknown samples should succeed each other within about 0.2 hours. This requirement may be somewhat relaxed for lower slopes and/or concentrations; on the other hand, the time which may be allowed to elapse between the observations must be further reduced for the slopes and absorbances quoted if one measures during the first two or three hours after switching on the instrument.

2.2.4 Some conclusions

1. For our instrument at least, a systematic error is introduced by the gradual heating of the prism and the consequent drift of the wavelength scale.
2. Even after several hours' operation of the instrument, absorbances may drift off to the extent of several per cent. per hour on steep slopes of (a,λ) curves.
3. Not measuring during the first few hours of operation and thereafter completing all observations at any given wavelength within say 0.2 hours will keep the resultant drift in absorbance down to an acceptable level.

2.3 The desirability of high absorbance values

We shall show in this section that the use of high absorbance values is desirable under the following assumptions:

1. the absorbances of the reference solutions are known exactly.
2. there is a working range of absorbances, say $0 \leq a \leq a_{max}$ such that, within this working range, the precision of an observation of a, say $\sigma(a)$, is essentially constant; for $a > a_{max}$, $\sigma(a)$ is assumed to be much higher.

   (For simplicity's sake we shall also assume all observations to be made at unit optical path length).

3. the spectra of the reference compounds are distinguishable (do not form a linearly dependent set).

We shall illustrate the case for n = 2 components; the proof can be generalized easily to arbitrary n. In some region of λ the molar absorptivities $\varepsilon_1(\lambda)$ and $\varepsilon_2(\lambda)$ of the species (compounds) concerned will be functions such as are shown in Fig. 2.4. We may use this figure equally well to represent the absorbances $a_1(\lambda)$ and $a_2(\lambda)$ as observed at unit cell length in suitable concentrations c_1 and c_2. Now if we choose two wavelengths, λ_1 and λ_2, at which to observe the absorbances of the pure species and their mixtures, we may represent these values in a two-dimensional absorbance space R_A, identifying each of the coordinate axes of R_A with the absorbance found at the corresponding wavelength. Thus (Fig. 2.5) C will represent the absorbance of the first compound, its coordinates being a_{11} (first wavelength, first compound) and a_{21} (second wavelength, first compound); likewise, A has coordinates a_{12}, a_{22}. By the assumed linearity and additivity the absorbances a_{10} and a_{20} of any mixture of these species of composition $0 < x_1' < c_1$, $0 < x_2' < c_2$ are represented by a point in R_A with coordinates a_{10}, a_{20}:

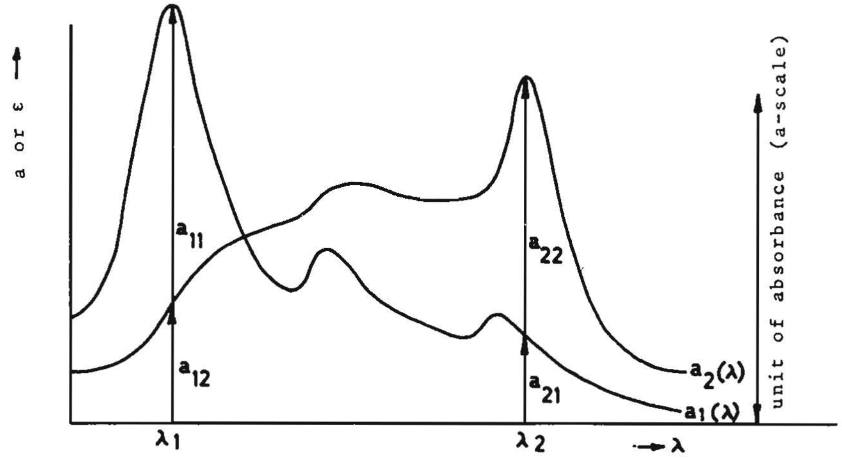

Fig. 2.4 A hypothetical pair of spectra $a_j(\lambda)$ to show the quantities needed for the construction of the absorbance vector diagram (Fig. 2.5). The ordinate may alternatively be taken to represent absorptivities ε, in which case the choice of wavelengths has been made so as to maximize $|\det \underline{E}|$.

$$\begin{cases} a_{10} = x_1a_{11} + x_2a_{12} = x_1'\varepsilon_{11} + x_2'\varepsilon_{12} \\ a_{20} = x_1a_{21} + x_2a_{22} = x_1'\varepsilon_{21} + x_2'\varepsilon_{22} \end{cases},$$

with the unprimed x_1 and x_2 introduced to represent relative concentrations, $x_j = x_j'/c_j$, and unit cell length being understood throughout.

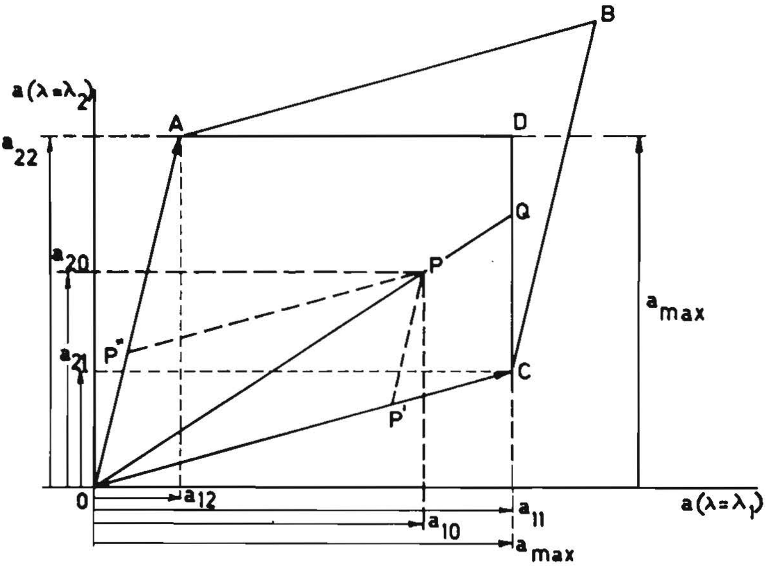

Fig. 2.5 The absorbance vector diagram constructed in R_A from the quantities shown in Fig. 2.4. OP represents the absorbance vector of a mixture, OP' and OP'' are its resolved components which allow the composition x_1, x_2 to be found by construction. The point Q gives the optimum concentration choice for a mixture which has the same ratio x_1/x_2 as P. The concave quadrilateral ABCD is the region of forbidden absorbances in which at least one of the maximum instrumentally admissible absorbances is exceeded.

By the inequalities imposed on x_1 and x_2, all such points are confined to the interior and boundary of the parallelogram OABC. Now we may also represent the point x_1', x_2' in a concentration space, or the point x_1, x_2 in an equivalent relative concentration space R_C (Fig. 2.6). By the restrictions imposed, the admissible points are contained within the interior and boundaries of the rectangle O'A'B'C' in concentration space. Clearly, points in R_A correspond uniquely to points in R_C, and conversely. In fact, the transformation from one space to the other is homogeneous and linear, as follows from the equations above and may be made even more manifest by rewriting them:

$$\underline{a}_o = x_1\underline{a}_1 + x_2\underline{a}_2 = x_1'\underline{\varepsilon}_1 + x_2'\underline{\varepsilon}_2 = \underline{Ax} = \underline{Ex}'$$

in which

$$\underline{a}_o = \begin{pmatrix} a_{10} \\ a_{20} \end{pmatrix}; \quad \underline{a}_1 = \begin{pmatrix} a_{11} \\ a_{21} \end{pmatrix}; \quad \underline{a}_2 = \begin{pmatrix} a_{12} \\ a_{22} \end{pmatrix} \quad ; \quad \underline{A} = \begin{pmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{pmatrix} \quad ;$$

$$\underline{x} = \begin{pmatrix} x_1 \\ x_2 \end{pmatrix} ; \underline{x}' = \begin{pmatrix} x_1' \\ x_2' \end{pmatrix} ; \underline{\varepsilon}_1 = \begin{pmatrix} \varepsilon_{11} \\ \varepsilon_{21} \end{pmatrix} ; \underline{\varepsilon}_2 = \begin{pmatrix} \varepsilon_{12} \\ \varepsilon_{22} \end{pmatrix} ; \underline{E} = \begin{pmatrix} \varepsilon_{11} & \varepsilon_{12} \\ \varepsilon_{21} & \varepsilon_{22} \end{pmatrix} .$$

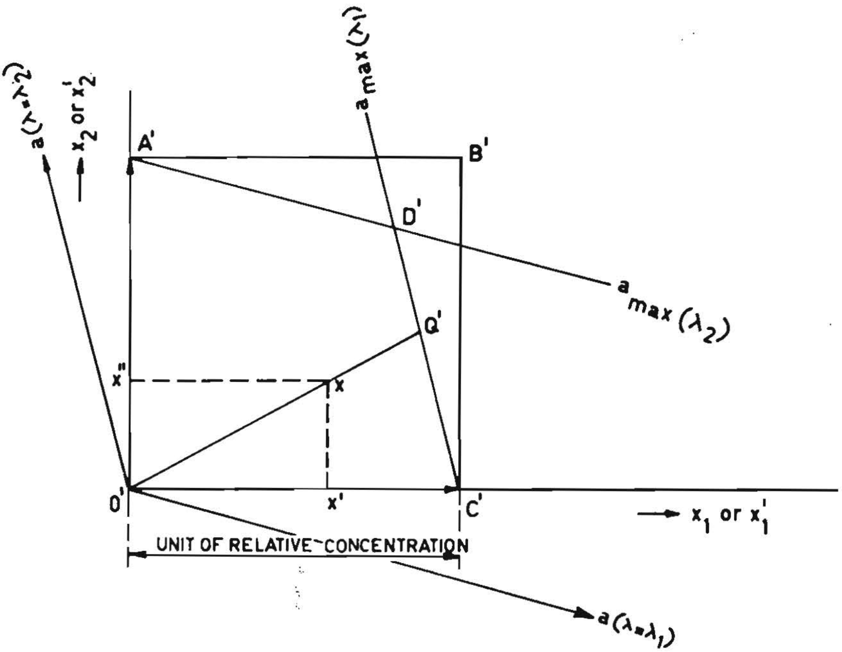

Fig. 2.6 The R_C analogue of Fig. 2.5. There are two distinct ways of labelling the orthogonal coordinate axes: as x_1, x_2, they stand for relative concentrations: the unit shown then represents unity on either axis; alternatively, as x_1', x_2', the pair of axes O'C' and O'A' indicate concentrations of the mixture directly. In that case, the unit distances O'C' and O'A' correspond to the (possibly different) concentrations of the first and second reference solutions respectively. The $a(\lambda = \lambda_1)$ and $a(\lambda = \lambda_2)$ axes are an oblique pair parallel to A'D' and D'C' respectively.

We may remark that $\underline{A}$ and $\underline{E}$ are related by $\underline{A} = \underline{EC}$, $\underline{C}$ being a diagonal matrix of concentrations,

$$\underline{C} = \begin{pmatrix} c_1 & 0 \\ 0 & c_2 \end{pmatrix};$$

it is also easily seen that $\underline{x}' = \underline{Cx}$.

For given $\underline{A}$ and $\underline{a}_o$, $\underline{x}$ is of course given by

$$\underline{x} = \underline{A}^{-1}\underline{Ax} = \underline{A}^{-1}\underline{a}_o$$

since $\underline{A}^{-1}$ exists by the assumed distinguishability of $\underline{a}_1$ and $\underline{a}_2$. For the rather special case of a two-dimensional vector $\underline{x}$ treated here, a graphical solution is available (Fig. 2.5): the observed absorbances of the mixture $P = (a_{10}, a_{20})$ are considered as a vector OP; this is resolved by oblique projection, parallel to OA and OC respectively, into its components OP' along OC and OP'' along OA. Then $x_1 = OP'/OC$ and $x_2 = OP''/OA$; for given $\underline{A}$, an oblique net may be constructed in advance (Fig. 2.7).

Now consider the observed point P: each of its coordinates is observable with limited precision; the instrumental error in a_{10} may be characterized perhaps by its standard deviation $\sigma(a_{10})$ or possibly by a 100 - α per cent. confidence interval, or even, ideally, by its probability distribution; analogous data will describe the error of observation in a_{20}. Clearly, the uncertain-

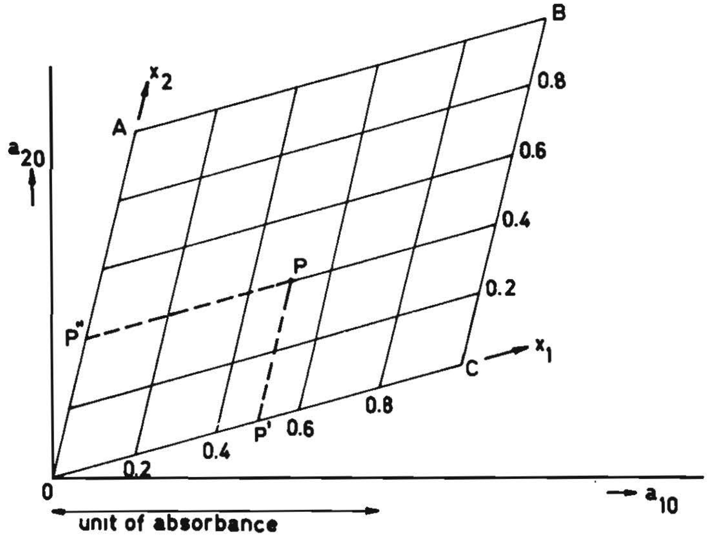

Fig. 2.7 The construction and use of an oblique net. The vectors OA and OC are constructed as in Fig. 2.5; they can then be subdivided as shown and a net of parallels can be drawn. To use the net for the graphical determination of $\underline{x}$, plot P (with the coordinates a_{10}, a_{20} as determined by observation) in the usual way, then read off x_1 and x_2 on the axes so labelled with the aid of the net.

ty in P will be, in some sense, a combination of the uncertainties in its components. With the latter supposed given, we can, if the law of combination is known, construct the resulting uncertainty in P. Rather generally, this will be some region in R_A surrounding P; let the equation of the boundary of any such region be $\phi(a_1, a_2) = 0$. Now we shall assume that the area of the region surrounding P is a reasonable measure of its uncertainty, just as the uncertainty in say a_{10} can be expressed in a measure of length, e.g. $\sigma(a_{10})$. Likewise, the resulting uncertainty in x will be taken to be represented by the area surrounding the point $\underline{x} = \underline{A}^{-1}\underline{a}_o$; the uncertainties will clearly be corresponding if the area, $\Sigma(\underline{x})$ say, in R_C arises from the area $\Sigma(\underline{a}_o)$ around P by a point to point transformation of its boundary according to $\underline{a}_o = \underline{Ax}$; this will result in an equation of the boundary in R_C, $\psi(x_1, x_2) = 0$.

Now
$$\Sigma(\underline{a}_o) = \iint_{\phi} da_1 da_2 \quad ; \quad \Sigma(\underline{x}) = \iint_{\psi} dx_1 dx_2 ,$$

the limits of integration being given by the functions ϕ and ψ in the usual manner. But, by the theory of transformation for multiple integrals:

$$\Sigma(\underline{a}_o) = \iint_{\phi} da_1 da_2 = \iint_{\psi} \begin{vmatrix} \frac{\partial a_1}{\partial x_1} & \frac{\partial a_1}{\partial x_2} \\ \frac{\partial a_2}{\partial x_1} & \frac{\partial a_2}{\partial x_2} \end{vmatrix} dx_1 dx_2, \text{ and } \frac{\partial a_i}{\partial x_j} = a_{ij} .$$

Now for this transformation the Jacobian is not a function of the x_j, therefore

$$\Sigma(\underline{a}_o) = \begin{vmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{vmatrix} \iint_{\psi} dx_1 dx_2$$

or
$$\Sigma(\underline{a}_o) = (\det \underline{A}) . \Sigma(\underline{x});$$

and similarly:
$$\Sigma(\underline{a}_o) = (\det \underline{E}) . \Sigma(\underline{x}').$$

The last formula may be taken to mean that for a given uncertainty in the coordinates of P, the resulting uncertainty in concentrations x_1' and x_2', considered jointly, is minimal for maximal det $\underline{E}$, that is for a choice of wavelengths λ_1 and λ_2 that maximizes

$$\varepsilon_{11}\varepsilon_{22} - \varepsilon_{12}\varepsilon_{21} ,$$

as was noted in passing by van Dranen 1958.

Actually, this statement is not quite exact: if one interchanges the designation of the indices on λ, det $\underline{E}$ changes its sign, whereas obviously no physical change is caused by merely redefining the former λ_1 as the new λ_2 and conversely. The difficulty, which is caused by the fact that $\underline{A}$ defines a relation between oriented spaces, whereas we disregard orientation when comparing areas, is most easily removed by substituting the moduli, $|\det \underline{A}|$ and $|\det \underline{E}|$, whenever the formulae above contain determinants.

Ideally then, the condition $|\det \underline{E}|$ = maximum should govern the selection of wavelengths for a sensitive analysis, i.e. when we wish to have the detectable amounts as low as possible.

Now likewise, for given $\Sigma(\underline{a}_o)$, the condition

$$|\det \underline{A}| \text{ maximal}$$

is, by the above, equivalent to requiring that a given uncertainty in $\underline{a}_o$ have minimal influence on the uncertainty in $\underline{x}$. Hence we require the reference spectra $\underline{a}_j$, which are the columns of $\underline{A}$, to consist of as high absorbances as are consistent with the working range.

To see that the same argument applies to the absorbances $\underline{a}_o$ of the mixture, we reason as follows. Given $\underline{A}$ and $\Sigma(\underline{a}_o)$, $\Sigma(\underline{x})$ is determined completely; this given error region in $\underline{x}$ will be least important relative to the values of $\underline{x}$ if $\underline{x}$ is maximal in each element. But the elements of $\underline{x}$ cannot be chosen to be arbitrarily high, because there is the requirement that $\underline{Ax}$ be at most equal to a_{max} for each element. Hence, for $\underline{a}_o$ too, the maximal element should be chosen to be at the top of the working range.

It will be seen that the derivation above, from the point where we introduce the matrix relations onward, is independent of the number of components, provided we consider $\Sigma(\underline{a}_o)$ and $\Sigma(\underline{x})$ as n-dimensional (hyper)volumes rather than as areas.

Likewise, the reasoning also applies to overdetermined systems, since, as we shall show (Section 3.1) the vector of estimated relative concentrations in that case is merely a weighted average of systems of the form $\underline{Ax} = \underline{a}_o$.

Similarly, assumption 1 of this section is redundant; the assumption of errors in the $\underline{a}_j$ ($j \neq 0$) will be shown (Section 3.1) to be very nearly equivalent to the assumption of increased errors in $\underline{a}_o$, so that the above theory still applies in essence.

We conclude:

Under the assumption that $\sigma(a)$ is independent of a in a certain working range $0 \leq a \leq a_{max}$, the concentrations should - if possible - be adjusted so that

$$\max_{i} a_{ij} = a_{max}, \quad j = 0, 1, \ldots, n,$$

i.e. so that the maximum absorbance of the sample and of each reference solution is at the top of the working range.

2.4 The feasibility of high absorbances.

That the precision of an observation of absorbance should be constant within a large interval of absorbance, say $0 < a < 1.6$, is undoubtedly a bold assumption. It will be argued in this section that the assumption is nevertheless justified by and large, at least for the instrument and procedure adopted in the present work.

The instrument is provided with a read-out facility in which a scale is optically projected onto a ground glass screen; the latter carries a hair line, 0.3 mm in width, which serves to read the scale. The scale itself, being essentially galvanometric, is linear in the transmittance (though it is graduated in both T and a) and has a length of 40 cm for the 0... 100% range of T. Of this scale, the range T = 10.97 .... 100% (a = 0.96 .... 0) is used for reading absorbances directly; for a > 0.96, actuating a lever introduces an additional amplification factor of 10, thus expanding the T-scale.

In the present work this expanded scale has been used in the range T = (10 ×) 2.51 .... 10.97% (a = 1.6 .... 0.96).

In all cases the observer was instructed to estimate on the absorbance scale and to report his estimate to the nearest thousandth in a.

This is quite comfortably possible around T = 50%, a = 0.301, where $\Delta a = 0.001$ corresponds to $\Delta T = 0.12\%$ or about 0.5 mm on the scale; it is definitely less than comfortable as we proceed up the a scale to a = 0.96, where $\Delta a = 0.001$ corresponds to $\Delta T = 0.025\%$ or $\frac{1}{20}$ of the distance between the ½ % graduations on the T-scale. This ΔT is equivalent to 0.1 mm on the scale and obviously is on the verge of what is observable by the unaided human eye. (The comparison with a standard burette, where 0.01 ml also corresponds to ∿ 0.1 mm is not valid, because the galvonometer scale is noisy, i.e. subject to random fluctuations). Vollbracht (1962:52), who applied the method, states that a can be read to within only 0.002 in the range 0.8 ... 0.96 and to within

0.001 elsewhere ($a \leq 1.6$); other observers, including the present author, feel themselves capable of estimating a to the nearest 0.001 even in this range.

A moderate to high degree of damping, i.e. imposing a higher time constant on the galvanometer, becomes advisable when observing at $a > 0.96$, because the increased amplification increases the noise in proportion. However, there is no doubt that it is possible to report a to 0.001 even in the presence of the greater noise level in the range $0.96 \leq a \leq 1.6$.

It is not remarkable that observations at $a > 0.8$, say, are apt to take appreciably longer than those in the 'easy' range of a ($a < 0.5$ say). This results either from the higher damping or from the observer's wish to average readings over a certain period of time; both are complementary aspects of the same fundamental phenomenon, viz. that the spectrophotometer and its associated amplifier constitute a noisy channel in the sense of information theory. The signal in our case can be identified with the transmittance T, which decreases with increasing a, the noise can assume two distinct levels according as the additional amplification factor of 10 has or has not been used. In the light of these facts it is inevitable that, for constant precision in a, the time to obtain a reading of a should depend on a.

It is plausible that by instructing the observer to report a to the nearest thousandth some information is lost in the 'easy' range; this is almost certainly true in the sense that, had a comparable amount of time and care been expended on the observations of $a < 0.5$, the absorbances in that region might have had an even better read-out precision. It is not difficult to show that such precision would be redundant: it will not contribute appreciably to the final analytical result $\hat{\underline{x}}$.

The practice to limit a to 1.6 is to some extent arbitrary. The criterion has been based on the observation that residuals r_i definitely tend to larger values when absorbance values $a \sim 1.7 \ldots$ .. 1.8 occur in the i-th equation. This is not to say that an occasional value $a = 1.65$ or so is unacceptable; for safety, though, the condition $a \leq 1.6$ should be observed.

It is easy to show that this read-out precision is quite realistic too, in the sense that it is comparable to the calculated standard deviation of an observation, $\sigma(a)$. Vollbracht, for instance, lists in an appendix (Vollbracht 1962:115) the root-mean-square values of the residuals per equation, $\hat{s}$, for 65 analyses of reaction mixtures in 82.4% sulfuric acid; the root-mean-square value of $\hat{s}$ is 0.0016 per equation. Now $\hat{s}$ is related to the estimated standard deviation, $\hat{\sigma}(a)$, of an observation of a by

$$\hat{s}^2 = \underline{\hat{\xi}}^\tau \underline{\hat{\xi}} . \hat{\sigma}^2(a).$$

It follows that for his data, $\hat{\sigma}(a)$ is slightly over one thousandth (0.0013) in absorbance with absorbances up to $a \sim 1.5$ (Vollbracht 1962:52).

Conclusions:

1. On our instrument, a careful observer can obtain absorbance values meaningfully to within 0.001 (or, at worst, to within 0.002 for the range $0.8 \leq a \leq 0.96$).
2. Such a read-out precision is obtained at the price of longer observation times for $a > 0.8$; it is essentially constant in the entire usable range, $a \leq 1.6$.
3. This read-out precision is justified by the comparable magnitude of the standard deviation of an observation of a.

2.5 The desirability of overdetermined systems.

Given n spectra, with $k >> n$ sampling points available, one may legitimately require that an analysis be based on the optimal choice of n sampling points; that is, for a given observational effort ($n(n + 1)$ absorbances) and a given precision of each observation, one requires that the resultant uncertainty in the estimated composition $\hat{\underline{x}}$ be minimal.

The optimality criterion is simple (Section 2.3): one only has to consider the $k \times n$ matrix $\underline{E}$ of molar absorptivities ε_{ij} and to construct all $\binom{k}{n}$ possible combinations $\underline{E}'$ of n rows at a time; the submatrix $\underline{E}'$ that has maximum $|\det \underline{E}'|$ is optimal. In practice, this is a fairly intractable problem, since there seems to be no strict algorithm that avoids calculating all $\binom{k}{n}$ determinants.

Now for $k = 30$ and $n = 6$, $\binom{k}{n} = 593{,}775$; even with an electronic computer, the evaluation of such a number of determinants is not trivial.

Fortunately, such a choice is not necessary; if the k absorbances $\underline{a}_o$ of the sample are determined along with those of the n references ($\underline{A}$), the resultant overdetermined system of equations,

$$\underline{A}\underline{x} = \underline{a}_o$$

may be solved for $\underline{x}$ by least squares. The solution, which we shall call $\hat{\underline{x}}$, has the important property that it represents a weighted average of all those solutions $\underline{x}'$ that can be formed by the choice of distinct, square submatrices $\underline{A}'$ (with $\det \underline{A}' \neq 0$);

the solutions $\underline{x}'$ are assigned weights proportional to the squares of the determinants of their submatrices $\underline{A}'$, i.e. inversely proportional to their expected variances. (For a proof, see Section 3.2).

As a corollary of the above we see that there need be no fear to include rows in $\underline{A}$ that, taken by themselves, would lead to small values of $|\det \underline{A}'|$; the weighting alluded to guarantees that 'bad' wavelengths cannot spoil the preciser results from 'good' wavelengths. We may also rephrase this to read that a solution by least squares utilizes all the information available from $\underline{A}$.

Conclusion:

At the cost of additional observations, the use of an overdetermined system of equations obviates the need to choose a best set of n wavelengths; the least squares solution completely utilizes the information presented.

2.6 Other advantages of overdetermined systems.

Anticipating the theoretical development given in Chapters III and IV, we mention here some practical advantages inherent in the use of overdetermined systems and their solution by least squares.

2.6.1 Increased precision.

For a given precision of observation of the absorbance - e.g. expressed as its standard deviation $\sigma(a)$ - it can be proved that the precision - e.g. expressed as a (hyper)volume $\Sigma(\underline{x})$ (section 2.3) - is always better ($\Sigma(\underline{x})$ is smaller) when using the matrix $\underline{A}$ of $k > n$ rows than when merely using any $n \times n$ square submatrix $\underline{A}'$ of $\underline{A}$.

When k is chosen between 20 and 40, as is usual in practice, the standard deviation for a single component $\hat{x}_j$ of $\hat{\underline{x}}$ is lowered by some factor between 2 and 4 when passing from the optimal $\underline{A}'$ to the rectangular $\underline{A}$. This seems to hold for most spectra investigated, independently of n. An example with two scatter diagrams is given in Herschberg and Sixma 1962.

2.6.2 Some assurance of accuracy.

A least-squares solution, which merely represents a weighted average of the solutions of the constituent equations with square matrices, is just as susceptible, in principle, to systematic error as are its constituent equations. However, the following argument will show that freedom from gross systematic error, i.e. the accuracy of the results, remains to some degree verifiable.

It has been established in the present work that $\sigma(a)$, the standard deviation of an observation of a, is constant under widely differing circumstances; in virtual independence of such variables as solvent, wavelength region and compounds analysed, $\sigma(a) = 0.001_2$. But each overdetermined system, on solution, yields from the residual vector $\underline{r}$ and $\hat{\underline{x}}$ a quantity $\hat{\varepsilon}$,

$$\hat{\varepsilon}^2 = \frac{\underline{r}^\tau \underline{r}}{\hat{\xi}^\tau \hat{\xi} \cdot (k-n)} ,$$

which represents an estimate $\hat{\sigma}(a)$, with k-n degrees of freedom, of $\sigma(a)$. Now the hypothesis $\hat{\sigma}(a) = \sigma(a)$ can be tested by standard statistical techniques; if the hypothesis is accepted, the result $\hat{\underline{x}}$ can be accepted too as accurate in the sense that it conforms reasonably to the model, since it is easily shown that deviations from the model will all tend to increase $\hat{\varepsilon}$.

If $\hat{\varepsilon}$ is significantly greater than $\sigma(a)$, it is natural to conclude that the assumptions of the model have been violated. Of the various deviations from the model, at least three manifest themselves in a sufficiently characteristic manner in the residual vector to be identifiable.

a) Sufficiently large read-out or clerical errors in the absorbances.

When manually recording values of a, an occasional error of these types is hardly avoidable. Such an error shows up in the residuals as a single, widely deviant element r_i; also, on removing the offending row of $\underline{A}$, the sum of squared residuals $\underline{r}^\tau\underline{r}$ falls sharply, often so much - e.g. by a factor of 3 or more - that one concludes that the single element r_i has contributed more than half of the variance of $\underline{r}$. In such cases there need be no hestitation to strike out the i-th row; in practice, $|r_i| > 0.005$ is highly suspect, $|r_i| > 0.01$ is 'impossible'. It is advisable to include a facility to reject such rows in the computer program; the rejection of the i-th row may be governed by some condition such as

$$'|r_i| > t\hat{s} \text{ and } |r_i| > 0.005 ' ,$$

where $t = 3$ seems safe (though its choice should be left to the user) and $\hat{s}$ is the root-mean-square value of an element of $\underline{r}$, $\hat{s}^2 = \underline{r}^\tau\underline{r}/(k-n)$.

b) Slit width error.

If the ratio $s/\Delta\nu_{\frac{1}{2}}$ of the optical slit width to the band half-width is not sufficiently small, absorbances cannot be expected to be proportional to concentration (Chapter V). The insufficient spectral purity of the incident radiation will affect the rows of $\underline{A}$ and $\underline{a}$ to varying degrees, which leads to a characteristic pattern in the residuals, which, in turn, allows this departure from the assumptions of the model to be identified with fair certainty. (see Section 5.5 and Figs. 5.10 and 5.11).

c) The presence of an extraneous component.

It may, of course, happen that an unanticipated component is present in the mixture to be analyzed; if that component contributes significantly to $\underline{a}$, its first effect will be to increase $\underline{r}^{\tau}\underline{r}$, since its contribution to the variance of $\underline{a}$ will be largely unexplained (unless the spectrum of the unanticipated component is a linear combination of the columns of $\underline{A}$ or very nearly so).

Rather remarkably, we found that a plot of $\underline{r}$ against λ shows at least some features of the spectrum of the unanticipated component. Notably, characteristic maxima will often be preserved, though they may be subject to distortion. Nevertheless, this may yield useful information about the nature of the unanticipated component.

For an illustration, we refer to Fig. 2.8. In the system naphthalene/1-methylnaphthalene/2-methylnaphthalene/2,3-dimethyl-

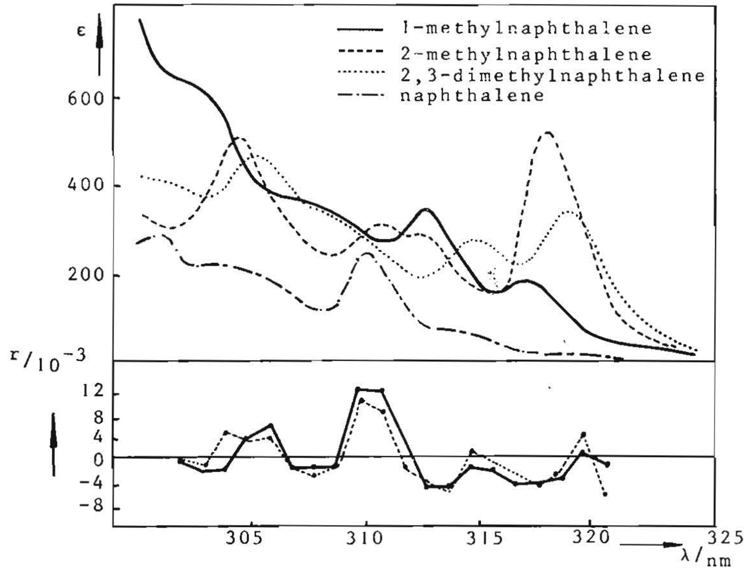

Fig. 2.8 Top: Spectra in a four-component system.
Bottom: Residual spectra of two analyses in this system, where naphthalene has incorrectly been assumed absent.

naphthalene, several synthetic mixtures were analyzed; these contained naphthalene in such concentration that its maximum absorbance in the mixture was 0.022. However, naphthalene was assumed not present; plots of $\underline{r}$ then clearly show the naphthalene maximum at $\lambda \sim 311$ nm as well as two lesser peaks ($\lambda \sim 307$ and $\lambda \sim 320$ nm) which should be classified as 'false detail'.

2.6.3 Insensitivity to differences in cell absorbance.

Any differences in cell absorbances will affect the observed values of $\underline{a}$ and $\underline{A}$. Clearly, this applies to square matrices A (and n-component $\underline{a}$-vectors) as well as to overdetermined systems. Such differences, if uncorrected, will distort the estimates of $\underline{x}$ in either case. However, it is possible to modify the computation of $\hat{\underline{x}}$ by least squares in such a way that such differences do not influence $\hat{\underline{x}}$ in so far as they are constant over the range of wavelengths considered. This advantage is offset by a moderate increase in the standard deviations of the elements of $\hat{\underline{x}}$. It still is true, of course, that, if the cell absorbances vary with λ, they will distort $\hat{\underline{x}}$.

The model usually assumed is that for any wavelength Beer's law and the additivity hold:

(Model I) $$\sum_j a_{ij}\, x_j = a_i \quad \text{for all } i;$$

the modification consists of assuming each absorbance a_{ij} to be affected by a cell absorbance c_j; the same applies to a_i, affected by c_o, say:

(Model II) $$\sum_j (a_{ij} + c_j)\, x_j = a_i + c_o$$

or $$\left(\sum_j c_j x_j - c_o\right) + \sum_j a_{ij}\, x_j = a_i \quad \text{for all } i.$$

The term in parentheses, which is independent of i, may be estimated by least squares as a resultant cell absorbance; it corresponds to the inclusion of a zeroth component in $\hat{\underline{x}}$.

In the earlier part of the work, Model I was assumed always; the observed cell absorbances were, of course, subtracted from the observed a_{ij}. It gradually became clear that Model II was slightly safer, since the observed own absorbances of the cells were only moderately reproducible. The own absorbances of the cells were still observed always; however, the adoption of Model II as the standard mode of computation of $\hat{\underline{x}}$ allowed us the freedom not to correct for them in so far as they did not vary significantly with λ. Since this, when applicable, reduced the clerical work and hence the chance of clerical errors, it was a strong supporting argument for choosing Model II (Cf. Section 3.5).

One would expect, when correcting for the cell absorbances, $\hat{x}_o$, which is the estimate of $\Sigma c_j . x_j - c_o$, to be quite small; this expectation was verified: $\hat{x}_o$ in such cases was usually smaller than its estimated standard deviation for synthetic mixtures. It may, however, be very significant when either the absorbances of the cells are not corrected for or when, as in Vollbracht's work on sulfonation reaction mixtures, a large 'grey' absorbance occurs in the contents of any of the cells; finding relatively large ($|\hat{x}_o| \leq 0.08$ in absorbance) 'backgrounds' is not entirely unexpected when working in 82% sulfuric acid or even in oleum containing 10% SO_3. To summarize: the computation by least squares according to Model II frees $\hat{\underline{x}}$ from all influence of grey (wavelength-independent) background, including differences in cell absorbances.

2.6.4 Availability of a measure of precision.

Not least among the additional advantages of overdetermined systems is the availability *from the data of each analysis*, of an estimate of the precision of any of its results $\hat{x}_j$. This estimate $\hat{\sigma}(\hat{x}_j)$, is given by

$$\hat{\sigma}^2(\hat{x}_j) = \frac{((\underline{A}^{*\tau}\underline{A}^{*})^{-1})_{jj}}{k - n^*} \cdot \underline{r}^{\tau}\underline{r}$$

where $\underline{r}$ is the residual vector $\underline{a}_o - \underline{A}^*\hat{\underline{x}}$; for Model I, $\underline{A}^* = \underline{A}$ and $n^* = n$, the number of components; for Model II, $\underline{A}^*$ is an $(n + 1) \times (n + 1)$ matrix which consists of the observed $\underline{A}$, extended on the left by a zeroth column vector of k unit elements:

$$\begin{pmatrix} 1 & \\ 1 & \\ \vdots & \underline{A} \\ 1 & \end{pmatrix}$$

and $n^* = n + 1$. (For a derivation, see Chapter IV).

The estimated standard deviation $\hat{\sigma}(\hat{x}_j)$ appears to be quite realistic, especially when it is in excess of 0.2% in relative concentration. We believe that $\hat{\sigma}(\hat{x}_j) < 0.2\%$ - which only occurs occasionally - is not to be relied on, since, despite all precautions, some systematic errors persist and these, being of the same order of magnitude, effectively prevent the results of the method to be consistently correct within less than 0.2%.

Commenting on the above formula for $\hat{\sigma}^2(\hat{x}_j)$, we remark that it contains $\underline{r}^{\tau}\underline{r}$, the sum of squares of the residual vector; this quantity is derived afresh for each analysis: hence, each analysis carries its own set of $\hat{\sigma}(\hat{x}_j)$. But, when there are no systematic disturbing causes (Section 3.6.2), the expected value of $\underline{r}^{\tau}\underline{r}$ is also known:

$$E(\underline{r}^{\tau}\underline{r}) = (k - n^*) \;.\; \hat{\underline{\xi}}^{\tau}\hat{\underline{\xi}} \;.\; \varepsilon^2$$

where ε^2 is the roughly constant error variance of an absorbance value and $\hat{\underline{\xi}} = \begin{pmatrix}\hat{\underline{x}}\\ -1\end{pmatrix}$. Then, since we know ε^2 and can usually estimate $\hat{\underline{\xi}}^{\tau}\hat{\underline{\xi}}$ (a rather rough estimate is sufficient) for a given type of analysis, we have, by combining the formulae above:

$$E(\hat{\sigma}^2(\hat{x}_j)) = ((\underline{A}^{*\tau}\underline{A}^*)^{-1})_{jj} \;.\; \hat{\underline{\xi}}^{\tau}\hat{\underline{\xi}} \;.\; \varepsilon^2$$

in which substituting a set of spectra $\underline{A}$ will enable us to calculate the expected value of $\hat{\sigma}(\hat{x}_j)$. Hence we can predict the precision expected from a knowledge of the spectra involved, the approximate relative composition of the analyte and the instrumental constant ε^2. This prediction of precision is, in our opinion, one of the most useful features of the method and obviates a great deal of the preliminary experimentation usually devoted to establishing the feasibility of a proposed method of analysis.

If one is willing to make some further assumptions about the errors in the elements of $\underline{a}$ and $\underline{A}$, an impressive array of statistical means for testing hypotheses about the true composition $\underline{y}$, given $\hat{\underline{x}}$, is also at one's disposal (Chapter IV).

To summarize this section in a few conclusions:

1. Each analysis by least squares carries an estimate of precision derived from the data of each analysis.
2. A knowledge of the instrumental precision and of the spectra of the components enables one in principle to predict the precision to be expected from the analytical results.

CHAPTER III

DATA REDUCTION : POINT ESTIMATION

3.0 Scope

After an introduction (Sec. 3.1), which states the assumptions and definitions that constitute the mathematical model, the classical least-squares point estimator $\hat{\underline{x}}$ is proposed as the preferred estimator, even though $\underline{A}$, the coefficient matrix, is subject to error. In Sec. 3.2, some reasonable properties of $\hat{\underline{x}}$ are discussed; these properties point to the use of $\hat{\underline{x}}$ under the assumption of 'small errors'. Section 3.3 exhibits some properties of $\hat{\underline{z}}$, the theoretically preferable estimator. Section 3.4 discusses the relation between $\hat{\underline{x}}$ and $\hat{\underline{z}}$ in the light of the 'small error' characteristics of the spectrophotometric material, provides some numerical illustrations and presents an efficient algorithm for $\hat{\underline{z}}$. Section 3.5 finally deals with a slight variant of the model (Model II), in which the columns of $\underline{A}$ are referred to their means.

It is regretted that it proved impossible to distinguish in typography between stochastic and non-stochastic variables. It is hoped this important distinction will be apparent from the context.

3.1 The model

The following assumptions (A) are made and the following definitions (D) introduced:

(A1) The mixture is taken to contain at most n known, absorbing species.

Comment: The presence of unanticipated species is briefly discussed in Section 2.6.2(b). The species will also be referred to as components or compounds.

(A2) Each of the n absorbing species is available in (assumed) pure form.

Comment: It may suffice for some purposes that the species are available in a standard form, not necessarily pure. Analyses then will simply be reported relative to that standard.

(A3) A solution of known content c_j is available for each species
Comment: These solutions will be called the reference solutions.

(A4) A solution of known content c_o is available for the mixture.
Comment: In (A4), 'of known content' does not apply when the concentrations sought need not be on an absolute scale - e.g. when the percentage composition only is required; in (A3) this phrase does not apply when only concentrations relative to these of the (standard) references are desired. For some purposes, e.g. in exploratory work, neither quantitative requirement need be met.

(A5) For each species the absorbance at any wavelength is proportional to the concentration of that species.
Comment: (A5) expresses Beer's Law. Chapter V discusses a certain type of departure from (A5); Chapter VI indicates a device that allows valid results even when (A5) is violated.

(A6) For a mixture, the absorbance at any wavelength is the sum of the absorbances of its constituents.
Comment: (A6), which states the additivity of partial absorbances in a mixture, is tantamount to the assumption of absence of physical and/or chemical interaction among the components. It also assumes no interaction of the solutes and the solvent.

(A7) The absorbances of the mixture and of the n constituent species are observed at k wavelengths under identical conditions at each wavelength.
Comment: Identical conditions are taken to include identity of solvent, cell length, slit width and generally all instrumental settings.

(D1) The observed absorbances of the constituent species form a matrix $\underline{A}$ of k rows and n columns, with elements a_{ij}; those of the mixture form a vector $\underline{a}_o$ with k elements a_{io}.

(D2) The relative concentration y_j of the j-th component of the mixture is defined as

$$y_j = \frac{\text{concentration of the j-th component in the mixture}}{\text{concentration of the j-th reference solution}}$$

The y_j form an n-component vector $\underline{y}$.

(A8) An <u>observed</u> absorbance a_{ij} $(j = 0,1,...n)$ is considered a stochastic quantity, and represents the sum of a non-stochastic <u>true</u> absorbance b_{ij} and a stochastic <u>error</u> ε_{ij}:

$$a_{ij} = b_{ij} + \varepsilon_{ij} \; ; \quad \begin{cases} i = 1,\ldots,k \\ j = 0,1,\ldots,n \end{cases} \qquad (3.1{:}1)$$

or, in array form:

$$\begin{cases} \underline{A} = \underline{B} + \underline{E} \\ \underline{a}_o = \underline{b}_o + \underline{\varepsilon}_o \end{cases} \qquad (3.1{:}2)$$

We assume the following about the ε_{ij}:

(A9) They have zero expected value:

$$E(\varepsilon_{ij}) = 0,$$

or, in array notation,

$$E(\underline{\varepsilon}_o) = \underline{0} \quad \text{and} \quad E(\underline{E}) = \underline{0}.$$

(A10) They are statistically independent:

$$E(\varepsilon_{kl}\varepsilon_{mn}) = 0 \text{ unless } k = m \text{ and } l = n.$$

(A11) They are homoscedastic; specifically, the variance of ε_{ij}: does not depend on i or j:

$$E(\varepsilon_{ij}^2) = \varepsilon^2. \qquad (3.1{:}3)$$

(A12) They are small relative to the a_{ij} and the b_{ij}:

$$E_{ij} << b_{ij},$$

or, more weakly,

$$\sum_i \varepsilon_{ij}^2 << \sum_i b_{ij}^2.$$

Comment: We shall be able to formulate (A12) more precisely later, viz. as (A14), Sec. 3.3.

From (A5), (A6), (D2) and (A8), we have

$$\underline{B}\,\underline{y} = \underline{b}_o \qquad (3.1{:}4)$$

as an ideal relation between unobservables. Given the observables $\underline{A}$ and $\underline{a}_o$, the problem is to estimate $\underline{y}$. We propose to use

$$\hat{\underline{x}} \equiv (\underline{A}^\tau \underline{A})^{-1} \underline{A}^\tau \underline{a}_o \;, \; k \geq n, \qquad (3.1{:}5)$$

the classical least squares estimator, as an estimator for $\underline{y}$.

The remainder of this chapter will be concerned with a justification of this estimator. The justification of $\hat{\underline{x}}$ depends on certain properties of our observational material, chiefly (A12).

3.2 Some properties of $\hat{\underline{x}}$

The present section seeks to show that $\hat{\underline{x}}$, under assumption (A12), has some intuitively reasonable properties.
We know by (3.1:2) and (A8) that

$$\begin{cases} E(\underline{A}) = \underline{B} \\ E(\underline{a}_o) = \underline{b}_o . \end{cases} \qquad (3.2{:}1)$$

Now consider the case k = n, that is, a system of n linear equations derived from observations at n wavelengths.

Then, the estimator $\hat{\underline{x}}$ of (3.1:5) takes the form

$$\hat{\underline{x}} = (\underline{A}^{\tau}\underline{A})^{-1}\underline{A}^{\tau}\underline{a}_o = \underline{A}^{-1}\underline{a}_o, \qquad k = n \qquad (3.2{:}2)$$

(The existence of $\underline{A}^{-1}$ and $\underline{B}^{-1}$ can be assumed safely, since the experimenter will have been at some pains to maximize $|\det \underline{A}|$, cf. Sec. 2.3).
This estimator may be seen to be reasonable by the following argument: to the extent that $\underline{E}$ is small relative to $\underline{B}$ (A12), we may write:

$$\underline{A}^{-1} = (\underline{B} + \underline{E})^{-1} \doteq \underline{B}^{-1} - \underline{B}^{-1}\underline{E}\,\underline{B}^{-1},$$

neglecting higher powers of $\underline{E}$. Then, since, by (3.1:4) $\underline{B}\underline{y} = \underline{b}_o$, we have

$$\hat{\underline{x}} = \underline{A}^{-1}\underline{a}_o = (\underline{B}+\underline{E})^{-1}(\underline{b}_o+\underline{\varepsilon}_o)$$

$$= \underline{y} + \underline{B}^{-1}\underline{\varepsilon}_o - \underline{B}^{-1}\underline{E}\,\underline{y}$$

$$\hat{\underline{x}} = \underline{y} + \underline{B}^{-1}(\underline{\varepsilon}_o - \sum_j y_j\underline{\varepsilon}_j), \qquad (3.2{:}3)$$

where $\underline{\varepsilon}_j$ is the j-th column of $\underline{E}$, so, by (A9):

$$E(\hat{\underline{x}}) = \underline{y}\ , \ k = n.$$

Hence we have established:

<u>Property 1</u>: For non-overdetermined systems and to the extent that we may neglect terms in ε^2,

(a) $\hat{\underline{x}}$ is unbiased;

(b) errors ε_{ij}, $j > 0$ affect $\hat{\underline{x}}$ in the same manner as errors $-\varepsilon_{io}y_j$.

Remark that (b) may be interpreted to mean that errors in $\underline{A}$ induce errors in $\underline{a}_o$ in a similar way as in the Berkson model (Mandel 1964:295, referring to Berkson 1950).

For k > n, we next show that $\hat{\underline{x}}$ is a reasonably weighted mean of estimators having Property 1.

The vector $\hat{\underline{x}}$ in (3.1:5) is the solution of

$$(\underline{A}^{\tau}\underline{A})\hat{\underline{x}} = \underline{A}^{\tau}\underline{a}_o. \qquad (3.2:4)$$

For the j-th element of $\hat{\underline{x}}$, we may write, by Cramer's rule:

$$\hat{x}_j = \frac{\det((\underline{A}^{\tau}\underline{A})_j)}{\det(\underline{A}^{\tau}\underline{A})} \qquad (3.2:5)$$

where $(\underline{A}^{\tau}\underline{A})_j$ denotes the matrix obtained from $\underline{A}^{\tau}\underline{A}$ on replacement of its j-th column by $\underline{A}^{\tau}\underline{a}_o$.

<u>Lemma</u>: Let $\underline{F}$ be an n × k matrix and $\underline{G}$ a k × n matrix, k > n; consider square submatrices $\underline{F}_s$ that arise from $\underline{F}$ by striking out a set of k - n columns and square submatrices $\underline{G}_s$ obtained by striking out the corresponding set of rows from $\underline{G}$: submatrices so obtained are said to be corresponding. Then we have: $\det(\underline{FG})$ equals the sum of the determinants of corresponding submatrices $\underline{F}_s\underline{G}_s$, the summation to be taken over all $\binom{k}{n}$ corresponding $\underline{F}_s\underline{G}_s$. (Heyting 1946:55).

By this lemma, the denominator of (3.2:5) can be written

$$\det(\underline{A}^{\tau}\underline{A}) = \sum_s (\det \underline{A}_s)^2.$$

Now, in the numerator, $(\underline{A}^{\tau}\underline{A})_j$ is equal to the product of $\underline{A}^{\tau}$ and $\underline{A}_j$, the latter being obtained from $\underline{A}$ on replacement of its j-th column by $\underline{a}_o$; therefore $\det((\underline{A}^{\tau}\underline{A})_j) = \sum_s \det \underline{A}_s \cdot \det(\underline{A}_j)_s$. Also, neither numerator nor denominator will be changed if we omit all those terms in either for which $\det \underline{A}_s = 0$.

Hence

$$\hat{x}_j = \frac{\sum_s{}' (\det \underline{A}_s) . (\det(\underline{A}_j)_s)}{\sum_s{}' (\det \underline{A}_s)^2}$$

$$= \frac{\sum_s{}' (\det \underline{A}_s)^2 \dfrac{\det(\underline{A}_j)_s}{\det(\underline{A}_s)}}{\sum_s{}' (\det \underline{A}_s)^2}$$

$$= \sum_s{}' w_s \frac{\det (\underline{A}_j)_s}{\det \underline{A}_s} \Big/ \sum_s{}' w_s,$$

where $w_s = (\det \underline{A}_s)^2$ and $\sum_s{}'$ denotes summation over all submatrices $\underline{A}_s$ except those with zero determinant.

But

$$\frac{\det\,(\underline{A}_j)_s}{\det\,\underline{A}_s}$$

is, by Cramer's rule, the j-th element of the solution vector $\hat{\underline{x}}_s$ of an estimator of type (3.2:2) obtained from a non-singlar submatrix $\underline{A}_s$ of $\underline{A}$ and the corresponding subvector $(\underline{a}_o)_s$ of $\underline{a}_o$. (This estimator $\hat{\underline{x}}_s$ has Property 1).

Hence we may write

$$\hat{x}_j = \Sigma_s{}' \; w_s (\hat{\underline{x}}_s)_j \Big/ \Sigma_s{}' \; w_s,$$

but this holds for all $j > 0$ and thus we have established:

Property 2: For $k > n$, $\hat{\underline{x}}$ represents a weighted mean of the solutions of all $\binom{k}{n}$ linear systems that can be obtained from $\underline{Ax} = \underline{a}_o$ by considering its combinations of n rows at a time. The weight assigned to each such solution is (proportional to) the square of the determinant of its coefficients.

Now this weighting is eminently reasonable. We rewrite (3.2:3)

$$\hat{\underline{x}} - \underline{y} = \underline{B}^{-1}(\underline{\varepsilon}_o - \Sigma_j \; y_j \underline{\varepsilon}_j)$$

as

$$\Delta \underline{x} = \underline{B}^{-1} \Delta \underline{a} \; .$$

Hence

$$\Delta \underline{x} \Delta \underline{x}^{\tau} = \underline{B}^{-1} \Delta \underline{a} \Delta \underline{a}^{\tau} (\underline{B}^{-1})^{\tau} .$$

On taking expected values, we remember that $E(\Delta \underline{a} \Delta \underline{a}^{\tau})$ has the form $\nu \underline{I}$ (by (A11), $\nu = \varepsilon^2 + \Sigma_j y_j^2 . \varepsilon^2$), so

$$E(\Delta \underline{x} \Delta \underline{x}^{\tau}) = \nu (\underline{B}^{\tau} \underline{B})^{-1} \tag{3.2:6}$$

In the above, the left hand side is the covariance matrix of the errors. Taking determinants, we have

$$\det(E(\Delta \underline{x} \Delta \underline{x}^{\tau})) = \nu^n (\det\,\underline{B})^{-2} \;;$$

the left hand side is the generalized error variance of $\hat{\underline{x}}$ resulting from a given error variance ε^2 in the observations for fixed $\underline{y}$. Assignment of weights w_s to the $(\underline{x}_s)_j$ above <u>should</u> have been made in inverse proportion to their (generalized) variance, i.e. in proportion to $(\det\,\underline{B}_s)^2$; we find it <u>has</u> been made proportionally to $(\det\,\underline{A}_s)^2$. We conclude that, <u>to the extent that</u>

$$(\det\,\underline{A}_s)^2 = (\det\,\underline{B}_s)^2,$$

the weighting of the solutions $\hat{\underline{x}}_s$ of the square subsystems contained in $\underline{Ax} = \underline{a}_o$, $k > n$, is optimal, and therefore presumably near-optimal for small errors.

As an important corollary of Property 2, we have: the inclusion of rows in $\underline{A}$ that, taken by themselves, would only lead to ill-conditioned submatrices $\underline{A}_s$, is harmless. The resultant $\hat{\underline{x}}$ is dominated almost completely by those submatrices $\underline{A}_s$ that have large squared determinants. We have already enunciated this "robustness" in sec. 2.5.

3.3 The estimator $\hat{\underline{z}}$ and some of its properties

As is well known, the estimator $\hat{\underline{x}}$ is derived by minimizing a residual sum of squares $\underline{r}^T\underline{r}$,

$$\underline{r} \equiv \underline{a}_o - \underline{Ax} \tag{3.3:1}$$

the underlying assumption being that only $\underline{a}_o$, not $\underline{A}$, is subject to error. Therefore, $\hat{\underline{x}}$ does not seem applicable to our case.

For those cases in which $\underline{A}$ is known to be subject to experimental error, there are three approaches: (We specialize at once to the particular case defined by assumptions (A10) and (A11), stating the independence and common variance of all ε_{ij}.)

1. The plane of closest fit (Pearson 1901):
   The problem is stated in terms of finding a (hyper)plane in R^{n+1} such that the sum of the squared distances from the k points defined by the rows of $(\underline{A},\underline{a}_o)$ is minimal.
2. The principal component of minimal variance:
   In this approach, a vector $\underline{v}_{n+1}$ in R^{n+1} is sought; this is to have unit length and should be orthogonal to a sequence of n mutually orthogonal vectors $\underline{v}_j$, $j = 1,\dots,n$, each of which is required to have maximal variance at unit length, thus minimizing the residual variance of $\underline{v}_{n+1}$.
3. The data adjustment method (Deming 1943):
   The problem here is cast in terms of the existence of k relations, assumed to be satisfied exactly by the unobservables $(\underline{B},\underline{b}_o)$. A minimization of the following sum of squares

   $$\sum_{j=0}^{n}\sum_{i=1}^{k}(a_{ij} - \hat{b}_{ij})^2,$$

   under the condition:

   $$\hat{\underline{B}}\hat{\underline{z}} - \hat{\underline{b}}_o = \underline{0}$$

   then leads to estimates $\hat{\underline{B}}$, $\hat{\underline{b}}_o$ and $\hat{\underline{z}}$.

Remarkably, these three conceptually different approaches yield the identical estimator $\hat{\underline{z}}$ for $\underline{y}$:

Let $\hat{\underline{\zeta}}$ be an eigenvector of the symmetric (n+1)-matrix

$$\overset{+}{\underline{A}}{}^{\tau}\overset{+}{\underline{A}} \equiv \begin{pmatrix} \underline{A}^{\tau}\underline{A} & \underline{A}^{\tau}\underline{a}_o \\ \underline{a}_o{}^{\tau}\underline{A} & \underline{a}_o{}^{\tau}\underline{a}_o \end{pmatrix} = (\underline{A},\underline{a}_o)^{\tau}(\underline{A},\underline{a}_o) \tag{3.3:2}$$

such that the associated eigenvalue λ_s is minimal among the eigenvalues of (3.3:2). Then $\hat{\underline{z}}$ consists of the first n elements of $\hat{\underline{\zeta}}$, provided $\hat{\underline{\zeta}}$ is normalized to make $\hat{\zeta}_{n+1} = -1$.

We note, for further reference:

3.3.1 $\overset{+}{\underline{A}}{}^{\tau}\overset{+}{\underline{A}}$ is positive definite, hence:

3.3.2 $\lambda_s > 0$.

3.3.3 λ_s is the estimated variance of the linear combination $\overset{+}{\underline{A}}\underline{\eta} = (\underline{A},\underline{a}_o)\underline{\eta}$ of the columns of $\overset{+}{\underline{A}}$, where $\underline{\eta}$ is parallel to $\hat{\underline{\zeta}}$ but $\underline{\eta}^{\tau}\underline{\eta} = 1$. (This standard result of principal component analysis may be found in any text dealing with the subject, e.g. Anderson 1958). Now $\hat{\underline{\zeta}}^{\tau}\hat{\underline{\zeta}}=1+\hat{\underline{z}}^{\tau}\hat{\underline{z}}$. Hence the estimated variance of $\overset{+}{\underline{A}}\hat{\underline{\zeta}}$ is $(1 + \hat{\underline{z}}^{\tau}\hat{\underline{z}}).\lambda_s = \underline{\zeta}^{\tau}\underline{\zeta}.\lambda_s$.

3.3.4 As defined above, $\underline{\eta}$ is a maximum likelihood estimator (Anderson 1958).

3.3.5 Writing - introducing $\underline{a} \equiv \underline{a}_o$ for brevity -

$$\begin{pmatrix} \underline{A}^{\tau}\underline{A} & \underline{A}^{\tau}\underline{a} \\ \underline{a}^{\tau}\underline{A} & \underline{a}^{\tau}\underline{a} \end{pmatrix}\begin{pmatrix} \hat{\underline{z}} \\ -1 \end{pmatrix} = \lambda_s\begin{pmatrix} \hat{\underline{z}} \\ -1 \end{pmatrix}$$

We have

$$\begin{cases} \underline{A}^{\tau}\underline{A}\hat{\underline{z}} - \underline{A}^{\tau}\underline{a} = \lambda_s\hat{\underline{z}} \\ \underline{a}^{\tau}\underline{A}\hat{\underline{z}} - \underline{a}^{\tau}\underline{a} = -\lambda_s, \end{cases} \tag{*}$$

whence $\{\underline{A}^{\tau}\underline{A} - (\underline{a}^{\tau}\underline{a} - \underline{a}^{\tau}\underline{A}\hat{\underline{z}})\,\underline{I}\}\hat{\underline{z}} = \underline{A}^{\tau}\underline{a}.$

But we may rewrite $\lambda_s = \underline{a}^{\tau}\underline{a} - \underline{a}^{\tau}\underline{A}\,\hat{\underline{z}}$ so that it gives some insight into the relation of $\hat{\underline{z}}$ and $\hat{\underline{x}}$:

Consider the residual vector $\underline{u}$,

$$\underline{u} \equiv \underline{a} - \underline{A}\,\hat{\underline{z}} \tag{3.3:3}$$

defined by analogy with $\underline{r}$ (3.3:1). Then $\underline{u}^{\tau}\underline{u} = (\underline{a} - \underline{A}\,\hat{\underline{z}})^{\tau}(\underline{a} - \underline{A}\,\hat{\underline{z}}) = \underline{a}^{\tau}(\underline{a} - \underline{A}\hat{\underline{z}}) - \underline{z}^{\tau}\underline{A}^{\tau}(\underline{a} - \underline{A}\hat{\underline{z}})$. The first term equals λ_s; by premultiplication of (*) by $\hat{\underline{z}}$ we find the second term to be equal to $-\hat{\underline{z}}^{\tau}\hat{\underline{z}}\cdot\lambda_s$. Hence

$$\underline{u}^{\tau}\underline{u} = (1 + \hat{\underline{z}}^{\tau}\hat{\underline{z}})\lambda_s$$

$$\lambda_s = \frac{\underline{u}^{\tau}\underline{u}}{\underline{\zeta}^{\tau}\underline{\zeta}}$$

Hence we can find $\hat{\underline{z}}$ from

$$(\underline{A}^\tau\underline{A} - \frac{\underline{u}^\tau\underline{u}}{\hat{\underline{\zeta}}^\tau\hat{\underline{\zeta}}}\underline{I})\hat{\underline{z}} = \underline{A}^\tau\underline{a}, \qquad (3.3:4)$$

while $\hat{\underline{x}}$ is estimated from (3.1:5):

$$(\underline{A}^\tau\underline{A})\hat{\underline{x}} = \underline{A}^\tau\underline{a}$$

The difference then consists in the correction, for $\hat{\underline{z}}$, of the main diagonal elements of $\underline{A}^\tau\underline{A}$ by the term $-(\underline{u}^\tau\underline{u})/(\underline{\zeta}^\tau\underline{\zeta})$; this ought to be a small term relative to the diagonal elements when the residuals have a small squared sum ($\underline{u}^\tau\underline{u}$ or $\underline{r}^\tau\underline{r}$) and $\underline{A}^\tau\underline{A}$ is well-conditioned.

3.3.6. We also remark that, by (A10),

$$E((\underline{A}^\tau\underline{A})_{ij}) = (\underline{B}^\tau\underline{B})_{ij}, \quad i \neq j,$$

so the off-diagonal elements of $\underline{A}^\tau\underline{A}$ are unbiased estimates of those of $\underline{B}^\tau\underline{B}$, whereas, by (A11),

$$E((\underline{A}^\tau\underline{A})_{jj}) = (\underline{B}^\tau\underline{B})_{jj} + k\varepsilon^2,$$

so the elements on the main diagonal have an expected bias of k times the common error variance of an observation.

Now, <u>for given</u> $\hat{\underline{z}}$, the expected value of $\underline{u}^\tau\underline{u}$ is

$$E(\underline{u}^\tau\underline{u}) = E((\underline{a} - \underline{A}\hat{\underline{z}})^\tau(\underline{a} - \underline{A}\hat{\underline{z}})) = (1 + \hat{\underline{z}}^\tau\hat{\underline{z}}) . k\varepsilon^2$$

whence, by $\lambda_s = (\underline{u}^\tau\underline{u})/(\hat{\underline{\zeta}}^\tau\hat{\underline{\zeta}})$,

$$E(\lambda_s) = k\varepsilon^2 \text{ for any given } \hat{\underline{z}}. \qquad (3.3:5)$$

In other words: $\hat{\underline{z}}$ is obtained by prior removal of $\lambda_s\underline{I}$ from $\underline{A}^\tau\underline{A}$. The expected value of λ_s is precisely the expected bias in a main diagonal element of $\underline{A}^\tau\underline{A}$.

3.4 The advantages of $\hat{\underline{x}}$ as an estimator

3.4.0 Some characteristics of the observations

Before justifying our preference for $\hat{\underline{x}}$ above $\hat{\underline{z}}$ as an estimator of $\underline{y}$, we shall state some characteristics of our spectrophotometric observations. This will allow us to sharpen assumption (A12), which stated the "smallness" of $\underline{E}$ relative to $\underline{B}$.

a) Experimentally, we arrange that

(A13) $$0 \leq y_j \leq 1$$

Comment: This follows naturally from the arrangement (Sec. 2.3) that both $\underline{a}_o$ and the n columns of $\underline{A}$ have approximately equal maximum absorbances; when we use mixtures as references (Chapter VI), there are other good reasons for not allowing y_j to exceed unity (Sec. 6.6., ex. 3). In fact, $\underset{j}{\Sigma}\, y_j \doteq 1$ usually holds.

b) From the observations, we find that $\underline{a}_o$ is resolvable as a linear combination of the columns of $\underline{A}$ with a very unusual goodness of fit. In a typical analysis the estimate $\hat{R}$ of the multiple correlation coefficient,

$$\hat{R}^2 \equiv 1 - \frac{\underline{r}^{\tau}\underline{r}}{\tilde{\underline{a}}^{\tau}\tilde{\underline{a}}} \cdot \frac{k-1}{k-n-1} \qquad (3.4:1)$$

is found to be $\hat{R} \doteq 1 - 10^{-5}$ or better and certainly $\hat{R} > 1-2\times10^{-5}$. This means that at most $1 - \hat{R}^2 < 4\times10^{-5}$ or 0.004% of the variance of $\underline{a}_o$ is not accounted for by the spectra of the reference solutions. (A few cases where the linearity was known not to hold are exceptions; even so, for the first example of Sec. 6.5, $\hat{R}=0.9998$ was found).

c) As against this, no column of $\underline{A}$ can be written as a linear combination of the remaining columns with a comparable goodness of fit.

In terms of regression analysis, the residual sum of squares is 100 times as large (at the very least) as that obtained from an analysis of $\underline{a}_o$ in terms of $\underline{A}$. In terms of principal component analysis, the variance, say, of the principal component of minimum variance and unit length of $\underline{A}^{\tau}\underline{A}$ is at least 100 times as large as λ_s, which is the variance of the corresponding principal component of $\overset{+}{\underline{A}}^{\tau}\overset{+}{\underline{A}}$.

We now recast this as assumption (A14):

(A14). Let λ_s be the smallest eigenvalue of $\overset{+}{\underline{A}}^{\tau}\overset{+}{\underline{A}}$ and μ be the smallest eigenvalue of $\underline{A}^{\tau}\underline{A}$; then, for the spectrophotometic application,

$$\lambda_s/\mu \leq 0.01.$$

Comment: 1. Since λ_s and μ are available quantities, the validity of (A14) is verifiable for any given case, in contrast to that of such assumptions as (A10).

2. (A14) represents a sharpening of the rather vague assumption (A12).

3. Since μ represents the (estimated) variance of the minimum variance linear combination of the columns of $\underline{A}$, it furnishes us with a measure of distinguishability of the reference spectra. But since λ_s has a known expected value, (3.3:5):

$$E(\lambda_s) = k\varepsilon^2,$$

λ_s (for given k) may be estimated a priori, provided we know the error variance ε^2 of an observation. Now the best estimate of ε^2, $\hat{\varepsilon}^2$ is about $(1.2 \times 10^{-3})^2$ for the usual case. With $20 \leq k \leq 40$, $E(\lambda_s)$ is roughly equal to 4×10^{-5}.

Sufficient distinguishability of the reference spectra $\underline{A}$ then simply means

$$\mu > 4 \times 10^{-3}$$

or better; more generally

$$\mu > 100\ k\varepsilon^2.$$

3.4.1 The relation between $\hat{\underline{x}}$ and $\hat{\underline{z}}$

In this subsection, we shall derive some strict upper bounds for some simple functions involving the difference between the estimators $\hat{\underline{x}}$ and $\hat{\underline{z}}$.

By their defining equations,

$$\hat{\underline{x}} = (\underline{A}^\tau \underline{A})^{-1} \underline{A}^\tau \underline{a}\ , \tag{3.1:5}$$

$$\hat{\underline{z}} = (\underline{A}^\tau \underline{A} - \lambda_s \underline{I})^{-1} \underline{A}^\tau \underline{a}\ . \tag{3.3:4}$$

Then

$$\hat{\underline{x}} - \hat{\underline{z}} = \quad (\underline{A}^\tau \underline{A})^{-1} - (\underline{A}^\tau \underline{A} - \lambda_s \underline{I})^{-1} \underline{A}^\tau \underline{a}\ ,$$

and, by a well-known series expansion (Bodewig 1959:164):

$$(\underline{A}^\tau \underline{A} - \lambda_s \underline{I})^{-1} = (\underline{A}^\tau \underline{A})^{-1} + \lambda_s (\underline{A}^\tau \underline{A})^{-2} + \lambda_s^2 (\underline{A}^\tau \underline{A})^{-3} + \ldots \tag{3.4:2}$$

$$= \sum_{n=0}^{\infty} \lambda_s^n\ (\underline{A}^\tau \underline{A})^{-(n+1)}$$

or

$$\hat{\underline{x}} - \hat{\underline{z}} = \left\{ - \sum_{n=1}^{\infty} \lambda_s^n\ (\underline{A}^\tau \underline{A})^{-(n+1)} \right\} \underline{A}^\tau \underline{a}_o\ ,$$

which, with $\hat{\underline{x}} = (\underline{A}^\tau \underline{A})^{-1} \underline{A}^\tau \underline{a}_o$, reduces to

$$\hat{\underline{x}} - \hat{\underline{z}} = - \sum_{n=1}^{\infty} \lambda_s^n\ (\underline{A}^\tau \underline{A})^{-n}\ \hat{\underline{x}}\ . \tag{3.4:3}$$

For brevity, we introduce the operator $\underline{T}$,

$$\underline{T} \equiv \lambda_s (\underline{A}^\tau \underline{A})^{-1} \equiv \underline{T}^\tau. \tag{3.4:4}$$

Then

$$(\hat{\underline{x}} - \hat{\underline{z}})^\tau (\hat{\underline{x}} - \hat{\underline{z}}) = \sum_{n=1}^{\infty} (-\underline{T}^n \hat{\underline{x}})^\tau \sum_{n=1}^{\infty} (-\underline{T}^n \hat{\underline{x}}) = \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} \hat{\underline{x}}^\tau \underline{T}^{n+m} \hat{\underline{x}} \tag{3.4:5}$$

Now for any integer p

$$\frac{\hat{\underline{x}}^{\tau}\underline{T}^{p}\hat{\underline{x}}}{\hat{\underline{x}}^{\tau}\hat{\underline{x}}} \leq \lambda_{max} \text{ of } \underline{T}^{p} \qquad (3.4:6)$$

$$\begin{aligned}
\text{But } \lambda_{max} \text{ of } \underline{T}^{p} &= \lambda_{s}^{p} \,.\, (\lambda_{max} \text{ of } (\underline{A}^{\tau}\underline{A})^{-p}) \\
&= (\lambda_{s} . \lambda_{max} \text{ of } (\underline{A}^{\tau}\underline{A})^{-1})^{p} \\
&= (\lambda_{s} . (\lambda_{min} \text{ of } \underline{A}^{\tau}\underline{A})^{-1})^{p} \\
\lambda_{max} \text{ of } \underline{T}^{p} &= (\lambda_{s}/\mu)^{p} \qquad (3.4:7)
\end{aligned}$$

Now since $\underline{A}^{\tau}\underline{A}$, like $\underline{A}^{+\tau}\underline{A}^{+}$, is positive definite, $\mu > 0$. But $\underline{A}^{+\tau}\underline{A}^{+}$ arises from $\underline{A}^{\tau}\underline{A}$ by bordering, therefore we can apply a theorem on bordered matrices, which states (Householder 1953:173) that $\mu > \lambda_{s}$, therefore $0 < \lambda_{s}/\mu < 1$.
Combining (3.4:5), (3.4:6) and (3.4:7), we have

$$(\hat{\underline{x}} - \hat{\underline{z}})^{\tau}(\hat{\underline{x}} - \hat{\underline{z}}) \leq \hat{\underline{x}}^{\tau}\hat{\underline{x}} \sum_{n=1}^{\infty}\sum_{m=1}^{\infty} (\lambda_{s}/\mu)^{n+m}$$

and the double sum converges because $0 < \lambda_{s}/\mu < 1$. The double sum is seen to be equal to

$$\frac{(\lambda_{s}/\mu)^{2}}{(1 - \lambda_{s}/\mu)^{2}}\text{ , therefore we have found:}$$

$$(\hat{\underline{x}} - \hat{\underline{z}})^{\tau}(\hat{\underline{x}} - \hat{\underline{z}}) \leq \hat{\underline{x}}^{\tau}\hat{\underline{x}} . \frac{(\lambda_{s}/\mu)^{2}}{(1 - \lambda_{s}/\mu)^{2}} \quad , \qquad (3.4:8)$$

giving an upper bound for $\sum_{j}(\hat{x}_{j} - \hat{z}_{j})^{2}$.

From (3.4:8), we can also derive an upper bound for $|\hat{x}_{j} - \hat{z}_{j}|$:

$$|\hat{x}_{j} - \hat{z}_{j}| \leq ((\hat{\underline{x}} - \hat{\underline{z}})^{\tau}(\hat{\underline{x}} - \hat{\underline{z}}))^{\frac{1}{2}} \leq \frac{\lambda_{s}/\mu}{1 - \lambda_{s}/\mu} \qquad (3.4:9)$$

Likewise, we can compare the residual sum of squares $\underline{r}^{\tau}\underline{r}$ with the analogously defined residual sum of squares $\underline{u}^{\tau}\underline{u}$ (3.3:3).

It is immediate that $\underline{u}^{\tau}\underline{u} > \underline{r}^{\tau}\underline{r}$, because $\underline{r}^{\tau}\underline{r}$ has been minimized exactly by the application of (3.1:5). Also, $\underline{r}$ is orthogonal to the columns of $\underline{A}$:

$$\underline{r} \equiv \underline{a}_{o} - \underline{A}\hat{\underline{x}} = \underline{a}_{o} - \underline{A}(\underline{A}^{\tau}\underline{A})^{-1}\underline{A}^{\tau}\underline{a}_{o},$$

hence $\underline{A}^{\tau}\underline{r} = \underline{A}^{\tau}\underline{a}_{o} - \underline{A}^{\tau}\underline{A}\,(\underline{A}^{\tau}\underline{A})^{-1}\underline{A}^{\tau}\underline{a}_{o} = \underline{0}\ ;\ \underline{r}^{\tau}\underline{A} = \underline{0}^{\tau}$.

Now we can write, using the above orthogonality:

$$\underline{u} - \underline{r} = (\underline{a}_o - \underline{A}\underline{\hat{z}}) - (\underline{a}_o - \underline{A}\underline{\hat{x}}) = \underline{A}(\underline{\hat{x}} - \underline{\hat{z}})$$

$$(\underline{u}-\underline{r})^{\tau}(\underline{u}-\underline{r}) = (\underline{\hat{x}}-\underline{\hat{z}})^{\tau}\underline{A}^{\tau}\underline{A}(\underline{\hat{x}}-\underline{\hat{z}}) = \underline{u}^{\tau}\underline{u} - \underline{r}^{\tau}\underline{u} - \underline{u}^{\tau}\underline{r} + \underline{r}^{\tau}\underline{r}.$$

But $\underline{r}^{\tau}\underline{u} = \underline{r}^{\tau}(\underline{a}_o - \underline{A}\underline{\hat{z}}) = \underline{r}^{\tau}\underline{a}_o = \underline{r}^{\tau}(\underline{r} + \underline{A}\underline{\hat{x}}) = \underline{r}^{\tau}\underline{r} = \underline{u}^{\tau}\underline{r},$

hence $(\underline{u}-\underline{r})^{\tau}(\underline{u}-\underline{r}) = \underline{u}^{\tau}\underline{u} - \underline{r}^{\tau}\underline{r} = (\underline{\hat{x}}-\underline{\hat{z}})^{\tau}\underline{A}^{\tau}\underline{A}(\underline{\hat{x}}-\underline{\hat{z}}).$ (3.4:10)

Again using (3.4:3) for $(\underline{\hat{x}} - \underline{\hat{z}})$, we find from (3.4:10)

$$\begin{aligned}\underline{u}^{\tau}\underline{u} - \underline{r}^{\tau}\underline{r} &= (\underline{\hat{x}}-\underline{\hat{z}})^{\tau}\underline{A}^{\tau}\underline{A}(\underline{\hat{x}}-\underline{\hat{z}}) = \lambda_s(\underline{\hat{x}}-\underline{\hat{z}})^{\tau}\underline{T}^{-1}(\underline{\hat{x}}-\underline{\hat{z}}) \\ &= \lambda_s \sum_{n=1}^{\infty} (\underline{T}^n\underline{\hat{x}})^{\tau}\underline{T}^{-1} \sum_{n=1}^{\infty} \underline{T}^n\underline{\hat{x}} \\ &\leq \lambda_s\, \underline{\hat{x}}^{\tau}\underline{\hat{x}} \sum_{n=0}^{\infty} \sum_{m=1}^{\infty} (\lambda_s/\mu)^{n+m}\end{aligned}$$

Then, working out the double sum, we have

$$\underline{u}^{\tau}\underline{u} - \underline{r}^{\tau}\underline{r} \leq \lambda_s .\, \underline{\hat{x}}^{\tau}\underline{\hat{x}} .\, \frac{(\lambda_s/\mu)}{(1 - \lambda_s/\mu)^2} \qquad (3.4:11)$$

We remark that the equality signs in (3.4:8) and (3.4:11) will hold if and only if $\underline{\hat{x}}$ is parallel to the eigenvector $\underline{v}$ defined by $\underline{A}^{\tau}\underline{A}\underline{v} = \mu\underline{v}$.

We <u>conclude</u>:

The estimator $\underline{\hat{x}}$ yields a closer approximation to the theoretically correct $\underline{\hat{z}}$ as λ_s/μ decreases, that is (given the approximately constant value of λ_s) as μ, which measures the distinguishability of the reference spectra, increases.

3.4.2 Some numerical experience with $\underline{\hat{x}}$ and $\underline{\hat{z}}$

In a few cases, both $\underline{\hat{x}}$ and $\underline{\hat{z}}$ were calculated. We cite some results: <u>Example 1</u>: Analysis no. 105, benzene and 5 alkyl-substituted benzenes, 30 sampling points in the range 250 to 275 nm. The relevant quantities of this analysis have been tabulated in Table 3.1. Similarly, Table 3.2 summarizes data and results for analysis no. 112, naphthalene and various substituted naphthalenes, which may serve as <u>Example 2.</u>

A glance at the Tables shows at once that $\underline{\hat{x}}-\underline{\hat{z}}$ is strictly negligible relative to $\underline{y}-\underline{\hat{x}}$; even in these precise analyses (chemically speaking), y_j-x_j, which is of the same order of magnitude as the estimated standard deviations $\hat{\sigma}(\hat{x}_j)$, is about 10^3 times as large as the difference vector elements $\hat{x}_j-\hat{z}_j$.

However, the spectra that served to construct $\underline{\overset{+}{A}}^{\tau}\underline{\overset{+}{A}}$ for these examples were at least moderately well distinguishable. In order

Table 3.1

Data and results for analysis No. 105 (Model I)

$$\overset{+}{\underline{A}}{}^{\tau}\overset{+}{\underline{A}} = \begin{pmatrix} 8.355\,361 & 9.029\,099 & 10.822\,923 & 9.042\,642 & 7.386\,232 & 5.158\,696 & 7.601\,683 \\ 9.029\,099 & 17.679\,474 & 19.896\,801 & 18.444\,493 & 16.558\,432 & 13.083\,676 & 13.802\,390 \\ 10.822\,923 & 19.896\,801 & 23.051\,567 & 21.045\,637 & 18.758\,745 & 14.484\,186 & 15.689\,808 \\ 9.042\,642 & 18.444\,493 & 21.045\,637 & 21.662\,658 & 20.038\,156 & 15.380\,324 & 14.735\,396 \\ 7.386\,232 & 16.558\,432 & 18.758\,745 & 20.038\,156 & 19.845\,261 & 15.528\,349 & 13.342\,305 \\ 5.158\,696 & 13.083\,676 & 14.484\,186 & 15.380\,324 & 15.528\,349 & 15.579\,686 & 10.704\,135 \\ 7.601\,683 & 13.802\,390 & 15.689\,808 & 14.735\,396 & 13.342\,305 & 10.704\,135 & 10.972\,159 \end{pmatrix}$$

j	component	$\hat{x}_j$	$\hat{\zeta}_j$	$\hat{x}_j-\hat{z}_j$	y_j (Taken)	$\hat{\sigma}(\hat{x}_j)$
1	benzene	0.194 586	0.194 591	-0.000 005	0.1948	0.0006
2	toluene	0.463 631	0.463 673	-0.000 042	0.4588	0.0015
3	ethylbenzene	0.048 896	0.048 859	-0.000 037	0.0538	0.0014
4	<u>o</u>-xylene	0.058 126	0.058 123	+0.000 003	0.0616	0.0013
5	<u>m</u>-xylene	0.027 625	0.027 626	-0.000 001	0.0224	0.0011
6	<u>p</u>-xylene	0.102 900	0.102 898	+0.000 002	0.1022	0.0006
7	-	-	-1.000 000	-	-	-

$\lambda_s = 4.56 \times 10^{-5}$ $\underline{r}^{\tau}\underline{r} = 5.79 \times 10^{-5}$ $\mu = 2.46 \times 10^{-1}$

Table 3.2

Data and results for analysis no. 112 (Model I)

$$\overset{+}{\underline{A}}{}^{\tau}\overset{+}{\underline{A}} = \begin{pmatrix} 12.895\,088 & 12.092\,431 & 13.926\,265 & 16.481\,358 & 13.736\,083 & 13.394\,609 & 13.704\,468 \\ 12.092\,431 & 12.689\,275 & 14.899\,145 & 17.401\,531 & 14.296\,180 & 13.758\,503 & 14.234\,747 \\ 13.926\,265 & 14.899\,145 & 23.446\,885 & 24.777\,811 & 17.378\,831 & 19.352\,903 & 19.283\,110 \\ 16.481\,358 & 17.401\,531 & 24.777\,811 & 28.160\,880 & 20.359\,008 & 22.873\,713 & 22.184\,098 \\ 13.736\,083 & 14.296\,180 & 17.378\,831 & 20.359\,008 & 16.591\,995 & 15.931\,561 & 16.482\,482 \\ 13.394\,609 & 13.758\,503 & 19.352\,903 & 22.873\,713 & 15.931\,561 & 20.654\,000 & 18.471\,305 \\ 13.704\,468 & 14.234\,747 & 19.283\,110 & 22.184\,098 & 16.482\,482 & 18.471\,305 & 17.810\,346 \end{pmatrix}$$

j	component	$\hat{x}_j$	$\hat{\zeta}_j$	$\hat{x}_j-\hat{z}_j$	y_j (Taken)	$\hat{\sigma}(\hat{x}_j)$
1	naphthalene	0.034 586	0.034 579	+0.000 007	0.0362	0.0013
2	-, 1-methyl-	0.083 203	0.083 186	+0.000 017	0.0820	0.0024
3	-, 2-methyl-	0.165 309	0.165 322	-0.000 013	0.1609	0.0014
4	-,2,3-dimethyl-	0.045 686	0.045 651	+0.000 035	0.0487	0.0028
5	-, 1-chloro-	0.297 724	0.297 758	-0.000 034	0.2960	0.0029
6	-, 2-chloro-	0.381 323	0.381 339	-0.000 016	0.3785	0.0014
7	-	-	-1.000 000	-	-	-

$\lambda_s = 2.62 \times 10^{-5}$ $\underline{r}^{\tau}\underline{r} = 3.17 \times 10^{-5}$ $\mu = 9.56 \times 10^{-2}$

to verify the tentative hypothesis that $\hat{\underline{x}}$ is indistinguishable from $\hat{\underline{z}}$ for all practical purposes, we now consider, as <u>Example 3</u>, a case where $\underline{A}^{\tau}\underline{A}$ has intentionally been chosen to be ill-conditioned, that is, to have low μ. The analysis in this example was in terms of mixtures. The pure components were (A), 1.01 mole/l of ethylbenzene and (B), 1.03 mole/l of <u>n</u>-propylbenzene, CCl_4 being the solvent. Observations were made in the near infra-red.

$\overset{+}{\underline{A}} = (\underline{a}_1, \underline{a}_2, \underline{a}_0)$ represents the following set of mixtures of these stock solutions (A) and (B):

$$\underline{C} = \begin{pmatrix}0.6057 & 0.8007 & 0.7064\\ 0.3943 & 0.1993 & 0.2936\end{pmatrix}\begin{matrix}(A)\\ (B)\end{matrix} \quad = (\underline{c}_1, \underline{c}_2, \underline{c}_0)$$

All relevant data have been collected in Table 3.3, the observed spectra of the reference solutions $\underline{a}_1$ and $\underline{a}_2$ are shown in Fig. 3.1, which demonstrates to what remarkable degree the spectra are parallel.

Table 3.3

Data and results for a near infra-red analysis.

Wavelength λ/nm	$\overset{+}{\underline{A}}/10^{-3}$		
	$\underline{a}_1$	$\underline{a}_2$	$\underline{a}_o$
2220	224	219	223
2230	205	199	223
2240	220	210	215
2250	321	306	313
2260	570	568	569
2270	856	829	843
2280	918	864	890
2290	967	919	941
2300	1315	1234	1275
2310	1047	1002	1026
2320	999	939	968
2330	960	927	944
2340	1101	1034	1068
2350	1104	1036	1069
2360	915	860	887
2370	891	867	879
2380	774	719	748
2390	880	816	849
2400	769	701	735
2410	691	609	649
2420	677	619	647

$$\overset{+}{\underline{A}}{}^{\tau}\overset{+}{\underline{A}} \text{ (Model I)} = \begin{pmatrix}14.786\,896 & 13.944\,383 & 14.364\,557\\ 13.944\,383 & 13.156\,031 & 13.549\,258\\ 14.364\,557 & 13.549\,258 & 13.955\,911\end{pmatrix}$$

j	$\hat{x}_j$	$\hat{\zeta}_j$	$\hat{x}_j-\hat{z}_j$	y_j (Taken)	$\hat{\sigma}(\hat{x}_j)$
1	0.448 927	0.488 832	0.000 095	0.4836	0.0118
2	0.511 665	0.511 766	-0.000 101	0.5164	0.0125
3	-	-1.000 000	-	-	-

$\lambda_s = 1.22 \times 10^{-5}$ $\quad$ $\underline{r}^{\tau}\underline{r} = 1.83 \times 10^{-5}$ $\quad$ $\mu = 3.26 \times 10^{-3}$

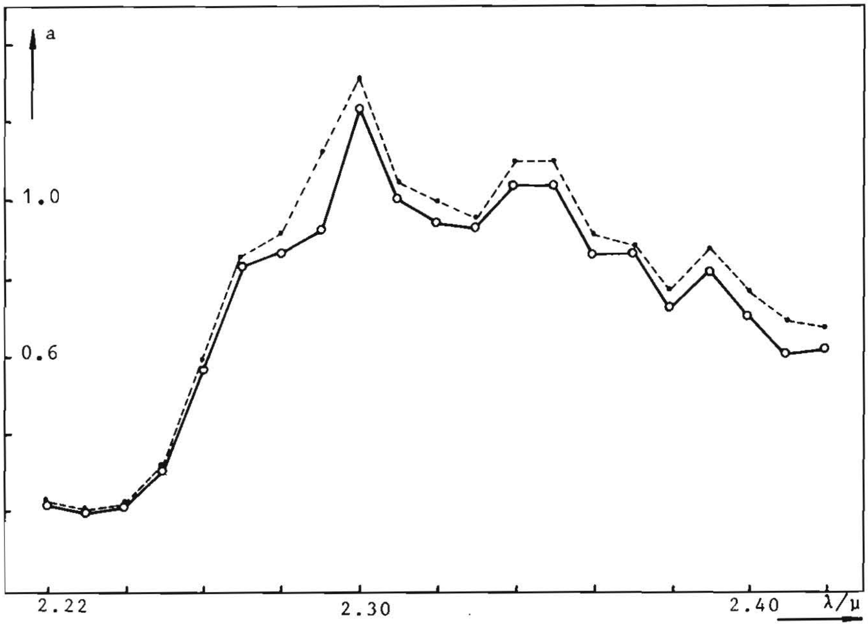

Fig. 3.1 A pair of strongly dependent reference spectra.
----- 0.610 mole/l ethylbenzene + 0.406 mole/l n-propylbenzene($\underline{a}_1$)
——— 0.805 mole/l ethylbenzene + 0.206 mole/l n-propylbenzene($\underline{a}_2$)
Solvent: carbon tetrachloride.

In this deliberate attempt to construct an ill-conditioned matrix $\underline{A}^T\underline{A}$ - it has $\lambda_{max} = 27.940$ and $\lambda_{min} = 3.26 \times 10^{-3} = \mu$ - we still find that $\hat{\underline{x}} - \hat{\underline{z}}$ is negligible when compared with either $\hat{\underline{x}} - \underline{y}$ or the vector of estimated standard deviations in $\hat{\underline{x}}$. This may be brought out even more clearly by expressing $\hat{\underline{x}}$ and $\hat{\underline{x}} - \hat{\underline{z}}$ in terms of the pure components (A) and (B): We find for the estimated composition $\hat{\underline{c}}_o = (\underline{c}_1,\underline{c}_2)\hat{\underline{x}}$:

$$\hat{\underline{c}}_o = \begin{pmatrix} 0.6057 & 0.8007 \\ 0.3943 & 0.1993 \end{pmatrix} \begin{pmatrix} 0.4889 \\ 0.5117 \end{pmatrix} = \begin{pmatrix} 0.7058 \\ 0.2948 \end{pmatrix} \; ; \; \underline{c}_o = \begin{pmatrix} 0.7064 \\ 0.2936 \end{pmatrix} \; ;$$

similarly expressing $\hat{\underline{x}} - \hat{\underline{z}}$, we have $(\underline{c}_1,\underline{c}_2)(\hat{\underline{x}}-\hat{\underline{z}}) \doteq \begin{pmatrix} -0.00002 \\ +0.00002 \end{pmatrix}$

which is, of course, negligible relative to $\hat{\underline{c}}_o - \underline{c}_o$.

Finally, we show that even for this case of poorly distinguishable reference spectra,

$$\lambda_s/\mu = 1.22 \times 10^{-5}/(3.26 \times 10^{-3}) \doteq 0.0037,$$

the values of $(\hat{\underline{x}}-\hat{\underline{z}})^{\tau}(\hat{\underline{x}}-\hat{\underline{z}})$, $\underline{u}^{\tau}\underline{u} - \underline{r}^{\tau}\underline{r}$ etc. are far below their upper bounds:

	Upper bound	Found by direct calculation
$(\hat{\underline{x}}-\hat{\underline{z}})^{\tau}(\hat{\underline{x}}-\hat{\underline{z}})$	7.0×10^{-6}	1.9×10^{-8}
$\max_j \lvert\hat{x}_j-\hat{z}_j\rvert$	2.7×10^{-3}	1.0×10^{-4}
$\underline{u}^{\tau}\underline{u} - \underline{r}^{\tau}\underline{r}$	2.3×10^{-8}	8.0×10^{-10}
$(\underline{u}^{\tau}\underline{u} - \underline{r}^{\tau}\underline{r})/\underline{r}^{\tau}\underline{r}$	1.001 3	1.000 045

It is particularly gratifying to note that, even if $\max_j \lvert\hat{x}_j-\hat{z}_j\rvert$ had reached its bound, 2.7×10^{-3}, it would still be small relative to $\max \hat{\sigma}(\hat{x}_j)$, which turns out to be 1.25×10^{-2}. We conclude that even for this case the hypothesis that $\hat{\underline{x}}$ is virtually indistinguishable from $\hat{\underline{z}}$ is not contradicted.

We summarize the results of this subsection as follows: To all practical intents and purposes, the estimators $\hat{\underline{x}}$ and $\hat{\underline{z}}$ are equivalent under assumptions (A13) and (A14).

3.4.3 The preference for $\hat{\underline{x}}$

We have demonstrated that $\hat{\underline{x}}$ and $\hat{\underline{z}}$ are equivalent for the purpose of spectrophotometry; our stated preference for $\hat{\underline{x}}$ as an estimator is based on the following two points:

1. The numerical calculation of $\hat{\underline{x}}$ is considerably simpler than that of $\hat{\underline{z}}$ by conventional methods.
2. The fact that $\hat{\underline{x}}$ is the classical least squares estimator for linear regression allows us to derive a theory of (approximate) interval estimation, based on the similarity of our case to that in which $\underline{A}$ is known exactly (Chapter IV).

The first point deserves some comment: we know that $\overset{+}{\underline{A}}{}^{\tau}\overset{+}{\underline{A}}$, of which $\hat{\underline{\zeta}}$ is an eigenvector, is nearly singular. In fact, this matrix becomes more nearly singular as the fit of $\underline{a}_o$ to the subspace spanned by the columns of $\underline{A}$ improves. Thus, paradoxically, the numerical calculation of $\hat{\underline{\zeta}}$ becomes more difficult in proportion to the improvement of the precision of the analysis. Therefore we propose the following practical algorithm for $\hat{\underline{z}}$:

a) Obtain $\hat{\underline{x}}$ from $\hat{\underline{x}} = (\underline{A}^{\tau}\underline{A})^{-1}\underline{A}^{\tau}\underline{a}_o$.

b) Calculate $\underline{r}^{\tau}\underline{r}$ - preferably directly by

$$\underline{r}^{\tau}\underline{r} = \sum_i (a_{oi} - \sum_j a_{ij}\hat{x}_j)^2,$$

since the short-cut method ($\underline{r}^{\tau}\underline{r} = \underline{a}_o{}^{\tau}\underline{a}_o - \hat{\underline{x}}^{\tau}\underline{A}^{\tau}\underline{A}\hat{\underline{x}}$, which exploits the orthogonality relation $\underline{r}^{\tau}\underline{A} = \underline{0}^{\tau}$) is likely to lead to a considerable loss of calculating precision when $n > 4$ say.

c) Subtract an approximation λ_s^* to λ_s:

$$\lambda_s = \frac{\underline{u}^{\tau}\underline{u}}{\hat{\underline{\zeta}}^{\tau}\hat{\underline{\zeta}}} \doteq \frac{\underline{r}^{\tau}\underline{r}}{1+\hat{\underline{x}}^{\tau}\hat{\underline{x}}} = \lambda_s^*$$

from the main diagonal elements of $\underline{A}^{\tau}\underline{A}$. Call the resulting matrix $\underline{A}^*$.

d) Obtain the approximation $\hat{\underline{z}}^*$ to $\hat{\underline{z}}$ from

$$\hat{\underline{z}}^* = (\underline{A}^*)^{-1}\underline{A}^{\tau}\underline{a}_o.$$

e) If $\hat{\underline{z}}^* - \hat{\underline{x}}^*$ is of considerable magnitude, iterate from a), with $\underline{A}^*$ substituted for $\underline{A}^{\tau}\underline{A}$; else accept $\hat{\underline{z}}^*$ as $\hat{\underline{z}}$.

Note: In practice, the iteration has never been found necessary; to give an example, for the matrix, of Table 3.3, λ_s^* was 12.215×10^{-6}, which agrees to all five significant figures with the true λ_s.

The chief advantage of this algorithm is the fact that it avoids all operations on the ill-conditioned matrix $\overset{\ddagger}{\underline{A}}{}^{\tau}\overset{\ddagger}{\underline{A}}$ but, instead, works on $\underline{A}^{\tau}\underline{A}$ which, under (A14), is of better condition. Also, for an automatic computer, no major subroutines are required except for the solution of the linear systems in steps a) and d).

3.5 An alternative model (Model II)

Instead of assuming, with (A5), the absorbance to be strictly proportional to (relative) concentration, we may prefer a slight modification of this assumption:

(A5) states that, for any i ($i = 1,\dots,k$),

$$b_{io} = \sum_{j=1}^{n} b_{ij}\, y_j. \qquad (3.5:1)$$

The alternative is

(A5'): $$b_{io} + c_o = \sum_{j=1}^{n} (b_{ij} + c_j)\, y_j \text{ for all } i, \qquad (3.5:2)$$

in which the c_j ($j=0,\dots,n$) do not depend on i. We may regard $-c_j$ as a correction to be applied to all b_{ij} of the j-th column; it is natural to think of the $-c_j$ as cell absorbances which have to be allowed for before linearity can be assumed. We shall designate (A5) = (3.5:1), the case treated so far, as Model I, the modified model (A5') = (3.5:2) as Model II.

If the cell absorbances are not known to be reproducible, Model II, which allows for their presence, is obviously the safer model to employ. Indeed, analysis by the more comprehensive Model II has become standard practice for all but the earliest experiments (cf. Sec. 2.6.3).

Rewriting (3.5:2) as

$$b_{io} = (\sum_{j=1}^{n} c_j y_j - c_o) + \sum_{j=1}^{n} b_{ij}\, y_j$$

$$b_{io} = y_o + \sum_{j=1}^{n} b_{ij}\, y_j, \qquad (3.5:3)$$

we remark that, passing from the unobservables ($\underline{B}$, $\underline{b}_o$) to the observable arrays ($\underline{A}$, $\underline{a}_o$), classical least squares theory can still be applied to

$$a_{io} = y_o + \sum_{j=1}^{n} a_{ij} y_j + r_i \text{ for all } i \qquad (3.5:4)$$

to yield an estimate $\hat{\underline{x}}_e$,provided we assign a constant coefficient of unity to y_o, i.e. define an (n+1)-column matrix $\underline{A}_e$ which is $\underline{A}$ extended on the left by a vector consisting of k elements unity. In $\hat{\underline{x}}_e$, found from $\hat{\underline{x}}_e = (\underline{A}_e^{\tau} \underline{A}_e)^{-1} \underline{A}_e^{\tau} \underline{a}_o$, the first element, $(\hat{\underline{x}}_e)_o$ say, then estimates

$y_o = (\sum_{j=1}^{n} c_j y_j) - c_o$, the remaining elements

$(\hat{\underline{x}}_e)_j$, j > 0, estimate the y_i as before.

In this approach, $\hat{\underline{x}}_e$ is obtained from an (n+1) square matrix and has n+1 elements. In a second approach, the extension of $\hat{\underline{x}}$ to n+1 elements is avoided by the following device: the elements of $\underline{A}$, $\underline{a}_o$ are "referred to their (column) means", i.e. $\underline{A}$, $\underline{a}_o$ are replaced by $\tilde{\underline{A}}$, $\tilde{\underline{a}}_o$, where

$$\tilde{a}_{ij} = a_{ij} - (\sum_{i=1}^{k} a_{ij})/k, \; j=0,1,\ldots,n$$

and $\tilde{\underline{x}}$ (of n elements) is found from

$$\tilde{\underline{x}} = (\tilde{\underline{A}}^{\tau} \tilde{\underline{A}})^{-1} \tilde{\underline{A}}^{\tau} \tilde{\underline{a}}_o .$$

The equivalence of the approaches is readily shown: consider the p,q-th element of $\tilde{\underline{A}}^{\tau}\tilde{\underline{A}}$ or, (q=0), the p-th element of $\tilde{\underline{A}}^{\tau}\tilde{\underline{a}}_o$:

$$(\tilde{\underline{A}}^{\tau}\tilde{\underline{A}})_{pq} = \sum_{r=1}^{k} \tilde{a}_{rp}\, \tilde{a}_{rq}; \; p=1,\ldots,n; \; q=0,1,\ldots n$$

$$= \sum_{r=1}^{k} (a_{rp} - (\sum_{i=1}^{k} a_{ip})/k)(a_{rq} - (\sum_{i=1}^{k} a_{iq})/k)$$

$$= \sum_{r=1}^{k} a_{rp}\, a_{rq} - \frac{1}{k} (\sum_{i=1}^{k} a_{ip})(\sum_{i=1}^{k} a_{iq}) .$$

So we find:

$$\left\{\begin{array}{l} \tilde{\underline{A}}^{\tau}\tilde{\underline{A}} = \underline{A}^{\tau}\underline{A} - \underline{s}\underline{s}^{\tau}/k \\ \tilde{\underline{A}}^{\tau}\tilde{\underline{a}}_o = \underline{A}^{\tau}\underline{a}_o - \alpha\underline{s}/k \end{array}\right. \tag{3.5:5}$$

with $\underline{s}$, the vector of column sums of $\underline{A}$, defined by $s_j \equiv \sum_i a_{ij}$ and $\alpha \equiv \sum_i a_{io}$.

Hence
$$\tilde{\underline{x}} = (\tilde{\underline{A}}^{\tau}\tilde{\underline{A}})^{-1}\,\tilde{\underline{A}}^{\tau}\tilde{\underline{a}}_o = (\underline{A}^{\tau}\underline{A} - \underline{s}\underline{s}^{\tau}/k)^{-1}(\underline{A}^{\tau}\underline{a}_o - \alpha\underline{s}/k).$$

On the other hand,
$$\underline{A}_e^{\tau}\underline{A}_e = \begin{pmatrix} k & \underline{s}^{\tau} \\ \underline{s} & \underline{A}^{\tau}\underline{A} \end{pmatrix}$$

and one verifies, by direct multiplication, that

$$(\underline{A}_e^{\tau}\underline{A}_e)^{-1} = \begin{pmatrix} \delta & -\delta\underline{s}^{\tau}\underline{R} \\ -\delta\underline{R}\underline{s} & (\underline{A}^{\tau}\underline{A} - \underline{s}\underline{s}^{\tau}/k)^{-1} \end{pmatrix}$$

with $\underline{R} \equiv (\underline{A}^{\tau}\underline{A})^{-1}$ and $\delta \equiv (k - \underline{s}^{\tau}\underline{R}\underline{s})^{-1}$

Then
$$\hat{\underline{x}}_e = (\underline{A}_e^{\tau}\underline{A}_e)^{-1}\underline{A}_e^{\tau}\underline{a}_o = (\underline{A}_e^{\tau}\underline{A}_e)^{-1}\begin{pmatrix} \alpha \\ \underline{A}^{\tau}\underline{a}_o \end{pmatrix}.$$

Now comparing

$$\tilde{\underline{x}} = (\underline{A}^{\tau}\underline{A} - \underline{s}\underline{s}^{\tau}/k)^{-1}\underline{A}^{\tau}\underline{a}_o - (\underline{A}^{\tau}\underline{A} - \underline{s}\underline{s}^{\tau}/k)^{-1}\alpha\underline{s}/k$$

with the last n elements, say $\hat{\underline{x}}_{e|n}$, of $\hat{\underline{x}}_e$:

$$\hat{\underline{x}}_{e|n} = (\underline{A}^{\tau}\underline{A} - \underline{s}\underline{s}^{\tau}/k)^{-1}\underline{A}^{\tau}\underline{a}_o - \delta\underline{R}\underline{s}\alpha\ ,$$

one finds, after some algebraic reduction, that

$$(\underline{A}^{\tau}\underline{A} - \underline{s}\underline{s}^{\tau}/k)^{-1}\underline{s}/k = \delta\underline{R}\underline{s},$$

which proves that the last n elements of $\hat{\underline{x}}_e$ are identical to the vector $\tilde{\underline{x}}$ and therefore that Model II analyses may be calculated at will either from $(\underline{A}_e, \underline{a}_o)$ or from $(\tilde{\underline{A}}, \tilde{\underline{a}}_o)$.

In practice, we prefer the approach that starts from $\underline{A}_e, \underline{a}_o$, because it most easily yields $(\hat{\underline{x}}_e)_o$, the estimator of y_o, as well as $\hat{\sigma}((\hat{\underline{x}}_e)_o)$, its estimated standard deviation.

When using Model II, the assertions of the previous sections remain true, provided $\underline{A}$, $\underline{a}_o$, $\hat{\underline{x}}$ etc. are replaced by $\tilde{\underline{A}}$, $\tilde{\underline{a}}_o$, $\tilde{\underline{x}}$ etc., as one verifies easily. Likewise, n should then be replaced by n+1; we shall henceforth write n^*, meaning n for Model I and n+1 for Model II. For simplicity's sake, however, we shall continue to write $\underline{A}$, $\underline{a}_o$ etc. for both models: the context will remove any ambiguity by stating the model used.

Finally, we remark that numerical experience indicates that the smallness of $\hat{\underline{x}} - \hat{\underline{z}}$, and hence the legitimacy of the use of $\hat{\underline{x}}$, is not lost when using Model II; e.g., for Ex. 3, (Sec. 3.4.2) we find:

$$\tilde{\underline{A}}^{\tau}\tilde{\underline{A}} = \begin{pmatrix} 1.97302858 & 1.85463502 \\ 1.85463502 & 1.74948202 \end{pmatrix}$$

as against

$$\underline{A}^{\tau}\underline{A} = \begin{pmatrix} 14.786896 & 13.944383 \\ 13.944383 & 13.156031 \end{pmatrix} ;$$

though λ_{max} is only 3.72 for $\tilde{\underline{A}}^{\tau}\tilde{\underline{A}}$ (27.94 for $\underline{A}^{\tau}\underline{A}$), the critical quantity $\lambda_{min} = \mu$ has virtually the identical value:

$$3.26 \times 10^{-3} \text{ for } \underline{A}^{\tau}\underline{A},\ 3.25 \times 10^{-3} \text{ for } \tilde{\underline{A}}^{\tau}\tilde{\underline{A}}.$$

CHAPTER IV

DATA REDUCTION : INTERVAL ESTIMATION

4.0 Scope

The present chapter deals with measures of uncertainty associated with the estimator $\hat{\underline{x}}$. The covariance matrix of $\hat{\underline{x}}$ is found to contain the unknown parameters $\underline{y}$; its estimation is nevertheless possible for known $\underline{y}$ and with the substituion $\underline{A}^\tau\underline{A} \rightarrow \underline{B}^\tau\underline{B}$, which is shown to be reasonable. Some uses are indicated for the estimated covariance matrix and the estimated error variance. After a brief account of some interval estimators in the classical regression case which assume normality of the error distribution, the analogy between the classical case and ours is used to draw up hypotheses on the distribution of interval estimators connected with $\hat{\underline{x}}$, likewise assuming a normal distribution of errors. The factual behaviour of these estimators is investigated by a Monte Carlo method and found not to lead to a rejection of the hypotheses. Finally, the use of these estimators, leading to tests and confidence intervals based on χ^2 and F, is described; a short note on their relevance to practice is appended.

4.1 The covariance matrix of $\hat{\underline{x}}$

The assumptions previously introduced:

(A9) $E(\varepsilon_{ij}) = 0$ for all i and j
(A10) $E(\varepsilon_{ij}\varepsilon_{hk}) = 0$ if $i \neq n$ or $j \neq k$
(A11) $E(\varepsilon_{ij}^2) = \varepsilon^2$ for all i and j

suffice to calculate the covariance matrix of

$$\hat{\underline{x}} = (\underline{A}^\tau\underline{A})^{-1}\underline{A}^\tau\underline{a}_o,$$

which is defined as $E((\hat{\underline{x}}-\underline{y})(\hat{\underline{x}}-\underline{y})^\tau)$. Starting from

$$\underline{A} = \underline{B} + \underline{E},\ \underline{a}_o = \underline{b}_o + \underline{\varepsilon}_o \tag{3.1:2}$$

and the usual approximation

$$(\underline{A}^\tau\underline{A})^{-1} \doteq (\underline{B}^\tau\underline{B})^{-1} - (\underline{B}^\tau\underline{B})^{-1}(\underline{B}^\tau\underline{E}+\underline{E}^\tau\underline{B}+\underline{E}^\tau\underline{E})(\underline{B}^\tau\underline{B})^{-1},$$

one finds, after considerable, but straightforward algebraic reduction, that, neglecting terms in ε^3 and ε^4:

$$\underline{\Sigma} \equiv E((\hat{\underline{x}}-\underline{y})(\hat{\underline{x}}-\underline{y})^\tau) = (1+\underline{y}^\tau\underline{y})(\underline{B}^\tau\underline{B})^{-1}.\varepsilon^2 \,. \qquad (4.1:1)$$

It is instructive to compare (4.1:1) with the covariance matrix for the classical regression ($\underline{A}\equiv\underline{B}$), which reads:

$$\underline{\Sigma}_{class} = (\underline{B}^\tau\underline{B})^{-1}.\varepsilon^2 \text{ (where } \varepsilon^2 \equiv E(\varepsilon_{io}^2)). \qquad (4.1:2)$$

One remarks that the shapes are identical (the hyperellipsoid $\underline{v}^\tau\underline{\Sigma}\underline{v} = c$ is similar to and homothetical with $\underline{v}^\tau\underline{\Sigma}_{class}\underline{v} = c$), but that for our case each element of $\underline{\Sigma}$ has "expanded" in the ratio $1 + \underline{y}^\tau\underline{y}$. Closer analysis reveals that in (4.1:1) the term

$$\underline{y}^\tau\underline{y}\ (\underline{B}^\tau\underline{B})^{-1}.\varepsilon^2$$

arises from $\underline{E}$, the error matrix of $\underline{B}$, whereas the "classical" term $(B^\tau B)^{-1}.\ \varepsilon^2$ is due to the error vector $\underline{\varepsilon}_o$ of $\underline{b}_o$.

The expansion of the elements of $\underline{\Sigma}$ in the ratio $1 + \underline{y}^\tau\underline{y}$ again strongly suggests a treatment of our problem as a variant of classical regression in which the errors in $\underline{B}$ induce additional errors in $\underline{a}_o$; the induced errors equal

$$-\sum_{j=1}^{n} y_j\ \varepsilon_{ij}$$

for the i-th row, or, more generally, $-\underline{Ey}$ for $\underline{a}_o$. (Cf. Sec. 3.2, Property 1.).

Again, consider the expected value of $\underline{r}^\tau\underline{r}$, the sum of squared residuals, for given $\underline{y}$; for the classical case, we have:

$$E(\underline{r}^\tau\underline{r}) = E((\underline{a}_o-\underline{By})^\tau(\underline{a}_o-\underline{By})) = E(\underline{\varepsilon}_o{}^\tau\underline{\varepsilon}_o) = k\varepsilon^2, \qquad (4.1:3)$$

whereas for our case we find:

$$E(\underline{r}^\tau\underline{r}) = E((\underline{a}_o-\underline{Ay})^\tau(\underline{a}_o-\underline{Ay})) = E((\underline{a}_o-(\underline{B}+\underline{E})\underline{y})^\tau(\underline{a}_o-(\underline{B}+\underline{E})\underline{y}))$$

$$= E((\underline{\varepsilon}_o-\underline{Ey})^\tau(\underline{\varepsilon}_o-\underline{Ey})) = (1+\underline{y}^\tau\underline{y}).\ k\varepsilon^2 \qquad (4.1:4)$$

Again, the comparison of (4.1:4) and (4.1:3) strongly suggests a treatment of our case as a variant of classical regression with induced error in $\underline{a}_o$.

Summarizing these findings, we conclude:

The elements of $\underline{\Sigma}$ and $E(\underline{r}^\tau\underline{r})$ have expanded in the same ratio $1+\underline{y}^\tau\underline{y}$, when comparing our case ($\underline{A} = \underline{B}+\underline{E}$) to the classical regression ($\underline{A}\equiv\underline{B}$).

Note: The above is valid for both Model I and Model II, provided we take $\underline{A}$, $\underline{a}_o$ etc. to mean $\tilde{\underline{A}}$, $\tilde{\underline{a}}_o$ with the latter model.

4.2 The estimation of ε^2 and $\underline{\Sigma}$

We recall: if, in the classical case, ε^2 is not (assumed) known, it is estimated from the residual vector $\underline{r}$. Now the result $E(\underline{r}^\tau\underline{r}) = k\varepsilon^2$ was derived on the assumption of an independently given $\underline{y}$. When $\underline{y}$ must be estimated from $\underline{B}$, $\underline{a}_o$ by the estimator $\hat{\underline{x}}$, the estimator $\hat{\varepsilon}^2$,

$$\hat{\varepsilon}^2 = \frac{\underline{r}^\tau\underline{r}}{k-n^*} , \qquad (4.2:1)$$

should be used rather than $(\underline{r}^\tau\underline{r})/k$; the divisor $k-n^*$ arises from the loss of n^* degrees of freedom, corresponding to the estimation of n^* parameters $\hat{\underline{x}}$ (or $\hat{\underline{x}}_e$) from the same set of data.

Combining (4.1:2) and (4.2:1), one estimates $\underline{\Sigma}_{class}$ by

$$\hat{\underline{\Sigma}}_{class} = (\underline{B}^\tau\underline{B})^{-1} . \; (\underline{r}^\tau\underline{r})/(k-n^*). \qquad (4.2:2)$$

But, for our case, $(\underline{A} = \underline{B} + \underline{E})$,

$$\hat{\varepsilon}^2 = \frac{\underline{r}^\tau\underline{r}}{1 + \underline{y}^\tau\underline{y}} \cdot \frac{1}{k-n^*} + O(\varepsilon^4) \qquad (4.2:3)$$

can be derived from (4.1:4) under the same assumptions as were used to derive (4.2:1). Combination of (4.2:3) and (4.1:1) yields (neglecting $O(\varepsilon^4)$)

$$\hat{\underline{\Sigma}} = (\underline{B}^\tau\underline{B})^{-1} . \; (\underline{r}^\tau\underline{r})/(k-n^*) , \qquad (4.2:4)$$

which is formally identical with (4.2:2) and no longer contains the unknown true quantities $\underline{y}$.

As against this, $\hat{\underline{\Sigma}}$ unfortunately still contains the unobservable $\underline{B}$. In order to resolve this difficulty, we propose to use

$$\hat{\hat{\underline{\Sigma}}} \equiv (\underline{A}^\tau\underline{A})^{-1} . \; (\underline{r}^\tau\underline{r})/(k-n^*) \qquad (4.2:5)$$

as an estimator of $\underline{\Sigma}$ for our case. The substitution of $(A^\tau A)^{-1}$ for $(\underline{B}^\tau\underline{B})^{-1}$ is justified by the following argument.

a) Consider the matrix norm $||\underline{M}|| \equiv \max |\underline{v}^\tau\underline{M}\underline{v}/\underline{v}^\tau\underline{v}|$, $\underline{v} \neq \underline{0}$. Then $(\underline{A}^\tau\underline{A})^{-1}$ has norm

$$||(\underline{A}^\tau\underline{A})^{-1}|| = \lambda_{max} \text{ of } (\underline{A}^\tau\underline{A})^{-1} = \lambda_{min}^{-1} \text{ of } \underline{A}^\tau\underline{A} = \mu^{-1} .$$

But our estimator for $\underline{B}^\tau\underline{B}$ is $\underline{A}^\tau\underline{A} - \lambda_s\underline{I}$; this estimator has the minimal eigenvalue $\mu - \lambda_s$, hence, to the extent that we may replace $\underline{B}^\tau\underline{B}$ by its estimator, $||(\underline{B}^\tau\underline{B})^{-1}|| = (\mu - \lambda_s)^{-1}$. Therefore

$$||\hat{\underline{\Sigma}}|| \;/\; ||\hat{\hat{\underline{\Sigma}}}|| = \mu/(\mu-\lambda_s);$$

but by assumption (A14), $\lambda_s/\mu \leq 0.01$, hence $||\hat{\underline{\Sigma}}|| \hat{=} ||\hat{\hat{\underline{\Sigma}}}||$ to within 1%. We <u>conclude</u> that the approximation $(\underline{A}^\tau\underline{A})^{-1} \hat{=} (\underline{B}^\tau\underline{B})^{-1}$ will not distort measures of uncertainty derived from $\hat{\hat{\underline{\Sigma}}}$ by more the 1% when these measures are quadratic forms $\underline{v}^\tau\underline{\hat{\hat{\Sigma}}v}$.

b) The distortion by $\leq$ 1% arrived at above seems particularly unimportant relative to the uncertainty associated with the term $(\underline{r}^\tau\underline{r})/(k-n^*)$ in (4.2:4). Assuming for the moment that the residials are normally distributed, we find that for $k-n^* = 25$, the 95% confidence interval for ε^2, given $\hat{\varepsilon}^2$, extends from $0.54\hat{\varepsilon}^2$ to $1.67\hat{\varepsilon}^2$. In view of this uncertainty, which affects each element of both $\hat{\underline{\Sigma}}$ and $\hat{\hat{\underline{\Sigma}}}$, the minor distortion caused by using $\hat{\hat{\underline{\Sigma}}}$ fades into insignificance.

In our opinion, the arguments a) and b) sufficiently justify the use of (4.2:5) as an estimator of the covariance matrix of $\hat{\underline{x}}$, which we shall henceforth denote by $\hat{\underline{\Sigma}}$.

4.3 Suggested uses of $\hat{\varepsilon}^2$ and $\hat{\underline{\Sigma}}$

The following use is suggested for the formulae derived so far:

1. With ε^2 assumed known, we can, if provided with a set of spectra $\underline{A}$ and an approximate true composition $\underline{y}$, use (4.1:1) to calculate the covariance matrix and thus to predict the precision expected for a proposed analysis. Note that for this purpose a very rough (say ∿ 10%) estimate of the y_j is sufficient, since $\underline{\Sigma}$ depends only slightly on $\underline{y}$ under the assumptions (cf.(A13)) that $\max_j y_j \hat{=} 1$ and $\sum_j y_j \hat{=} 1$.

Since ε^2 is an instrumental constant (for our instrument $\varepsilon^2 = (1.2 \times 10^{-3})^2$), trial analyses can, strictly speaking,be replaced by such a calculation, provided the linearity (A5) and additivity (A6) can be assumed to hold.

2. With ε^2 <u>not</u> assumed known, we can use (4.2:3) to estimate ε^2 from $\underline{r}^\tau\underline{r}$ and a given composition $\underline{y}$ (e.g. for a trial analysis). This estimate gives a rough idea of instrumental precision for the analysis in question.

A rougher, but still practical estimate of instrumental precision is provided by $\hat{s}$, defined by

$$\hat{s}^2 \equiv \underline{r}^\tau\underline{r}/(k-n^*) = (1+\underline{y}^\tau\underline{y}).\hat{\varepsilon}^2, \qquad (4.3:1)$$

since $(1+\underline{y}^\tau\underline{y})^{\frac{1}{2}}$ varies only between 1.1 and 1.4, and can even be assumed constant for a series of analyses of roughly equal expected composition.

3. The most important application of the formulae is the use of (4.2:5), which estimates $\underline{\Sigma}$, to derive numerical estimates of

the variance of the elements of $\hat{\underline{x}}$ and/or of linear functions of these elements.

We recall the standard result that, for given $\hat{\underline{\Sigma}}$, the estimated variance $\hat{\sigma}^2(\underline{v}^\tau\hat{\underline{x}})$ of the linear combination

$$\underline{v}^\tau\hat{\underline{x}} = \Sigma v_j \hat{x}_j$$

is given by

$$\hat{\sigma}^2(\underline{v}^\tau\hat{\underline{x}}) = \underline{v}^\tau\hat{\underline{\Sigma}}\underline{v} \tag{4.3:2}$$

for arbitrary $\underline{v}$. As an important special case, choose $\underline{v} = \underline{e}_j$, the unit vector with 1 as its j-th element and zeros elsewhere; then,

$$\hat{\sigma}^2(\underline{e}_j^\tau\hat{\underline{x}}) = \hat{\sigma}^2(\hat{x}_j) = \underline{e}_j^\tau\hat{\underline{\Sigma}}\underline{e}_j = (\hat{\underline{\Sigma}})_{jj}$$

$$= ((\underline{A}^\tau\underline{A})^{-1})_{jj} \cdot \hat{s}^2$$

or

$$\hat{\sigma}(\hat{x}_j) = ((\underline{A}^\tau\underline{A})^{-1})_{jj}^{\frac{1}{2}} \cdot \hat{s} \ . \tag{4.3:3}$$

Thus we have, in (4.3:3), a particularly simple formula for the estimated standard deviation of an element of $\hat{\underline{x}}$. In our opinion, any computer program for least-squares analysis should, as a matter of course, print the $\hat{\sigma}(\hat{x}_j)$ automatically and obligatorily with the corresponding $\hat{x}_j$.

Incidentally, we note that (4.3:3) justifies our preference for Model II calculations by $\underline{A}_e$, not $\tilde{\underline{A}}$ (Sec. 3.5): in the former approach, an estimate of the standard deviation of the constant term $(\hat{\underline{x}}_e)_o$ is immediately available. Chemically, this estimate is valuable because it allows us to judge the magnitude of the estimated resultant cell correction.

We remark that the use of the formulae in this section does not imply any assumptions beyond those already introduced.

4.4 The assumption of normal errors and its consequences

The usefulness of the knowledge of $\hat{\underline{\Sigma}}$ and $\hat{\varepsilon}^2$ is enormously enhanced when a joint normal distribution of the ε_{ij} can be assumed. We recall the following results for the classical regression case, in which $\underline{A} \equiv \underline{B}$ and the errors ε_{io} are jointly normally and independently distributed with zero mean and variance ε^2:

C1: $\underline{r}^\tau\underline{r}/\varepsilon^2$ is distributed as χ^2 with k-n* degrees of freedom

C2: The quadratic form

$$Q = (\hat{\underline{x}} - \underline{y})^\tau(\underline{\Sigma}_o)^{-1}(\hat{\underline{x}} - \underline{y})/\varepsilon^2 \tag{4.4:1}$$

is distributed as χ^2 with m degrees of freedom, in which
(Model I) $m = n$ and $\underline{\Sigma}_o = (\underline{B}^\tau \underline{B})^{-1}$
(Model II) $m = n$ and $\underline{\Sigma}_o = (\hat{\underline{B}}^\tau \hat{\underline{B}})^{-1}$ or, equivalently, the $n \times n$ matrix obtained from $(\underline{B}_e^\tau \underline{B}_e)^{-1}$ by striking out the row and column corresponding to the sum row and column in $\underline{B}_e{}^\tau \underline{B}_e$.
Note that $\underline{\Sigma}_o$, by (4.1:2), equals $\underline{\Sigma}_{class}/\varepsilon^2$ in either case. (For $m < n$, see Sec. 4.6.3).

C3: These distributions are independent, hence

$$\frac{(\hat{\underline{x}} - \underline{y})^\tau (\underline{\Sigma}_o)^{-1} (\hat{\underline{x}} - \underline{y})}{\underline{r}^\tau \underline{r}} \cdot \frac{k-n^*}{m}$$

is distributed as F with m and $k-n^*$ degrees of freedom for numerator and denominator respectively.

With ε^2 known, C2 leads to a confidence interval for $\underline{y}$; for ε^2 estimated from the residuals, C3 will serve to derive such a confidence interval.

Let us introduce the assumption of normality, analogous to the classical case:

(A15) The errors ε_{ij}, $i=1,\ldots,k; j=0,1,\ldots n$, are jointly normally distributed.
($E(\varepsilon_{ij})=0$, the independence and the common error variance ε^2 have been assumed before).

Even with (A15), the statistical difficulties still seem insuperable: with ε^2 assumed known, we cannot, it appears, derive the distribution of $\underline{r}^\tau \underline{r}$, since even its expected value (4.1:4) depends on the true $\underline{y}$, which will be unknown except in special cases such as trial analyses. The difficulty is aggravated in the case of Q, (4.4:1), since its analogue would contain the inverse covariance matrix, and hence the unknown factor $(1+\underline{y}^\tau \underline{y})^{-1}$; in addition, $\underline{B}^\tau \underline{B}$ is not available. Finally, for the analogue of C3, we may expect the unknown factor $1 + \underline{y}^\tau \underline{y}$ to cancel in the quotient (cf. (4.2:4)), but again $\underline{B}^\tau \underline{B}$ is not available and the statistical independence now certainly cannot be proved.

Nevertheless, the close analogy of our case to the classical regression and the fact that, under (A14),

$$1 + \hat{\underline{x}}^\tau \hat{\underline{x}} \doteq 1 + \underline{y}^\tau \underline{y}$$

and

$$(\underline{A}^\tau \underline{A})^{-1} \doteq (\underline{B}^\tau \underline{B})^{-1}$$

are likely to be true to a very good approximation, prompts us to state three hypotheses:

(H1): $\rho \equiv \dfrac{\underline{r}^\tau\underline{r}}{(1+\hat{\underline{x}}^\tau\hat{\underline{x}})\,.\,\varepsilon^2}$ is distributed as χ^2 with $k-n^*$ d.f. to a good approximation;

(H2): $$Q' \equiv \frac{(\hat{\underline{x}}-\underline{y})^\tau(\hat{\underline{\Sigma}}_o)^{-1}(\hat{\underline{x}}-\underline{y})}{(1+\hat{\underline{x}}^\tau\hat{\underline{x}})\,.\,\varepsilon^2} \qquad (4.4:2)$$

is distributed as χ^2 with m degrees of freedom to a good approximation, where

(Model I): $m = n$ and $\hat{\underline{\Sigma}}_o = (\underline{A}^\tau\underline{A})^{-1}$

(Model II): $m = n$ and $\underline{\Sigma}_o = (\tilde{\underline{A}}^\tau\tilde{\underline{A}})^{-1}$ or, equivalently: the $n \times n$ matrix obtained from $(\underline{A}_e^\tau\underline{A}_e)^{-1}$ by striking out the row and column corresponding to the sum row and column in $\underline{A}_e^\tau\underline{A}_e$.

(H3): The distributions of (H1) and (H2) are independent to a good approximation, so that

$$\phi \equiv \frac{(\hat{\underline{x}}-\underline{y})^\tau(\hat{\underline{\Sigma}}_o)^{-1}(\hat{\underline{x}}-\underline{y})}{\underline{r}^\tau\underline{r}} \quad \frac{k-n^*}{m} \qquad (4.4:3)$$

is distributed as $F(m, k-n^*)$ to a good approximation.

For the purpose of testing (H1), (H2) and (H3), "to a good approximation" can be taken to mean "identically".

4.5 A test of the hypotheses

A computer program was written to test the above hypotheses. As a source of random normal deviates, we used the sum of 12 pseudo-random numbers, each rectangularly distributed in (0,1), diminished by 6; the result was multiplied by the chosen value of the error standard deviation, $\varepsilon = 10^{-3}$. The pseudo-random numbers ψ were drawn according to the formula

$$\psi_{n+1} \equiv 1001\psi_n + 1 \pmod{10^{10}}; \quad \psi = 10^{-10}\psi_{n+1}$$

(Allard, Dobell and Hull 1963).

The test was run as follows:

A pair of spectra (n=2) was read in; for greater verisimilitude, an observed set of moderately distinguishable spectra was chosen (*n*-propylbenzene and *i*-propylbenzene in *iso*-octane, $a_{max} \doteq 1.25$, 250(1)272 nm, hence k = 23). These spectra constituted the $\underline{B}$ matrix. From $\underline{y}=\binom{0.5}{0.5}$ and $\underline{B}$, $\underline{b}_o = \underline{B}\underline{y}$ was calculated. Then, for each sample:

a) $\underline{A}, \underline{a}_o$ was constructed by adding a random normal deviate to each element of $\underline{B}$, $\underline{b}_o$; of course, the original $\underline{B}$, $\underline{b}_o$ was not disturbed and fresh random normal deviates were used for each sample.

b) $\underline{A}, \underline{a}_o$ was solved, by Model II, for $\hat{\underline{x}}_e$, according to

$\hat{\underline{x}}_e = (\underline{A}_e^\tau \underline{A}_e)^{-1} \underline{A}_e{}^\tau \underline{a}_o$, inverting $\underline{A}_e{}^\tau \underline{A}_e$, and giving

$$\hat{\underline{x}}_e = \begin{pmatrix} \hat{x}_o \\ \hat{x}_1 \\ \hat{x}_2 \end{pmatrix}, \quad \hat{\underline{x}} = \begin{pmatrix} \hat{x}_1 \\ \hat{x}_2 \end{pmatrix} .$$

c) The residual vector $\underline{r}$ was calculated from $\underline{r} = \underline{a}_o - \underline{A}_e \hat{\underline{x}}_e$ and $\underline{r}^\tau \underline{r}$ made up; $\hat{s}$ was calculated by $\hat{s} = (r^\tau r/(k-n^*))^{\frac{1}{2}}$ and the standard deviations $\hat{\sigma}(\hat{x}_j)$, $j = 1,2$ obtained from

$$\hat{\sigma}(\hat{x}_j) = ((\underline{\tilde{A}}^\tau \underline{\tilde{A}})^{-1})^{\frac{1}{2}}_{jj} . \hat{s} \ .$$

d) $\hat{\underline{\xi}}^\tau \hat{\underline{\xi}} \equiv 1 + \hat{\underline{x}}^\tau \hat{\underline{x}}$ and the statistic $\rho = \underline{r}^\tau \underline{r}/(\hat{\underline{\xi}}^\tau \hat{\underline{\xi}} . \varepsilon^2)$ were calculated.

e) The $(\underline{\tilde{A}}^\tau \underline{\tilde{A}})^{-1}$ portion of $(\underline{A}_e^\tau \underline{A}_e)^{-1}$ was again inverted, yielding $\underline{\tilde{A}}^\tau \underline{\tilde{A}}$, and the statistic Q^1 calculated as

$$Q' = \frac{(\hat{\underline{x}} - \underline{y})^\tau \underline{\tilde{A}}^\tau \underline{\tilde{A}} \ (\hat{\underline{x}} - \underline{y})}{\hat{\underline{\xi}}^\tau \hat{\underline{\xi}} \ . \ \varepsilon^2}$$

f) The statistic ϕ was calculated from' $\phi = Q' . \ (k-n^*)/(\rho . n)$ with $n^* = 3$, $n = 2$.

g) The following quantities were printed:

$$\hat{x}_1 - y_1, \ \hat{\sigma}(\hat{x}_1), \ \hat{x}_2 - y_2, \ \hat{\sigma}(\hat{x}_2), \ \underline{r}^\tau \underline{r}, \ \hat{s}, \ Q', \ \rho, \ \phi.$$

In all, 1000 samples were obtained. The distribution of the statistics of interest, Q', ρ and ϕ, was then investigated in the following manner:

(H1):The hypothesis for ρ is that its distribution coincides with $\chi^2(k-n^*) = \chi^2(20 \text{ d.f.})$; accordingly, the 1000 values of ρ were scanned and the number of sample values falling in the classes below was tallied:

Class	Class limits	Number found	Number expected *
1	$28.41 \leq \rho < 31.41$	46	50
2	$31.41 \leq \rho < 34.17$	23	25
3	$34.17 \leq \rho < 37.57$	12	15
4	$37.57 \leq \rho < \infty$	11	10

$P(\chi^2(20) \geq 28.42) = 0.10$; $P(\chi^2(20) \geq 31.41) = 0.05$
$P(\chi^2(20) \geq 34.17) = 0.025$; $P(\chi^2(20) \geq 37.57) = 0.01.$

*) When (H1) is true

Testing the numbers found for goodness of fit, we calculate $\chi^2 = \frac{16}{50} + \frac{4}{25} + \frac{9}{15} + \frac{1}{10} = 1.16$, whereas we find from the tables of χ^2(4 d.f.): $P(\chi^2(4) \geq 1.04) = 0.9$; $P(\chi^2(4) \geq 1.92) = 0.75$.

Hence, the test does not contradict (H1).

(H2): The hypothesis for Q' is that its distribution coincides with χ^2(2 d.f.); accordingly, the 1000 values of Q' were scanned and the number of sample values falling in the classes below was tallied:

Class	Class limits	Number found	Number expected *
1	$4.605 \leq Q' < 5.991$	50	50
2	$5.991 \leq Q' < 7.824$	32	30
3	$7.824 \leq Q' < 9.210$	12	10
4	$9.210 \leq Q' < \infty$	10	10

$P(\chi^2(2) \geq 4.605) = 0.10$; $P(\chi^2(2) \geq 5.991) = 0.05$;
$P(\chi^2(2) \geq 7.824) = 0.02$; $P(\chi^2(2) \geq 9.210) = 0.01$

*) When (H2) is true

Testing the numbers found for goodness of fit, we calculate $\chi^2 = 0 + \frac{4}{30} + \frac{4}{10} + 0 \doteq 0.53$, whereas we find from the tables of χ^2(4 d.f.): $P(\chi^2(4) \geq 0.48) = 0.975$; $P(\chi^2(4) \geq 0.71) = 0.95$.

Hence, the test does not contradict (H2).

(H3): The hypothesis for ϕ is that its distribution coincides with $F(n, k-n^*) = F(2,20)$; accordingly, the 1000 values of ϕ were scanned and the number of sample values falling in the classes below was tallied:

Class	Class limits	Number found	Number expected*
1	$3.49 \leq \phi < 5.85$	40	40
2	$5.85 \leq \phi < \infty$	14	10
3	$0.010 < \phi \leq 0.052$	34	40
4	$0 < \phi \leq 0.010$	12	10

$P(F(2,20) \geq 3.49) = 0.05$; $P(F(2,20) \geq 5.85) = 0.01$;
$P(F(2,20) \leq 0.052) = 0.05$; $P(F(2,20) \leq 0.010) = 0.01$.

*) When (H3) is true

Testing the numbers found for goodness of fit, we calculate $\chi^2 = 0 + \frac{16}{10} + \frac{36}{40} + \frac{4}{10} = 2.90$, whereas we find from the tables of χ^2(4 d.f.): $P(\chi^2(4) \geq 1.92) = 0.75$; $P(\chi^2(4) \geq 3.36) = 0.50$.

Hence, the test does not contradict (H3).

We conclude that the statistics ρ, Q' and ϕ can be assumed to have the distributions (H1) to (H3) under (A13), (A14) and (A15) and that we can use these statistics in the same manner as their classical counterparts (C1) to (C3) of Sec. 4.4.

4.6 Suggested uses of ρ and ϕ

Before discussing the use of the statistics ρ and ϕ(Q' is of lesser importance), it is perhaps not superfluous to stress that ρ and ϕ have distributions known to a good approximation only under the explicit assumption of joint normality, independence and common variance of the errors, (A15). Also, the additivity and linearity assumptions (A5, A6) are implied to be strictly true.

4.6.1 The length of the residual vector

Let us assume that ε^2, the population value, is known from a long series of well-behaved, well-conducted analyses. For our case, ε^2 has been found to be $(1.2 \times 10^{-3})^2$ (Sec. 2.4).

Then, $\underline{r}^\tau\underline{r}/(\varepsilon^2 . \hat{\underline{\xi}}^\tau\hat{\underline{\xi}})$, being distributed as $\chi^2(k-n^*)$ when the assumptions hold, can be stated to satisfy

$$\chi^2(k-n^*;1-\alpha/2) \leq \frac{\underline{r}^\tau\underline{r}}{\varepsilon^2 . \hat{\underline{\xi}}^\tau\hat{\underline{\xi}}} \leq \chi^2(k-n^*;\alpha/2) \qquad (4.6{:}1)$$

with probability $1-\alpha$, where $\hat{\underline{\xi}}^\tau\hat{\underline{\xi}} \equiv 1 + \hat{\underline{x}}^\tau\hat{\underline{x}}$ and $\chi^2(k-n^*;\beta)$ denotes the value of χ^2 with $k-n^*$ degrees of freedom such that

$$P(\chi^2(k-n^*) \geq \chi^2(k-n^*;\beta)) = \beta.$$

(4.6.1) clearly provides us with a confidence interval for $\underline{r}^\tau\underline{r}$. However, we wish to use $\underline{r}^\tau\underline{r}$ (or $\hat{\varepsilon}^2$, or $\hat{s}$) to detect departures from "good behaviour" of the method or from the assumptions made, notably "blunders" in read-out, departure from linearity and the presence of extraneous components, (Sec. 2.6), all of which tend to <u>increase</u> $\underline{r}^\tau\underline{r}$; hence, a confidence interval giving a <u>lower</u> limit on $\underline{r}^\tau\underline{r}$ is not appropriate. When interested only in positive deviations of $\underline{r}^\tau\underline{r}$ from its expected value, the relation

$$\frac{\underline{r}^\tau\underline{r}}{\varepsilon^2 . \hat{\underline{\xi}}^\tau\hat{\underline{\xi}}} \leq \chi^2(k-n^*;\alpha) \qquad (4.6{:}2)$$

satisfied by $\underline{r}^\tau\underline{r}$ (when the assumptions hold) with probability $1-\alpha$

is more pertinent. The hypothesis that the assumptions made are valid should be rejected when the observ~~ed valu~~e of $\underline{r}^{\tau}\underline{r}$ does not satisfy (4.6:2); one runs a risk α that such a rejection is not, in fact, justified.

Combining (4.6:2) and the estimator $\hat{\varepsilon}^2 = \underline{r}^{\tau}\underline{r}/((k-n^*).\ \hat{\underline{\xi}}^{\tau}\hat{\underline{\xi}})$, we find

$$\frac{\hat{\varepsilon}^2}{\varepsilon^2} \leq \frac{\chi^2(k-n^*;\alpha)}{k-n^*} . \tag{4.6:3}$$

For a practical example, take $k - n^* = 25$ and $\alpha = 0.01$; then:

$$\hat{\varepsilon}^2 : \varepsilon^2 \leq 1.77, \text{ or } \hat{\varepsilon} \leq 1.33 \times 1.2 \times 10^{-3} \leq 1.6 \times 10^{-3}.$$

In practice, this value of $\hat{\varepsilon}$, which, when the assumptions hold, has a probability of being exceeded of 0.01, is exceeded rather more often. This is not surprising, since (4.6:3) assumes, among other things, strict linearity and we know that for narrow absorption bands some non-linearity must be accepted (Sec. 5.3); also, for bands of any shape, strict linearity of detector response over wide ranges of transmittance may not be safely assumed.

In view of this fact, we shall adopt a cautious attitude: if (4.6:3) holds, we shall accept that the observations are consonant with the model; if it does not hold, we ought at least to examine the residual vector for certain types of pattern in the r_i (Sec. 2.6) and accept the analysis only when no indication of abnormalities is found, though very gross violations of (4.6:3), say those for which $\hat{\varepsilon} > 2\varepsilon$, can still be regarded as prima facie evidence that something is amiss with the observations, the model or both.

4.6.2 Confidence intervals for $\underline{y}$

Since ϕ is distributed as $F(n,k-n^*)$, we can state that, when the assumptions hold, the relation

$$\frac{(\hat{\underline{x}}-\underline{y})^{\tau}(\hat{\underline{\Sigma}}_o)^{-1}(\hat{\underline{x}}-\underline{y})}{\underline{r}^{\tau}\underline{r}} \cdot \frac{k-n^*}{n} \leq F(n,k-n^*;\alpha) \tag{4.6:4}$$

holds with probability $1 - \alpha$, where $\hat{\underline{\Sigma}}_o$ is as defined in Sec. 4.4, (H2), and $F(n,\kappa-n^*;\beta)$ is the value of F with n degrees of freedom for the numerator and $k-n^*$ degrees of freedom for the denominator such that

$$P(F(n,k-n^*) \geq F(n,k-n^*;\beta)) = \beta .$$

Considered as an inequality on $\underline{y}$, (4.6:4) provides us with a confidence interval for the true relative composition $\underline{y}$, consisting of the interior and boundary of a hyperellipsoid in R^n; for $n > 2$, the usefulness of (4.6:4) is somewhat limited.

A more generally applicable use of the distribution of ϕ is for the test of the hypothesis H_o that the composition found, $\hat{\underline{x}}$, is identical with an assumed composition $\underline{x}_o$ and that $\underline{x}_o - \hat{\underline{x}}$ is "attributable to chance". For a test of H_o, the statistic

$$F' \equiv \frac{(\hat{\underline{x}} - \underline{x}_o)^\tau (\hat{\underline{\Sigma}}_o)^{-1} (\hat{\underline{x}} - \underline{x}_o)}{\underline{r}^\tau \underline{r}} \; \frac{k - n^*}{n} \qquad (4.6:5)$$

is calculated and a confidence level α chosen. Then, if

$$F' < F(n, k-n^*; \alpha),$$

the hypothesis H_o is accepted; H_o is rejected otherwise; one runs a risk α that such a rejection is not, in fact, justified.

4.6.3 Confidence intervals for subvectors of $\underline{y}$

Sometimes confidence intervals and/or tests are required in which the quantities of interest are some, but not all elements of $\underline{y}$ or $\hat{\underline{x}}$, say $m < n$ elements. Following Mood (1950:305), who treats the problem for the classical regression case, we state :

If we construct subvectors of $\hat{\underline{x}}$, $\underline{y}$ and $\underline{x}_o$ by striking out the elements not of interest, giving $\hat{\underline{x}}_m$, $\underline{y}_m$ and $\underline{x}_{om}$ say, and likewise delete from $\hat{\underline{\Sigma}}_o$ the corresponding rows and columns, giving $\hat{\underline{\Sigma}}_{om}$, then

$$F'_m \equiv \frac{(\hat{\underline{x}}_m - \underline{x}_{om})^\tau (\hat{\underline{\Sigma}}_{om})^{-1} (\hat{\underline{x}}_m - \underline{x}_{om})}{\underline{r}^\tau \underline{r}} \cdot \frac{k - n^*}{m} \qquad (4.6:6)$$

is distributed as F with m and $k-n^*$ degrees of freedom for numerator and denominator respectively. Therefore

$$F_m \leq F(m, k-n^*; \alpha)$$

provides us, when considered as an inequality on $\underline{y}_m$, with a confidence interval for $\underline{y}_m$, whereas the hypothesis H_o: $\hat{\underline{x}}_m = \underline{x}_{om}$ is tested by calculating

$$F'_m \equiv \frac{(\hat{\underline{x}}_m - \underline{x}_{om})^\tau (\hat{\underline{\Sigma}}_{om})^{-1} (\hat{\underline{x}}_m - \underline{x}_{om})}{\underline{r}^\tau \underline{r}} \cdot \frac{k - n^*}{m} .$$

Then, with chosen α, if

$$F'_m \leq F\ (m, k-n^*; \alpha),$$

H_o is accepted; H_o is rejected otherwise.

4.6.4 Confidence intervals for an element of $\underline{y}$

When m = 1, the case of 4.6.3 simplifies considerably: let us consider the j-th element of $\underline{\hat{x}}$ and $\underline{y}$; (4.6:6) then reduces to $(\hat{x}_j - y_j)^2 . ((\underline{A}^\tau\underline{A})^{-1}{}_{jj})^{-1} . (k-n^*) . (\underline{r}^\tau\underline{r})^{-1}$ for Model I and to a similar expression (with $\underline{\tilde{A}}^\tau\underline{\tilde{A}}$ for $\underline{A}^\tau\underline{A}$) for Model II. But, by (4.3:3)

$$\hat{\sigma}^2(\hat{x}_j) = (\underline{A}^\tau\underline{A})^{-1}_{jj} . \hat{s}^2, \qquad (\hat{s}^2 \equiv \underline{r}^\tau\underline{r}/(k-n^*)),$$

so

$$F_1' = (\hat{x}_j - y_j)^2/\hat{\sigma}^2(\hat{x}_j).$$

Using the fact that

$$F(1,k-n^*;\alpha) = (t(k-n^*;\alpha/2))^2,$$

a confidence interval for y_j is given by

$$|\hat{x}_j - y_j| \le t(k-n^*;\alpha/2) . \hat{\sigma}(\hat{x}_j),$$

where $t(k-n^*;\beta)$ is the value of Student's t for $k - n^*$ degrees of freedom such that

$$P(t(k-n^*) \ge t(k-n^*;\beta)) = \beta .$$

Similarly, testing $H_o : \hat{x}_j = (\underline{x}_o)_j$, amounts to calculating $|\hat{x}_j - (\underline{x}_o)_j|/\hat{\sigma}(\hat{x}_j)$ and choosing α. If

$$|\hat{x}_j - (\underline{x}_o)_j|/\hat{\sigma}(\hat{x}_j) \le t(k-n^*;\alpha/2),$$

H_o is accepted; H_o is rejected otherwise.

4.6.5 Confidence intervals for a linear function of $\underline{y}$

Since $\hat{\sigma}^2(\underline{v}^\tau\underline{\hat{x}}) = \underline{v}^\tau\underline{\hat{\Sigma}}\underline{v}$ (4.3:2) , calculating a confidence interval for the linear function $\underline{v}^\tau\underline{y}$ and testing the hypothesis H_o: $\underline{v}^\tau\underline{x}_o = \underline{v}^\tau\underline{\hat{x}}$ can be performed in complete analogy to the technique outlined in 4.6.4, provided that one first obtains the scalar quantity

$$\hat{s}^2 . \underline{v}^\tau\underline{\Sigma_o}\underline{v} = \hat{\sigma}^2(\underline{v}^\tau\underline{\hat{x}}).$$

This procedure is particularly useful for the important special case that the linear function takes the form of the sum and/ or difference of some components, i.e. when the v_j are -1, 0 or 1.

4.7 Concluding remarks

1. The most generally useful procedures of the preceding section are based on the distribution of ϕ; we note that, <u>formally</u>, these procedures are identical to the classical regression procedures using F;we shall refer to them as 'use of the F-ratio' in the following chapters. Their formal identity with the procedures in the classical case implies that any computer program for linear regression, if provided with facilities for testing hypotheses by means of the F-ratio, is suitable for the treatment of our special case without adaptation. Unfortunately, this facility is usually lacking, though its availability seems a logical and necessary extension of a program estimating the unknown parameters $\hat{\underline{x}}$.

2. In view of the drastic assumptions made, it is legitimate to inquire to what extent tests of hypotheses and confidence intervals are suitable in practice, i.e. whether trial analyses (with known $\underline{y}$) give acceptable results when testing the hypothesis $\hat{\underline{x}} = \underline{y}$.

To answer this question, we must first remark that minor departures from linearity are known to occur in certain cases, (one part in 1000 has been assumed acceptable in Sec. 5.3),and cannot be excluded for certain instrumental conditions (Cf. 4.6.1). The bias so introduced in the $\hat{x}_j$ is estimated, however, to be in the order of magnitude of say 0.002 in the $\hat{x}_j$ or less. Next, we note that the assumption of the normality and independence of the errors has not been tested - indeed, it is difficult to devise such a test, seeing that the a_{ij} are not reproducible in time (Sec. 2.2.3). Any positive correlation between the errors would lead to underestimating the $\hat{\sigma}(\hat{x}_j)$, i.e. to overestimating the precision of the $\hat{x}_j$.

Because of the possible bias of $\sim$ 0.002 or so in the $\hat{x}_j$, values $\hat{\sigma}(\hat{x}_j)$ of the order of magnitude 0.002 or less, which occur with compounds with very characteristic spectra, have no practical meaning: the random variability of the $\hat{x}_j$ is dominated by the effects of non-linearity. However, such bias will be insignificant when considered relative to those $\hat{x}_j$ for which $\hat{\sigma}(\hat{x}_j)$ is of the order 0.004 or more.

Therefore, the following position seems reasonable: if the hypothesis $\hat{\underline{x}} = \hat{\underline{x}}_o$ is accepted by the procedures of Sec. 4.6, we shall accept it. If it is rejected, it is legitimate to scan the $\hat{\sigma}(\hat{x}_j)$: if any $\hat{\sigma}(\hat{x}_j) < 0.002$, the hypothesis $\hat{\underline{x}}_s = (\underline{x}_o)_s$ is tested, where the subvectors $\hat{\underline{x}}_s$, $(\hat{\underline{x}}_o)_s$ now exclude the components with low

$\hat{\sigma}(\hat{x}_j)$. If this hypothesis is accepted, we still accept $\underline{\hat{x}} = \underline{x}_o$, though with some misgiving; otherwise we reject $\underline{\hat{x}} = \underline{x}_o$.

The procedure, though objectionable statistically by the sequential element it contains, still seems the best we can offer. For the difficult cases, it need not be invoked often, since with complicated mixtures spectral similarities will usually cause the $\hat{\sigma}(\hat{x}_j)$ to be well in excess of 0.002, even with $k-n^* > 30$.

Finally, we point out that cases of clearly failing linearity can always be treated by the method of Chapter VI.

CHAPTER V

LINEARITY

5.0 Motivation of the study and the model

Since much of the present work is based on the linear dependence of absorbance (a) on concentration (c), some inquiry into the conditions governing the validity of this linear dependence is in order. It is well known that, strictly speaking, Beer's Law, which expresses the linear dependence, is a limiting law, being valid, if at all, for absorbances at a single, definite frequency ν_o only.

In practice, the limit of a single frequency cannot even be approached very closely: all measurements of a must, of necessity, be obtained with a band of frequencies,

$$\nu_o - \Delta\nu \leq \nu \leq \nu_o + \Delta\nu, \quad \text{say,}$$

since in the limit $\Delta\nu \rightarrow 0$, zero radiant energy would be transmitted, and a fair amount of radiant energy is necessary for a reliable measurement.

Assuming a to be proportional to c as $\Delta\nu \rightarrow 0$, we should investigate the degree to which the apparent absorbance $a' \equiv a'(\nu_o, \Delta\nu)$, as measured in a band of frequencies $\Delta\nu$ centering on ν_o, deviates from linearity with c. It is to this investigation that the present chapter is devoted.

Instead of determining the a',c relation experimentally, it was preferred to survey a model of the same by numeric methods. The model consists of an idealized monochromator being applied to an idealized absorber.

The monochromator

Following Dennisson 1928 and Williams 1948, we assume

1) that the energy incident on the monochromator is constant in the interval $(\nu_o - \Delta\nu, \nu_o + \Delta\nu)$
2) that the dispersion $dn/d\nu$ is likewise constant in this interval
3) that entrance and exit slits are equal
4) that diffraction effects are negligible

Under these assumptions, the energy leaving a monochromator slit is given by

$$E(\nu) = \frac{s - |\nu_o - \nu|}{s} \qquad (5.1:1)$$

for $\nu_o - s \leq \nu \leq \nu_o + s$, $E(\nu)$ being zero outside this interval. $E(\nu)$ represents an isosceles triangle with $E(\nu_o) = 1$ and half-width s.

The absorber

The idealized absorber consists of a single absorption band, represented by a Lorentz resonance function,

$$a(\nu) = \frac{d}{(\nu-\nu_o)^2 + b^2} ,$$

(Lorentz 1906), shifted so that ν_o becomes the new origin, viz.

$$a(\nu) = \frac{d}{\nu^2 + b^2} . \qquad (5.1:2)$$

In this two-parameter curve, symmetrical about $\nu = 0$, the parameters d and b are connected to the physical parameters of an absorption band by

$$\begin{cases} a_{max} = a(\nu=0) = d/b^2 \\ \Delta\nu_{\frac{1}{2}} = 2b , \end{cases}$$

where $\Delta\nu_{\frac{1}{2}}$ is the band half-width:

$$a(\nu = \tfrac{1}{2}\Delta\nu_{\frac{1}{2}}) = a(\nu = -\tfrac{1}{2}\Delta\nu_{\frac{1}{2}}) = \tfrac{1}{2}a_{max} \qquad \text{(cf. Fig. 5.1).}$$

The full model consists of the absorber as 'seen' by the monochromator. We assume in this, and in the following, that equation (5.1:2) holds for the chemically commonest definition of absorbance, viz. as the negative decimal logarithm of the transmittance. The point is unimportant, since $\Delta\nu_{\frac{1}{2}}$ is independent of the base of logarithms chosen. The energy transmitted, $T(\nu)$, by the absorber (5.1:2) is, for unit incident energy,

$$T(\nu) = 10^{-\frac{d}{\nu^2 + b^2}}$$

In order to obtain the apparent absorbance $a'(\nu')$, i.e. the absorbance as seen by the slit, positioned at $\nu = \nu'$, we first take the convolution of $T(\nu)$ and $E(\nu)$, normalized on division by

$$\int_{-\infty}^{+\infty} E(\nu)d\nu = \int_{\nu'-s}^{\nu'+s} E(\nu)d\nu = s$$

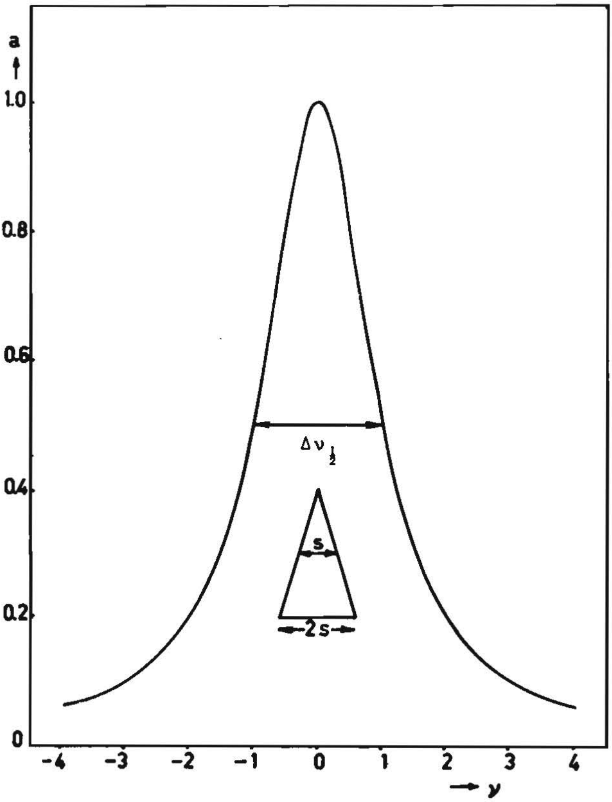

Fig. 5.1 The Lorentz curve of $a_{max} = 1, \Delta\nu_{\frac{1}{2}} = 2, \nu_0 = 0$. Also shown is a slit function, drawn to an arbitrary scale.

in which ν' is the frequency of maximum energy of (5.1:1), i.e. the nominal frequency setting of the scanning slit.

This normalized convolution $\frac{1}{s}\int E(\nu)T(\nu)$ is the energy transmitted by absorber and slit, expressed as a fraction of the incident energy; $a'(\nu)$ is its negative decimal logarithm. Since $E(\nu)$ is zero outside the interval

$$(\nu' - s, \nu' + s),$$

we obtain:

$$a'(\nu') = -{}_{10}\log\left\{\frac{1}{s}\int_{\nu'-s}^{\nu'+s}\frac{s - |\nu' - \nu|}{s}\cdot 10^{-\frac{d}{\nu^2 + b^2}}\,d\nu\right\}$$

Apparently, $a'(\nu')$ should be written as $a'(\nu',s,d,b)$, that is a' seems to involve a four-parameter family of integrals. However, by a slight recasting of the above formula we can express a' in terms of three parameters only. Consider two absorbers, A_1 and A_2, both having the same a_{max}. Let A_1 have $\Delta\nu_{\frac{1}{2}} = 2b$ and be observed at ν' through a slit s; let A_2 have $\Delta\nu_{\frac{1}{2}} = 2\lambda b$ $(\lambda \neq 0)$ and be observed at $\lambda\nu'$ through a slit λs. Then, clearly, the parameter d for A_1 equals $a_{max}.\ b^2$ while d for A_2 equals $a_{max}.\ \lambda^2 b^2$, Then, by substitution in the above formula,

$$a'(A_1) = a'(\nu',s,\ a_{max},b^2,b) = a'(\lambda\nu',\lambda s,a_{max},\lambda^2 b^2,\lambda b) = a'(A_2)$$

This is to say that for constant a_{max}, the function a' is unambiguously determined by ν'/b and s/b; putting $b = 1$, we have $a' = a'(a_{max},\ \nu'^*,\ s^*)$, where ν'^* is the frequency counting from the band centre, expressed in units of b and s^* is the slit width similarly expressed. Writing ν' and s for ν'/b and s/b respectively, we finally have:

$$a'(a_{max},\ \nu',s) = -{}_{10}\log\left\{\frac{1}{s}\int_{\nu'-s}^{\nu'+s} \frac{s-|\nu'-\nu|}{s}.\ 10^{-\frac{a_{max}}{\nu^2+1}}\ d\nu\right\}$$

By a standard interval-seeking Simpson integration program, numerical values of this function have been obtained for

$s = .1(.1)1.0$

$\nu' = 0(.2)2.0$

$a_{max} = .2(.2)2.0$

to within 10^{-6}; after rounding to 10^{-5} they are given here as Table 5.1 (p. 106 sqq.).

5.1 A test of the model

No literature data seem to be available on the correctness of the above model in the spectral region accessible to quartz prism instruments. Accordingly, as a test for the model assumed, the dependence of peak absorption on slit width was investigated for a few representative fine structure peaks in the ultra-violet region.

Some care had to be exercised in order to select absorption bands that conformed at least reasonably well to a representation as isolated bands, since such has been assumed explicitly to be the case in the model. Overlap with other bands, if present to any considerable extent (such as is often the case in the ultra-violet), invalidates to some degree the conclusions that may be drawn from the model. The optical slit width was read off from the slit width drum and converted to the appropriate units; it will be referred to as s, the computed slit width. Because of imperfections of read-out, accuracy of s is not thought to be better than a few per cent. Apparent half-width of the bands was estimated by directly reading on the wave number scale the spectral positions at which the observed absorbance equalled one half that at band centre. Again, accuracy was moderate, being limited by the necessity to estimate $\nu_{\frac{1}{2}}$ to a tenth of a division on the wavelength or wave number scale.

As a first example, we may consider the band occurring at about 319 nm in the spectrum of 2-methylnaphthalene in alcohol; the spectrum of the compound between 325 and 300 nm is shown in Fig. 5.2. Apparent absorbances at band maximum were measured at a number of slit widths; the apparent half-width of the absorption band was determined as 3.4 nm, using minimal slit width. Similar measurements were obtained on the band at about 274.5 nm in the

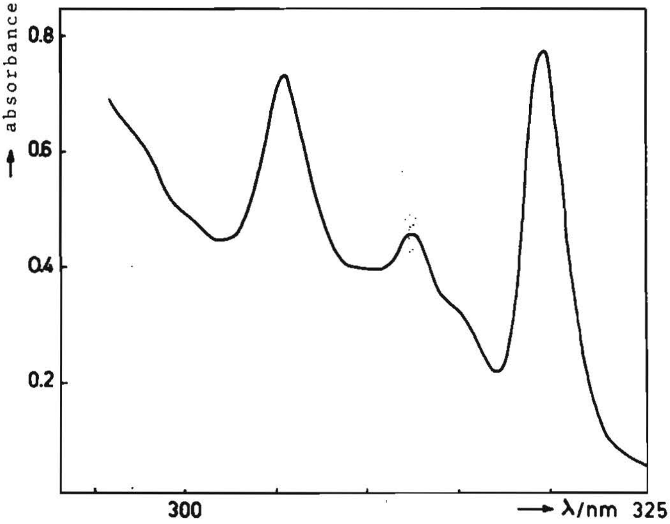

Fig. 5.2 Part of the absorption spectrum of 2-methylnaphthalene in 96% ethanol.
Concentration: 1.47 millimole/litre, optical path length d = 1.0 cm.

absorption spectrum of an alcoholic solution of p-xylene, a record of the spectrum of which is shown in Fig. 5.4. The experimentally determined half-width was 3.3 nm.

On comparing these experimental results with the theoretical apparent absorbances that were derived from Table 5.1, it was found that the experiment yielded an (a',s) relationship exhibiting a more rapid falling off of a' with increasing s than predicted by theory. The discrepancy was much more marked for p-xylene than for 2-methylnaphthalene. However, a very satisfactory fit was obtained when a smaller value for $\Delta\nu_{\frac{1}{2}}$ was assumed than that obtained from the experiment (Figs. 5.3 and 5.5). It is reasonable to suppose that the necessity for the downward revision of $\Delta\nu_{\frac{1}{2}}$ is caused by the interference from shorter wavelength bands, the wings of which cause an appreciable underlying absorption troughout the observed bands. Apart from causing a marked lack of symmetry in the apparent band shape, which necessitates half-width estimates to be obtained from the distance (absorption peak to longer wavelength point of half peak absorbance) only, thus reducing precision of the estimate of $(\Delta\nu_{\frac{1}{2}})_{app}$, the underlying absorption, being relatively more important at $\nu_{\frac{1}{2}}$ than at ν_{max}, will tend to displace $\nu_{\frac{1}{2}}$ outward from ν_{max}, thus leading to

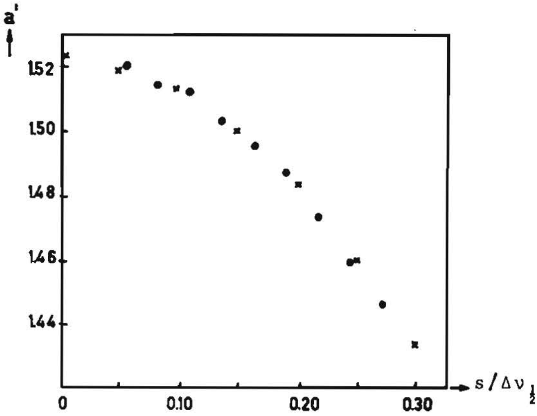

Fig. 5.3 The relation between the apparent absorbance and the slit width. Data are for the band at ∿ 319 nm in the absorption spectrum of an alcoholic solution of 2-methylnaphthalene.

O Observed

× Calculated from the Lorentz curve model for $\Delta\nu_{\frac{1}{2}}$ = 3.2 nm.

observed band half-widths that are in excess of the correct value. The observed half-widths, being too large, will cause $s/\Delta\nu_{\frac{1}{2}}$ to be underestimated, which in turn results in an underestimate of $|a - a'|$.

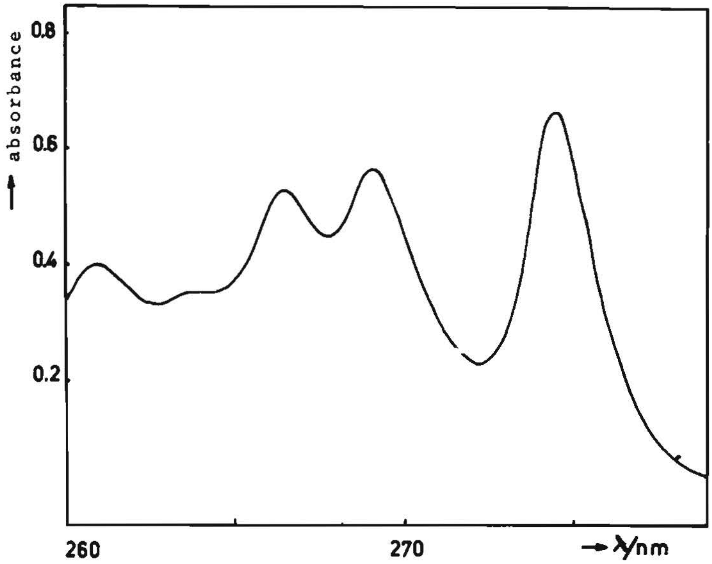

Fig. 5.4 Part of the absorption spectrum of p-xylene in 96% ethanol.

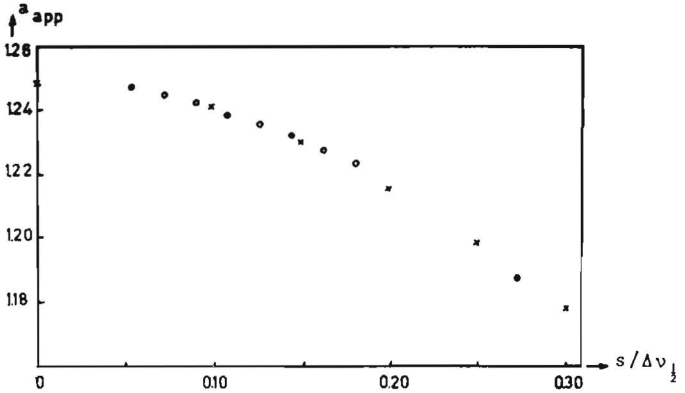

Fig. 5.5 The relation between the apparent absorbance and the slit width. Data are for the band at ∿ 274.5 nm in the absorption spectrum of an alcoholic solution of p-xylene.

o Observed

x Calculated from the Lorentz curve model for $\Delta\nu_{\frac{1}{2}}$=2.7nm.

The magnitudes of the revisions of $\Delta\nu_{\frac{1}{2}}$ which were applied agree with this explanation: the relatively well isolated band of 2-methylnaphthalene, $(\Delta\nu_{\frac{1}{2}})_{obs} = 3.4$ nm, required a revision to 3.2 nm only for the observed apparent absorbances to fit; the more strongly interfered with band of p-xylene, $(\Delta\nu_{\frac{1}{2}})_{obs} = 3.3$ nm, necessitated a downward revision of the estimate of this parameter to 2.7 nm.

The view given here is supported to some extent by an attempt to fit a Lorentz curve to the 2-methylnaphthalene band (Fig. 5.6). The curve was made to pass through the observed absorbances at 31200, 31300 and 31400 cm^{-1}, giving a calculated half-width of 332 cm^{-1} or about 3.35 nm. Comparison of the obser-

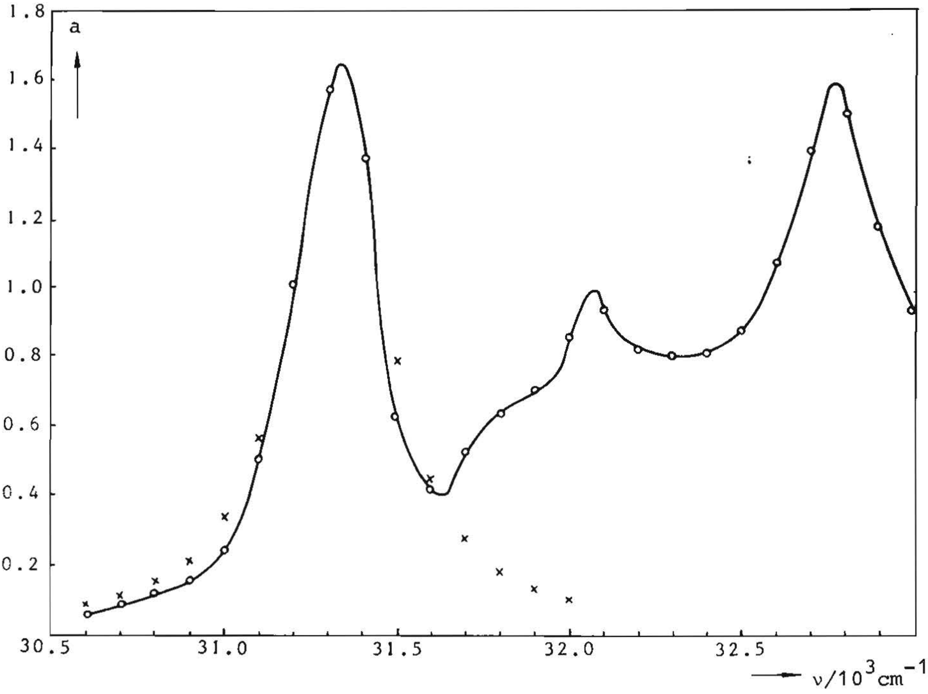

Fig. 5.6 Fitting a Lorentz curve to absorption data. The spectrum is that of an alcoholic solution of 2-methylnaphthalene.

○ Observed absorbances

× Absorbances as calculated from the Lorentz curve passing through the absorbances observed at 31200, 31300 and 31400 cm^{-1}.

The result tends to indicate that this method, too, overestimates $\Delta\nu_{\frac{1}{2}}$.

ved and calculated absorbances at other points in the band, however, seems to indicate a definitely too large width of the Lorentz curve, as well as giving clear indications of overlap.

In the near infra-red, experimental difficulties preclude the observation of the (a',s) relationship. From the similarity of band shapes between the ultra-violet and infra-red regions, we may expect a similar (a',s) relation to hold in the near infrared as well.

We conclude:

1. Lorentz curves provide a tolerable representation of band shapes;
2. Experimental determination of band half-width tends to overestimate $\Delta\nu_{\frac{1}{2}}$.

5.2 Failure of linearity

In order to set out clearly why we are concerned about possible failures of linearity, we shall now prove that, in principle, deviations from linearity always occur unless the transmittance T is a linear function of frequency.

Let the true transmittance of the sample near the spectral position chosen be represented by a power series in ν,

$$T(\nu) = b_o + b_1\nu + b_2\nu^2 + .. ;$$

for convenience, we may shift the origin to ν', the spectral position chosen, putting $\mu = \nu - \nu'$, and obtaining $T(\nu) = T'(\mu) = c_o + c_1\mu + c_2\mu^2 + ...$ We may note that a change of concentration will cause the coefficients c_i to change non-proportionally, since T' is not a linear, but an exponential function of concentration. The true transmittance at $\nu = \nu'$, $\mu = 0$, is evidently c_o. In order to obtain the apparent transmittance, we determine, as before

$T'_{app}(\mu=0) = \dfrac{\int T(\mu)E(\mu)\,d\mu}{\int E(\mu)\,d\mu}$, $E(\mu)$ being the slit function previously defined, the integration extending over the entire slit, $\mu = -s$ to $\mu = s$. On integration, T'_{app} is seen to be

$$T'_{app}(\mu=0) = c_o + \frac{2c_2}{3.4}s^2 + \frac{2c_4}{5.6}s^4 + ...,$$

the coefficients of the odd powers of μ yielding no contribution. Since, however, the absorbance, - log T, was assumed to be proportional to concentration, and a change of concentration will therefore cause the coefficients c_i to change non-proportionally to the change in c_o, we may conclude the apparent absorbances, $-\log T_{app}$, to be non-proportional to concentrations for all $s \neq 0$ unless c_2, c_4 etc. vanish identically for all μ, which implies that b_2, b_3, b_4 etc. do so vanish for all ν, that is to say, unless $T(\nu)$ is linear in ν or a constant over the entire slit width.

5.3 The extent of the non-linearity

Accepting the Lorentz curve as a working model for band shapes, and using Table 5.1, we can investigate quantitatively on our model to what degree the assumption of a linear (a,c) relation will be violated in various cases. In this section we shall confine ourselves to a single component; we shall take its relative concentration x_{calc} to be estimated from the ratio $a'_{sample}/a'_{reference}$.

Assuming $a' = 1.6$ to be the highest absorbance one is prepared to accept in the reference cell, one calculates for $\nu' = 0$, that is, at absorption maximum, for $x = 0.5$:

$s/\Delta\nu_{\frac{1}{2}}$	0	0.10	0.20	0.30	0.40
$a'_{reference}$	1.60000	1.58932	1.55730	1.50479	1.43493
a'_{sample}	0.80000	0.79471	0.77930	0.75522	0.72466
x_{calc}	0.5000	0.5000	0.5004	0.5019	0.5051

Other relative concentrations show a similar trend:

$s/\Delta\nu_{\frac{1}{2}}$	0	0.10	0.20	0.30	0.40
x_{calc}	0.2500	0.2500	0.2503	0.2513	0.2536
x_{calc}	0.7500	0.7500	0.7504	0.7514	0.7539

(The above values are at $\nu' = 0$ too, and with the true reference absorbance $a = 1.6$).

From calculations such as the above, we conclude that the above behaviour is typical in several respects:

1. The departure from linearity (slit width bias), as measured by $x_{calc} - x$, is negligible at low $s/\Delta\nu_{\frac{1}{2}}$ and increases rapidly with $s/\Delta\nu_{\frac{1}{2}}$. (Hence, an overestimate of $\Delta\nu_{\frac{1}{2}}$ will cause the bias to be underestimated).
2. The bias is such that $x_{calc} < 1$ will always be too high.
3. The bias goes through a maximum at $x \doteq 0.5$ (see Fig. 5.7). The relative bias, $(x_{calc} - x)/x$, may, of course, be more important at low x.

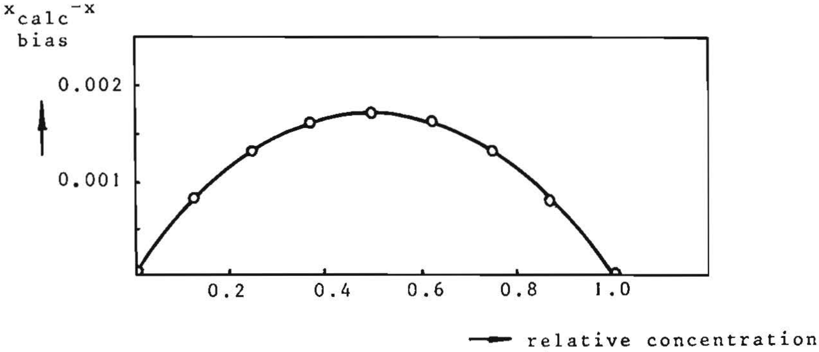

Fig. 5.7 The dependence of slit width bias on relative concentration. Data are for $\nu' = 0.8$ on a Lorentz curve with $s/\Delta\nu_{\frac{1}{2}} = 0.10$ and a true reference absorbance at maximum of 1.6.

All the above conclusions remain valid at off-peak positions in the absorption band, as may be verified from similar calculations on the apparent absorbances in Table 5.1 at $\nu' \neq 0$. The amount of bias, however, which is entirely acceptable at $s/\Delta\nu_{\frac{1}{2}} \leq 0.20$ and $\nu' = 0$, increases sharply as one moves outwards from the peak. This may be illustrated by the following table.

$s/\Delta\nu_{\frac{1}{2}} = 0.10$ $a_{ref} = 1.6$

ν'	0.0	0.2	0.4	0.6	0.8	1.0	1.2
x_{calc}	0.2500	0.2502	0.2509	0.2513	0.2513	0.2511	0.2509
	0.5000	0.5005	0.5012	0.5017	0.5017	0.5015	0.5012
	0.7500	0.7503	0.7509	0.7513	0.7513	0.7511	0.7509

Similar tables for larger value of $s/\Delta\nu_{\frac{1}{2}}$ confirm that the maximum bias is reached for some value of ν' between 0.6 and 0.8. Increasing $s/\Delta\nu_{\frac{1}{2}}$ will cause the bias to increase sharply as before; at $\nu' = 0.8$, the following may be considered representative:

$(a_{ref})_{max} = 1.6$	x_{calc}, $s/\Delta\nu_{\frac{1}{2}} = 0.10$	x_{calc}, $s/\Delta\nu_{\frac{1}{2}} = 0.20$
x		
0.2500	0.2513	0.2550
0.5000	0.5017	0.5067
0.7500	0.7513	0.7550

For $\nu' = 0.8$, $a_{max} = 2.0$ and $s/\Delta\nu_{\frac{1}{2}} = 0.5$, the non-linearity of the (a',x) relation is depicted in Fig. 5.8.

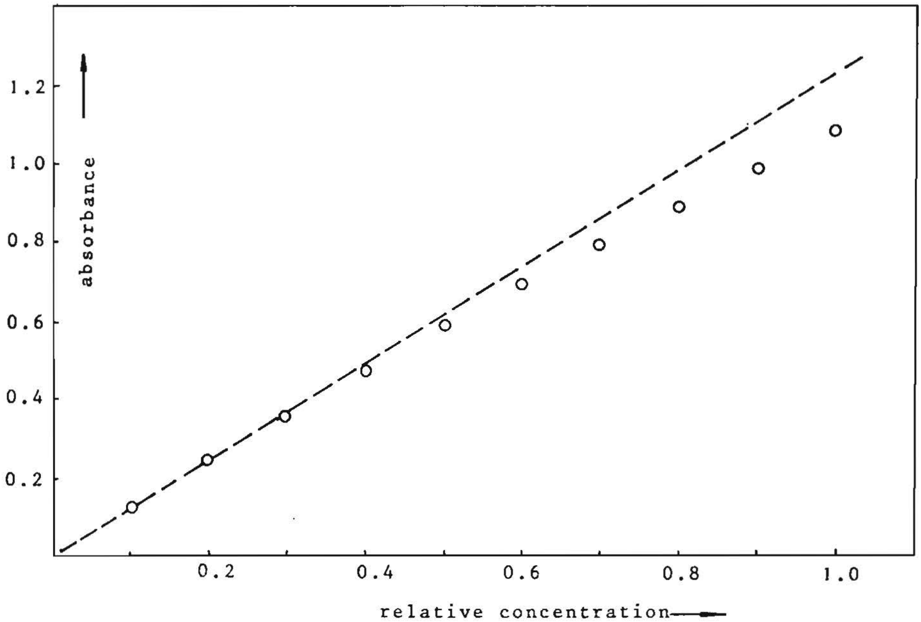

Fig. 5.8 An extreme case of departure from linearity of the apparent absorbance vs. concentration relation, due to slit width error. The plotted data are for $\nu' = 0.8$ on a Lorentz curve with $s/\Delta\nu_{\frac{1}{2}} = 0.50$. At relative concentration = 1.0, the true absorbance at $\nu' = 0$ would be 2.0. The straight line represents the relation predicted by Beer's law.

In connexion with the overall precision attainable, it seems reasonable to wish to limit the slit width bias to (say) one part in thousand of the reference concentration. From Table 5.1, we then calculate the following short table for a single component:

Table 5.2

Maximum allowable relative slit width $s/\Delta\nu_{\frac{1}{2}}$ for the bias not to exceed 0.001 in x

$a_{\text{reference}}$	For measurements at band centre $\nu' = 0$	For measurements anywhere in the band $\nu' \neq 0$
1.0	$s/\Delta\nu_{\frac{1}{2}} \leq 0.3$	$s/\Delta\nu_{\frac{1}{2}} \leq 0.1$
1.6	$s/\Delta\nu_{\frac{1}{2}} \leq 0.25$	$s/\Delta\nu_{\frac{1}{2}} \leq 0.08$

For single peaks in the ultra-violet, even $s/\Delta\nu_{\frac{1}{2}} \leq 0.08$ can nearly always be attained on our instrument. This will therefore keep the slit width bias within acceptable limits. Since, as we shall show in the following section, the effect of the bias is aggravated in the multicomponent case, the allowable reference absorbances should then possibly be lowered. This recommendation has to be weighed against the better precision generally resulting from the use of higher reference absorbances. Some compromise will obviously have to be found; there are indications that, occasionally, slit width bias is responsible for some deviating results obtained in practice.

When working out a compromise between the maintenance of linearity and the wish to use high reference absorbances, it should be remembered that the slit width bias vanishes both for $x = 0$ and for $x = 1$, that is, in practice, for low or near unit relative concentrations.

5.3.1 Systematic slit width error in multicomponent systems

Extension of the results of the preceding section to multicomponent systems requires knowledge of the inverse $\underline{A}^{-1}$ of the matrix of the reference absorbances. It will be convenient in this connexion to consider the bias to be caused by an axcess Δa_i of the sample absorbances over their values a_i, the latter being assumed equal to $\sum_j x_j (a_{ref})_j$. The bias in the j-th component will then be given by

$$\Delta x_j = (x_j)_{calc} - x_j = \sum_i k_{ji} \Delta a_i,$$

k_{ji} being the i-th element of the j-th row of $\underline{A}^{-1}$, the summation extending over a number of frequencies equal to the number of components.

As a special case of this result we may consider a system not infrequently with in practice, viz. a two-component system in which the bias is (almost) entirely due to non-linearity caused by one component at one sampling frequency. In this connexion it is important to point out that it seems to have been established that one may expect additivity of absorbances to hold, even when linearity of absorbances with concentration is departed from. Let the matrix of reference absorbances,

$$\underline{A} = \begin{pmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{pmatrix}$$

be such that $a_{11} \doteq a_{22} >> a_{12} \doteq a_{21} > 0$ and let $s/\Delta\nu_{\frac{1}{2}}$ for the first component at the first frequency be so large as to cause appreciable departure from linearity (cf. Fig. 5.9). For the mixture to be analysed the absorbances may then be represented by

$$\begin{pmatrix} a_1 + \Delta a_1 \\ a_2 \end{pmatrix}$$

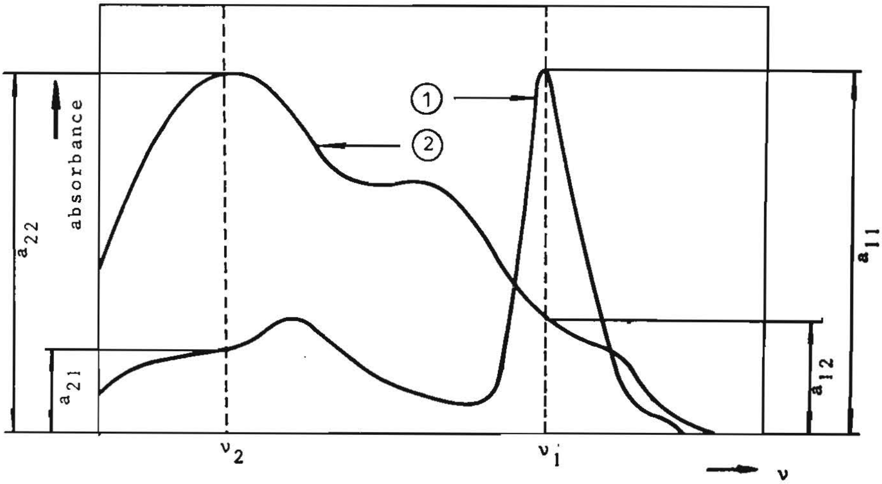

Fig. 5.9 Schematic of the absorption spectra of a two-component system, in which slit width error is apt to occur for a single component at a single frequency only.

The results of the biased analysis are

$$(x_1)_{calc} = k_{11}(a_1 + \Delta a_1) + k_{12}a_2 = x_1 + k_{11}\Delta a_1$$

$$(x_2)_{calc} = k_{21}(a_1 + \Delta a_1) + k_{22}a_2 = x_2 + k_{21}\Delta a_1$$

so

$$\Delta x_1 = k_{11}\Delta a_1 \quad ; \quad \Delta x_2 = k_{21}\Delta a_1 .$$

However,

$$\underline{A}^{-1} = \begin{pmatrix} k_{11} & k_{12} \\ k_{21} & k_{22} \end{pmatrix} = (\det \underline{A})^{-1} \begin{pmatrix} a_{22} & -a_{12} \\ -a_{21} & a_{11} \end{pmatrix} ,$$

hence

$$\Delta x_1 : \Delta x_2 = k_{11} : k_{21} = a_{22} : - a_{21} .$$

If no second component were included in the analysis, the bias in the first component would be equal to $\Delta x = \Delta a_1/a_{11}$. With the second component assumed present, this bias becomes

$$\Delta x_1 = k_{11}\Delta a_1 = \frac{a_{22}\Delta a_1}{\det A} = \frac{a_{22}\Delta a_1}{a_{11}a_{22}-a_{12}a_{21}} = \frac{\Delta a_1}{a_{11} - \dfrac{a_{12}a_{21}}{a_{22}}} > \frac{\Delta a_1}{a_{11}}$$

therefore

$$\Delta x_1 > \Delta x .$$

This result may be expressed as follows:

If, in a system of two components, bias is present in one component at one frequency, the resulting bias in the two-frequency two-component analysis will be such that the originally biased component will show a greater bias than if no other component were considered present; in the result for the originally unbiased component a bias of opposite sign will be found to be induced.

Since one strives in practice to make A as nearly diagonal as the spectra permit, a_{12} will usually be much smaller than a_{22}; in that case the induced bias will equally be much smaller than in the originally biased component. As a practical instance, we may mention the system (naphthalene/2-methylnaphthalene), where slit width bias is apt to be more easily introduced at the 2-methylnaphthalene peak at approx. 319 nm than at the naphthalene peak at about 310.5 nm (Fig. 5.12).

For systems of more than two components, somewhat similar results may be derived; since, however, the expressions obtained become rather cumbersome, the problem, if of interest for a particular case, may be more easily attacked by calculation of $\underline{A}^{-1}$.

this procedure is to be recommended also for the two component case if the bias involves more than a single element of $\underline{A}$.

5.4 Slit width error and least squares

Since, as we have established (Sec.5.2), the bias in a Lorentz curve is minimal for $\nu' = 0$, that is, at band centre, and increases considerably as one departs from the absorption maximum, it may be thought that inclusion of off-centre observations will produce an unfavourable effect on the accuracy, since bias is inevitably increased thereby. Such is indeed the case, as may be evinced by the following comparison:

a_{max} reference	a_{max} sample	$s/\Delta\nu_{\frac{1}{2}}$	Single point $\nu' = 0$ x_{calc}	Single point $\nu' = 0.8$ x_{calc}	Least squares 21 points $\nu' = -2(.2)+2$ $\hat{x}$
1.6	0.8	0.05	0.5000	0.5004	0.5003
1.6	0.8	0.10	0.5000	0.5017	0.5008
1.6	0.8	0.15	0.5002	0.5039	0.5023

The results for the 21 equidistant points have been obtained by rounding off the pertinent apparent absorbances of Table 5.1 to the nearest thousandth in a and treating these values straightforwardly by the least squares program; the rounding-off error in the data can have affected $\hat{x}$ only very slightly.

The least squares estimates $\hat{x}$ are seen to show a bias intermediate between that exhibited by the best ($\nu'=0$) and worst ($\nu'=0.8$) ordinates, as was to be expected. The exact amount of bias will depend to some extent on the choice of ordinates made; a preponderance of points near $\nu'=0$ will tend to lower $\hat{x} - x$, increasing the number of observations on the flanks of the absorption band will tend to raise it.

In spite of the definitely greater tendency to bias shown by the least squares treatment of single band data when compared to the best point in the band, a number of arguments can be put forward in favour of the least squares method:

I) In practice, measurements, especially in multicomponent systems, will hardly ever be confined to points within a single absorption band, or, if they are, instrumental conditions will nearly invariably be such, that $s/\Delta\nu_{\frac{1}{2}}$ takes on values so low as to make the bias insignificant. In the former case, the tendency to bias caused by the inclusion of sampling frequencies on the flanks of a band will be held in check to a considerable degree by the presumably unbiased observations outside the band in question.

II) Whenever significant bias is present, there will be an associated tendency toward an increased variance of the residuals, which in turn will cause the estimated variance of the estimate $\hat{x}$ to increase beyond the estimate of its variance in the unbiased case. Thus confidence limits or similar measures of reliability for $\hat{x}$ will be widened as the bias increases. Evidently the bias, though still present, will therefore be less likely to lead to an inappropriate interpretation of the estimated composition.

III) It should be pointed out that a biased estimate of high precision may often be preferable to an estimate that, though free from bias, shows a much larger random variability. In many cases the variance in $\hat{x}$ resulting from the least squares treatment, even when corrected for a relatively larger bias, is considerably smaller than the variance computed (from the same assumed distribution of the variance of an estimate of a_{ij}) for the estimate obtained from n equations solved for the n unknown concentrations. In those cases the least squares method cannot but be considered superior.

5.5 Detection of slit width bias

IV) In cases of serious bias, notably at large $s/\Delta\nu_{\frac{1}{2}}$ and not too small concentration x_j of the affected component, the distribution of the residuals will give a definite indication of the presence of bias due to slit width error. In order to see how this extremely characteristic distribution arises, we may consider the rather extreme case of two Lorentz curves for $s/\Delta\nu_{\frac{1}{2}} = 0.30$, of true peak absorbances of 2.0 and 1.0 respectively. If we consider the larger absorbance as a reference, we may calculate the excess in a of the lower absorbance over its theoretical value, which is one half the reference absorbance. On plotting these values against ν', a characteristic curve is seen to emerge, having two maxima at about $|\nu'| = 0.6$, separated by a sharp minimum at $\nu'=0$ (Fig. 5.10,A).

Expressing the lower curve, sampled at 21 equidistant points, in terms of the reference curve by least squares, one obtains $\hat{x} = 0.5105$ as against the theoretical 0.5000. The bias, 0.0105 of the reference concentration, is therefore very considerable, especially when compared to the value from the single point $\nu'=0$, which is $x_{calc} = 0.5024$. However, on plotting the residuals (Fig. 5.10, B), there is found to exist a remarkable correlation between the excesses $\Delta a(\nu')$ in absorbance and the residuals r_i, the latter being very nearly equal to the former after subtracting a constant amount.

Thus, the residuals exhibit a pattern that is easily recognised as that due to slit bias; the pattern is remarkably persistent: it will show in the residuals even when as few as four or five equally spaced observations in the band are included, provided the band maximum is among them; a considerable number of unbiased observations does not perceptibly obscure it; in the large majority of cases it may be observed in the presence of other components.

As a demonstration, we may analyse in some detail the results obtained on treating by least squares the observations obtained on three samples consisting of mixtures of solutions of naphthalene and 2-methylnaphthalene at wavelenghts 300(1)325 nm, employing various slit widths.
The following table summarizes the experiment:

Slit width at 300 nm (in mm)		0.022	0.050	0.150
	Taken	Found	Found	Found
No.		240.01	240.04	240.07
Naphthalene	0.8543	0.8550	0.8543	0.8506
2-Methylnaphthalene	0.1457	0.1456	0.1475	0.1520
$\hat{s}$		1.74	1.72	4.77
No.		240.02	240.05	240.08
Naphthalene	0.5022	0.5029	0.5024	0.4936
2-Methylnaphthalene	0.4978	0.4992	0.5004	0.5138
$\hat{s}$		1.98	2.13	8.94
No.		240.03	240.06	240.09
Naphthalene	0.1492	0.1495	0.1493	0.1444
2-Methylnaphthalene	0.8508	0.8513	0.8513	0.8590
$\hat{s}$		1.65	1.79	4.69

Number of equations: $k = 26$
Dimensionality of unknown vector $\underline{\hat{x}}$: $n^* = 3$
Concentrations of reference solutions:
Naphthalene: 5.69 millimole/litre
2-Methylnaphthalene : 2.84 millimole/litre
Solvent: ethanol 96%

The absorbance values were obtained under the usual experimental conditons; the cell corrections, being very small, were not allowed for explicitly: their effect is thought to be sufficiently taken into account by the added constant on the left side

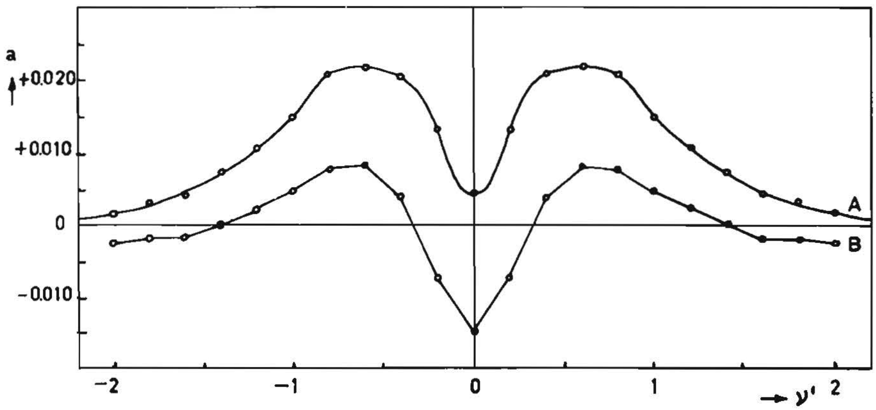

Fig. 5.10 Correlation between (A), the excess $\Delta a(\nu')$ of sample absorbances over their theoretical proportionality to concentration, due to slit error, and (B), the residuals from the least squares procedure, r_i. Data are for $s/\Delta\nu_{\frac{1}{2}} = 0.30$, derived from Lorentz curves for which true absorbance at maximum was 1.0 for the sample and 2.0 for the reference.

of the equations; the constants so determined were smaller than 0.0017 in absorbance for analyses numbers 240.01 to 240.06 inclusive. The entry on the line marked $\hat{s}$ is defined by

$$\hat{s}^2 = \frac{\underline{r}^{\tau}\underline{r}}{k-n^*}$$

$\underline{r}$ being the residual vector. Thus defined, $\hat{s}^2$ is the estimated variance of estimate of an element of $\underline{a}$, the vector of the absorbances of the mixture, assuming the absorbances of the reference solutions to be exact; the tabulated values of $\hat{s}$ have been multiplied by 1000.

Comparing the columns of the table, we note that the agreement between the concentration taken and that found is extremely satisfactory for the smallest slit width. At the intermediate width, 0.050 mm, there is seen a tendency for the 2-methylnaphthalene concentration to rise, whereas values for naphthalene are lowered; equally, $\hat{s}$ increases somewhat. Both tendencies, though, are of doubtful statistical significance.

Turning to the values for $\underline{\hat{x}}$ and $\hat{s}$ derived from the measurements at the widest slit, the concentrations found, $\hat{x}_1$, $\hat{x}_2$ are seen to be affected by gross errors; $\hat{x}_2$, the relative concentration of 2-methylnaphthalene, is considerably too high; $\hat{x}_1$ has an opposite, though smaller, deviation. The values of S, which may be considered an overall measure of the fit of the system of equations, rise to a multiple of the corresponding entries in the preceding columns of the table. On plotting the residuals (Fig. 5.11,B) for analysis no. 240.08, one immediately remarks (at H) a somewhat distorted, but still easily recognizable version of the pattern shown in Fig. 5.10. In conjunction with the intense and fairly narrow absorption peak centering on roughly 319 nm of 2-methylnaphthalene, (Fig. 5.12), the occurrence of this pattern may be regarded as prima facie evidence of slit bias.

Detection of slit bias is further facilitated by the fact that, even where the characteristic pattern is not present, there is a strong correlation between strongly positive values of the residuals on the one hand, and intense, rather narrow bands in the absorption spectra of the components concerned on the other. Examples are afforded by the regions at D and H in Fig. 5.11,B, which correspond to the bands at about 301 and 310 nm in the naphthalene spectrum. That these residuals owe their origin almost entirely to excesses Δa_i of the absorbances of the mixture

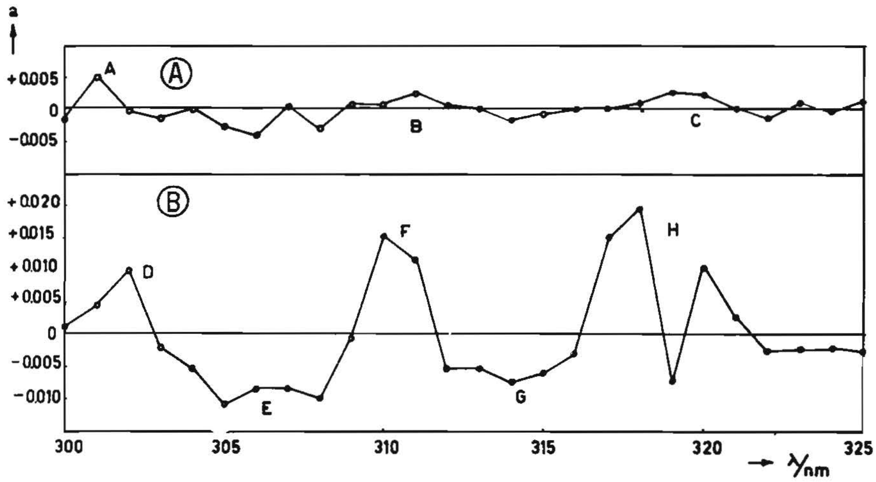

Fig. 5.11 The residuals r_i of the analysis of a mixture of solutions of naphthalene and 2-methylnaphthalene.

Ⓐ With the smallest slit width obtainable(s = 0.022mm, no. 240.02).

Ⓑ At a slit width showing considerable bias(s=0.150mm. no. 240.08).

Both reference solutions and mixture were identical for A and B.

over the values calculated from the known composition x is convincingly demonstrated by a comparison of the Δa_i (Fig. 5.14,A) for no. 240.08 with the r_i. From the experience gained in a fair number of comparable cases we may state:

If slit bias is present, it will tend to increase the length $(\underline{r}^T\underline{r})^{\frac{1}{2}}$ of the residual vector; if the sampling points in an affected absorption band are sufficiently dense, the elements of the residual vector will show a minimum at absorption maximum, flanked by two maxima; if, by reason of the lesser density of sampling points in the band , the absorption maximum or a frequency very near to it was not included among the points sampled in the analysis, the region corresponding to the flanks will show a more or less extended relative maximum in r_i.

For purposes of comparison plots of r_i are included for nos. 240.07 and 240.09 as well (Fig. 5.12). They indicate that even

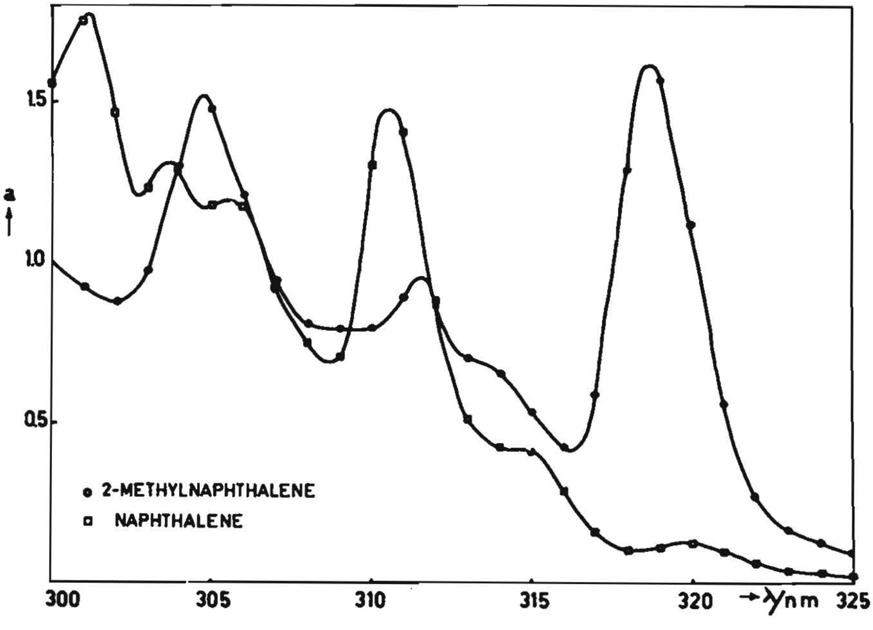

Fig. 5.12 The sampling points and the absorbances of the reference solutions in the part of the absorption spectra of naphthalene and 2-methylnaphthalene used for analyses nos. 240.01 to 240.03.

▫ Naphthalene, 5.69 millimole/litre

o 2-Methylnaphthalene, 2.84 millimole/litre.

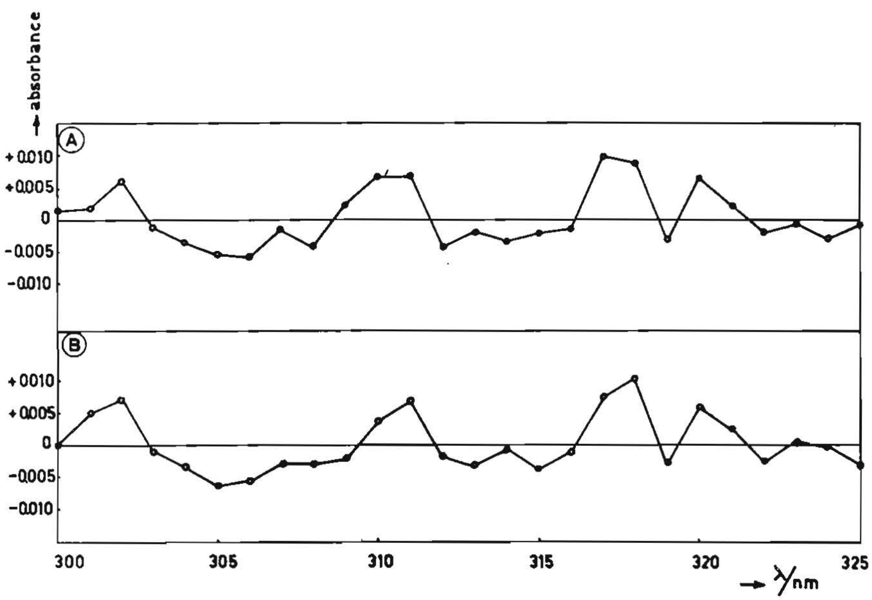

Fig. 5.13 The residuals r_i of the analyses of two further mixtures of solutions of naphthalene and 2-methylnaphthalene, at slit width s = 0.150 mm, with strong bias. Ⓐ Analysis number 240.07; Ⓑ Analysis number 240.09.

with a relative concentration of either component of only 0.15, essentially identical distributions of the r_i will be obtained, hence permitting detection of the bias present.

It is instructive to compare r_i with Δa_i for the smallest slit as well (Fig. 5.11,A and Fig. 5.14,B, resp.). Again, the correlation of the two vectors is striking. The observation marked **A** in both graphs deviates fairly strongly. Since it corresponds to a reference absorbance of 1.760 for naphthalene, which is well above the range usually considered reliable in the present work, and since it also is an off-peak sampling point, there is good reason to view the observation with extreme suspicion. In the residuals, the regions indicated by B and C seem, though faintly, to point to some slit width bias persisting even in this case. This would appear to be confirmed by the positive Δa_i's for the corresponding regions; it would almost certainly be correct to ascribe at least the greater part of the error in $\underline{\hat{x}}$ to the observation at 301 nm (A), which tends to increase $\hat{x}_1$, and the combined effects of the slit biases in bands B and C, the latter

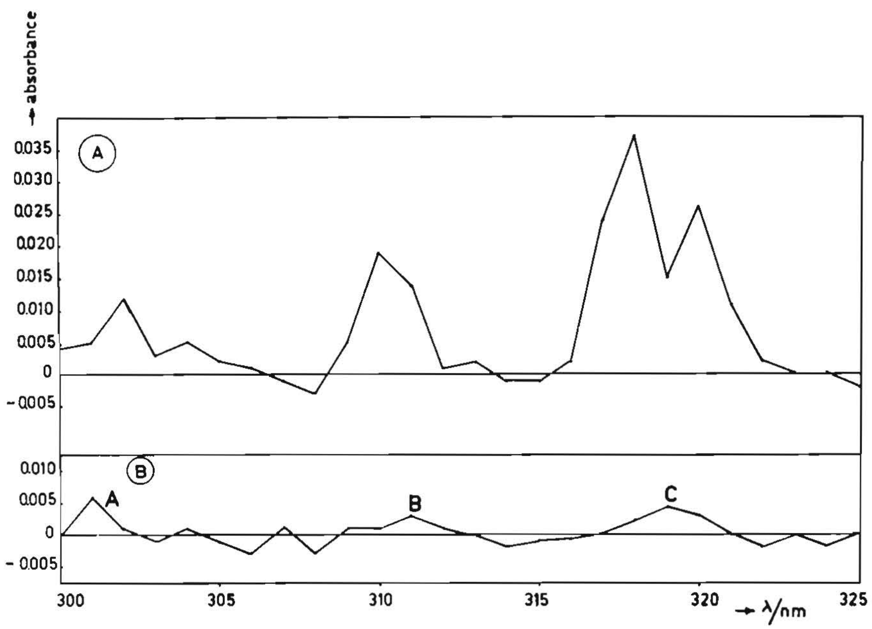

Fig. 5.14 The excesses Δa_i of the absorbances a_i of the mixture of solutions of naphthalene and 2-methylnaphthalene over their theoretical values, as calculated from

$$\Delta a_i = (a_i)_{obs} - \sum_j x_j (a_{ref})_{ij} .$$

Ⓐ For analysis no. 240.08, at the largest slit width, s = 0.150 mm.

Ⓑ For analysis no. 240.02, at the smallest slit width, s = 0.022 mm.

The composition x was equal for both samples, $x_1=0.5022$, $x_2=0.4978$.

band having the stronger effect, and tending to increase $\hat{x}_2$ at the expense of $\hat{x}_1$.

5.6 Prediction of the extent of the bias

The expression for the magnitude of the bias, which for the case of n observations was given by

$$\Delta x_j = \sum_i k_{ji} \Delta a_i ,$$

k_{ji} being the i-th element of the j-th row of $\underline{A}^{-1}$, may be immediately extended to the least squares case, provided we define a left-inverse $\underline{V}^{\tau}$ to matrix A by

$$\underline{V}^{\tau} \equiv (\underline{A}^{\tau}\underline{A})^{-1}\underline{A}^{\tau}.$$

We may then write at once

$$\Delta\ell_j = \sum_i v_{ji}\Delta a_i$$

in which v_{ji} is the i-th element of the j-th row of $\underline{V}^{\tau}$, or, in the more convenient matrix notation,

$$\Delta\ell_j = \underline{v}_j^{\tau}\Delta\underline{a}, \text{ in which } \underline{v}_j^{\tau} \text{ is the j-th row of } \underline{V}^{\tau}.$$

Knowledge of $\underline{V}^{\tau}$ will therefore immediately enable one to read off the effects of any change Δa_i on all elements of $\underline{\ell}$, the least squares approximation vector of the composition of the mixture.

As an application, we may now give a more rigorous discussion of the influence of $\Delta\underline{a}$ on $\underline{\ell}$, which has been treated more loosely above. A representation of the elements v_{1i} and v_{2i} is given in Fig. 5.15; indices 1 and 2 refer to naphthalene and 2-methylnaphthalene respectively. From the graph we see at first glance that Δa_{301} and Δa_{319} will cause relatively very large changes in $\underline{\ell}$. An excess of 0.001 in absorbance at 301 nm will produce an increase of 0.00039 in ℓ_1 jointly with a decrease of

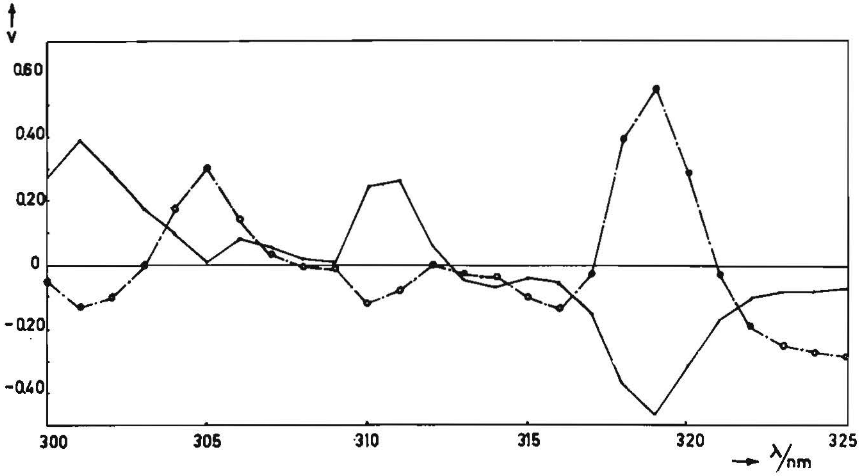

Fig. 5.15 A graphical representation of the elements v_{ji} of the left-inverse $\underline{V}^{\tau}$ for the two-component system (naphthalene/2-methylnaphthalene) for reference solutions and sampling points as shown in Fig. 5.12.

. ———————— . $\underline{v}_1^{\tau}$, naphthalene

o --------- o $\underline{v}_2^{\tau}$, 2-methylnaphthalene

($\underline{v}_3^{\tau}$ for the constant vector a_3, $a_{i3} = 1$, is not shown).

only one third of this amount in $\hat{x}_2$. At 319 nm the same increase in the absorbance of the mixture will, on the contrary, cause a decrease in $\hat{x}_1$ of magnitude $\Delta\hat{x}_1 = -0.00046$, compensated by an increase in $\hat{x}_2$ of $+0.00055$. The graph also allows us to conclude that roughly equal excesses Δa_i in the absorption bands at 310 and 319 nm respectively (cf. the graph of Δa_i, Fig. 5.14,B) will have far from equal effects on $\underline{\hat{x}}$: disturbance of the latter band will affect $\underline{\hat{x}}$ rather more seriously than similar errors in the former region. Such positive Δa_i in both bands as are shown in Fig. 5.14,B will therefore tend to make Δx_1 positive, as stated above.

We may also remark that the results $\underline{\hat{x}}$ are very insensitive to errors in absorbance of the mixture at about 308 and 313 nm. It is precisely at these points that the Δa_i are virtually zero even at the largest slit employed (Fig. 5.14,A). As against this we note that the regions that are most sensitive to error coincide with those most easily affected by departure from linearity of the (a',c) relation.

5.7 Reduction of slit bias

The preceding sections have dealt at some length with the detection of slit bias; a single run on a known mixture will, by the techniques set forth, give unequivocal evidence of the presence of such bias. Even if the composition of the mixture is known only qualitatively, that is, if the presence of absorbing, but unknown components is excluded, comparison of the spectra of the constituent components with the pattern exhibited by the residuals will usually give ample indication that such bias is present.

If the presence of slit bias has been detected and if its magnitude is objectionable for the specific purpose considered, one may always have recourse to at least one of the following four procedures.

(I) Reduction of slit width. If the available radiant energy is sufficient, the simplest means for reducing the slit bias will obviously consist in reducing the slit width. Since this entails a reduction of the power reaching the detector, the sensitivity of the detecting and amplifying parts of the apparatus must be increased, which in turn will lead to a greater random variability of the absorbance values read. However, this may often be compensated for by imposing a larger time constant on the read-out system.

The available energy will usually be such that in the ultraviolet region for $\lambda > 240$ nm values of $s/\Delta\nu_{\frac{1}{2}} = 0.10$ will not be exceeded at a mechanical slit width of 0.02 mm. This ensures that the amount of slit bias expected will nearly always be unobjectionable, whereas diffraction effects may safely be neglected. This estimate is based on the absorption spectra of organic compounds showing fine structure, and may not necessarily hold for certain other absorption bands, e.g. of the ions of the lanthanides. As one moves through the visible into the near infra-red, the ratio of mechanical slit width to the wavelength of the radiation diminishes from 80 at 250 nm to 8 at 2.5μ for $s = 0.02$mm; it is clear that the risk of unwanted diffraction is quite considerable at the red end of the spectral region accessible. In conjunction with the lower dispersion, narrower absorption bands, lower detector sensitivity and high concentration of solute generally encountered in the near infra-red, the diffraction effect will effectively preclude a reduction of s in this region. However, the much more general technique indicated below, (IV), will still be applicable.

(II) Increasing band width. It will be possible in some cases to lower $s/\Delta\nu_{\frac{1}{2}}$ by causing $\Delta\nu_{\frac{1}{2}}$ to increase. The improvement may be effected by changing to a more strongly polar solvent, e.g. by using ethanol instead of a hydrocarbon such as iso-octane. The spectra become somewhat more diffuse by the change; it follows that in general some loss of specificity (i.e. a relative increase of the covariances when compared to the variances) must be accepted in exchange for the reduction in bias.

(III) Reduction of concentration. Since lowering the absorbances of the reference solutions results in a decrease in bias, a reduction of all concentrations may be expected to have a beneficial effect. It should be realised that this advantage is partially offset by the lower precision resulting from the dilution, since the variance-covariance matrix of the $\hat{x}_j$, though unaffected in shape, is multiplied by the squared reciprocal of the dilution factor, at least when all reference concentrations are reduced by the same factor.

(IV) Analysis in terms of known mixtures. Instead of using solutions of the pure compounds as reference absorbances, one may employ, in the reference cells, solutions that contain known mixtures of the compounds required. For best results, these mixtures should be chosen so as to bracket the composition of the unknown mixture for all components. This technique, which requires some previous knowledge about the range of the x_j expected, is quite general and will successfully cope with all types of non-linearity. On account of its importance the method is dealt with more fully in Chapter VI, where an application to the system naphthalene/2-methylnaphthalene at the largest slit width (s = 0.150mm) is given.

Table 5.1

Apparent absorbances, obtained by scanning a family of Lorentz curves with a slit of triangular distribution of radiant energy. In this table,

a_{max}	is the *true* absorbance at absorbance maximum,
$s/\Delta\nu_{\frac{1}{2}}$	is the ratio of half-energy width of the slit to the *true* half-absorbance width of the Lorentz curve, while
$\nu' = \frac{1}{2}\nu/\Delta\nu_{\frac{1}{2}}$	is the distance of the spectral position from the position of maximum absorbance, expressed as the ratio of this distance to one half the *true* half-absorbance width of the Lorentz curve.

Absorbances are understood to be *decimal* logarithms.

$\frac{1}{2}\nu/\Delta\nu_{\frac{1}{2}}$	$s/\Delta\nu_{\frac{1}{2}}$ a_{max}	0.00	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50
0.0	0.2	0.20000	0.19967	0.19868	0.19709	0.19494	0.19232	0.18930	0.18596	0.18239	0.17866	0.17482
	0.4	0.40000	0.39934	0.39736	0.39415	0.38981	0.38447	0.37827	0.37140	0.36399	0.35621	0.34817
	0.6	0.60000	0.59900	0.59604	0.59119	0.58460	0.57644	0.56692	0.55628	0.54476	0.53259	0.51998
	0.8	0.80000	0.79867	0.79471	0.78820	0.77930	0.76822	0.75522	0.74060	0.72466	0.70775	0.69016
	1.0	1.00000	0.99834	0.99337	0.98518	0.97393	0.95982	0.94315	0.92428	0.90367	0.88165	0.85867
	1.2	1.20000	1.19800	1.19203	1.18214	1.16847	1.15124	1.13075	1.10742	1.08173	1.05422	1.02542
	1.4	1.40000	1.39767	1.39068	1.37907	1.36293	1.34246	1.31796	1.28990	1.25883	1.22543	1.19037
	1.6	1.60000	1.59733	1.58932	1.57597	1.55730	1.53348	1.50479	1.47171	1.43493	1.39522	1.35346
	1.8	1.80000	1.79700	1.78796	1.77284	1.75159	1.72429	1.69121	1.65286	1.60999	1.56357	1.51466
	2.0	2.00000	1.99666	1.98660	1.96968	1.94579	1.91490	1.87722	1.83330	1.78399	1.73044	1.67393
0.2	0.2	0.19231	0.19204	0.19123	0.18992	0.18814	0.18593	0.18337	0.18049	0.17738	0.17408	0.17066
	0.4	0.38462	0.38405	0.38238	0.37966	0.37595	0.37136	0.36600	0.36000	0.35348	0.34656	0.33936
	0.6	0.57692	0.57605	0.57345	0.56920	0.56341	0.55624	0.54786	0.53845	0.52821	0.51732	0.50598
	0.8	0.76923	0.76802	0.76443	0.75855	0.75053	0.74057	0.72891	0.71579	0.70150	0.68628	0.67040
	1.0	0.96154	0.95998	0.95532	0.94770	0.93728	0.92432	0.90911	0.89197	0.87327	0.85333	0.83251
	1.2	1.15385	1.15191	1.14613	1.13665	1.12367	1.10748	1.08843	1.06695	1.04345	1.01839	0.99221
	1.4	1.34615	1.34382	1.33685	1.32540	1.30967	1.29002	1.26684	1.24064	1.21196	1.18136	1.14941
	1.6	1.53846	1.53571	1.52749	1.51393	1.49529	1.47191	1.44429	1.41301	1.37874	1.34218	1.30402
	1.8	1.73077	1.72757	1.71803	1.70226	1.68050	1.65315	1.62075	1.58401	1.54373	1.50078	1.45600
	2.0	1.92308	1.91942	1.90848	1.89037	1.86530	1.83370	1.79619	1.75359	1.70688	1.65710	1.60530
0.4	0.2	0.17241	0.17228	0.17186	0.17117	0.17019	0.16895	0.16743	0.16567	0.16367	0.16146	0.15908
	0.4	0.34483	0.34450	0.34351	0.34188	0.33962	0.33677	0.33336	0.32944	0.32506	0.32028	0.31516
	0.6	0.51724	0.51667	0.51495	0.51213	0.50827	0.50345	0.49775	0.49126	0.48408	0.47632	0.46807
	0.8	0.68966	0.68878	0.68618	0.68193	0.67614	0.66896	0.66054	0.65105	0.64065	0.62949	0.61770
	1.0	0.86207	0.86084	0.85719	0.85125	0.84321	0.83328	0.82173	0.80879	0.79470	0.77970	0.76396
	1.2	1.03448	1.03285	1.02800	1.02011	1.00947	0.99640	0.98126	0.96441	0.94619	0.92689	0.90680
	1.4	1.20690	1.20480	1.19858	1.18850	1.17492	1.15830	1.13914	1.11791	1.09507	1.07104	1.04617
	1.6	1.37931	1.37670	1.36896	1.35642	1.33956	1.31898	1.29533	1.26925	1.24135	1.21214	1.18208
	1.8	1.55172	1.54854	1.53912	1.52387	1.50338	1.47844	1.44985	1.41845	1.38501	1.35020	1.31458
	2.0	1.72414	1.72033	1.70907	1.69084	1.66638	1.63666	1.60268	1.56551	1.52609	1.48527	1.44373
0.6	0.2	0.14706	0.14704	0.14697	0.14683	0.14662	0.14630	0.14586	0.14526	0.14449	0.14355	0.14243
	0.4	0.29412	0.29401	0.29368	0.29312	0.29230	0.29120	0.28981	0.28811	0.28607	0.28371	0.28102
	0.6	0.44118	0.44092	0.44014	0.43884	0.43703	0.43471	0.43187	0.42854	0.42473	0.42045	0.41575
	0.8	0.58824	0.58776	0.58635	0.58402	0.58083	0.57681	0.57204	0.56657	0.56047	0.55380	0.54662
	1.0	0.73529	0.73454	0.73231	0.72866	0.72369	0.71754	0.71034	0.70223	0.69334	0.68382	0.67373
	1.2	0.88235	0.88126	0.87801	0.87274	0.86563	0.85689	0.84679	0.83554	0.82338	0.81050	0.79704
	1.4	1.02941	1.02791	1.02347	1.01628	1.00664	0.99489	0.98142	0.96657	0.95067	0.93400	0.91679
	1.6	1.17647	1.17450	1.16867	1.15928	1.14674	1.13156	1.11427	1.09536	1.07529	1.05443	1.03309
	1.8	1.32353	1.32102	1.31363	1.30174	1.28595	1.26692	1.24537	1.22198	1.19733	1.17191	1.14610
	2.0	1.47059	1.46748	1.45834	1.44367	1.42426	1.40099	1.37479	1.34651	1.31690	1.28657	1.25600
0.8	0.2	0.12195	0.12199	0.12212	0.12231	0.12255	0.12282	0.12308	0.12331	0.12347	0.12353	0.12347
	0.4	0.24390	0.24393	0.24402	0.24414	0.24427	0.24437	0.24440	0.24432	0.24409	0.24366	0.24300
	0.6	0.36585	0.36582	0.36570	0.36549	0.36516	0.36467	0.36400	0.36309	0.36193	0.36048	0.35872
	0.8	0.48780	0.48765	0.48717	0.48638	0.48524	0.48375	0.48190	0.47968	0.47709	0.47412	0.47077
	1.0	0.60976	0.60942	0.60843	0.60679	0.60452	0.60163	0.59817	0.59417	0.58966	0.58470	0.57931
	1.2	0.73171	0.73115	0.72948	0.72675	0.72301	0.71834	0.71284	0.70662	0.69976	0.69238	0.68455
	1.4	0.85366	0.85282	0.85032	0.84624	0.84072	0.83390	0.82597	0.81711	0.80750	0.79730	0.78666
	1.6	0.97561	0.97443	0.97094	0.96528	0.95766	0.94834	0.93759	0.92572	0.91299	0.89963	0.88585
	1.8	1.09756	1.09599	1.09136	1.08388	1.07385	1.06168	1.04777	1.03254	1.01635	0.99952	0.98232
	2.0	1.21951	1.21750	1.21157	1.20202	1.18931	1.17397	1.15656	1.13764	1.11769	1.09712	1.07626
1.0	0.2	0.10000	0.10006	0.10025	0.10056	0.10098	0.10149	0.10208	0.10271	0.10337	0.10402	0.10463
	0.4	0.20000	0.20009	0.20035	0.20078	0.20135	0.20204	0.20281	0.20362	0.20443	0.20519	0.20585
	0.6	0.30000	0.30008	0.30030	0.30066	0.30112	0.30166	0.30223	0.30278	0.30327	0.30364	0.30387
	0.8	0.40000	0.40003	0.40010	0.40020	0.40030	0.40037	0.40037	0.40025	0.39999	0.39953	0.39886
	1.0	0.50000	0.49994	0.49974	0.49940	0.49889	0.49819	0.49727	0.49611	0.49469	0.49300	0.49102
	1.2	0.60000	0.59981	0.59923	0.59827	0.59691	0.59515	0.59299	0.59043	0.58749	0.58418	0.58054
	1.4	0.70000	0.69964	0.69858	0.69681	0.69436	0.69126	0.68754	0.68326	0.67847	0.67323	0.66760
	1.6	0.80000	0.79944	0.79777	0.79502	0.79126	0.78654	0.78098	0.77467	0.76773	0.76028	0.75239
	1.8	0.90000	0.89920	0.89681	0.89291	0.88760	0.88103	0.87334	0.86473	0.85537	0.84543	0.83507
	2.0	1.00000	0.99892	0.99571	0.99048	0.98342	0.97473	0.96467	0.95350	0.94148	0.92884	0.91578

$\frac{1}{2}\nu/\Delta\nu_{\frac{1}{2}}$	$s/\Delta\nu_{\frac{1}{2}}$ a_{max}	0.00	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50
1.2	0.2	0.08197	0.08203	0.08222	0.08253	0.08297	0.08351	0.08416	0.08490	0.08572	0.08658	0.08748
	0.4	0.16393	0.16404	0.16434	0.16484	0.16553	0.16639	0.16740	0.16854	0.16977	0.17106	0.17238
	0.6	0.24590	0.24602	0.24636	0.24693	0.24769	0.24864	0.24973	0.25095	0.25223	0.25355	0.25486
	0.8	0.32787	0.32797	0.32828	0.32879	0.32946	0.33027	0.33119	0.33218	0.33319	0.33417	0.33508
	1.0	0.40984	0.40990	0.41011	0.41042	0.41083	0.41131	0.41180	0.41228	0.41269	0.41301	0.41319
	1.2	0.49180	0.49181	0.49183	0.49184	0.49182	0.49175	0.49158	0.49129	0.49083	0.49019	0.48934
	1.4	0.57377	0.57369	0.57345	0.57304	0.57244	0.57162	0.57056	0.56925	0.56766	0.56580	0.56366
	1.6	0.65574	0.65555	0.65498	0.65402	0.65267	0.65092	0.64876	0.64620	0.64326	0.63994	0.63629
	1.8	0.73770	0.73738	0.73641	0.73479	0.73254	0.72967	0.72621	0.72220	0.71767	0.71270	0.70733
	2.0	0.81967	0.81919	0.81774	0.81535	0.81205	0.80789	0.80294	0.79727	0.79098	0.78415	0.77690
1.4	0.2	0.06757	0.06762	0.06779	0.06806	0.06844	0.06893	0.06952	0.07021	0.07099	0.07185	0.07278
	0.4	0.13514	0.13523	0.13551	0.13598	0.13662	0.13745	0.13844	0.13958	0.14086	0.14226	0.14375
	0.6	0.20270	0.20282	0.20317	0.20375	0.20455	0.20556	0.20676	0.20814	0.20967	0.21131	0.21304
	0.8	0.27027	0.27040	0.27077	0.27138	0.27223	0.27328	0.27452	0.27592	0.27745	0.27907	0.28075
	1.0	0.33784	0.33796	0.33830	0.33887	0.33965	0.34061	0.34172	0.34295	0.34426	0.34561	0.34697
	1.2	0.40541	0.40550	0.40578	0.40623	0.40683	0.40755	0.40837	0.40924	0.41013	0.41099	0.41179
	1.4	0.47297	0.47303	0.47319	0.47344	0.47376	0.47412	0.47449	0.47483	0.47510	0.47527	0.47531
	1.6	0.54054	0.54054	0.54053	0.54051	0.54045	0.54032	0.54009	0.53973	0.53921	0.53850	0.53760
	1.8	0.60811	0.60804	0.60782	0.60745	0.60690	0.60615	0.60518	0.60397	0.60250	0.60075	0.59874
	2.0	0.67568	0.67552	0.67505	0.67425	0.67311	0.67163	0.66979	0.66758	0.66500	0.66207	0.65881
1.6	0.2	0.05618	0.05622	0.05636	0.05658	0.05689	0.05729	0.05779	0.05837	0.05903	0.05979	0.06062
	0.4	0.11236	0.11244	0.11268	0.11307	0.11362	0.11433	0.11519	0.11620	0.11735	0.11864	0.12006
	0.6	0.16854	0.16864	0.16895	0.16947	0.17019	0.17111	0.17222	0.17351	0.17498	0.17660	0.17835
	0.8	0.22472	0.22484	0.22519	0.22578	0.22660	0.22764	0.22888	0.23032	0.23193	0.23370	0.23559
	1.0	0.28090	0.28102	0.28139	0.28201	0.28285	0.28392	0.28519	0.28664	0.28825	0.28998	0.29182
	1.2	0.33708	0.33720	0.33756	0.33814	0.33895	0.33995	0.34114	0.34248	0.34394	0.34548	0.34708
	1.4	0.39326	0.39336	0.39368	0.39419	0.39489	0.39575	0.39674	0.39785	0.39902	0.40023	0.40145
	1.6	0.44944	0.44952	0.44976	0.45015	0.45067	0.45130	0.45201	0.45276	0.45353	0.45427	0.45495
	1.8	0.50562	0.50567	0.50580	0.50602	0.50630	0.50662	0.50694	0.50724	0.50747	0.50762	0.50763
	2.0	0.56180	0.56180	0.56181	0.56181	0.56178	0.56171	0.56155	0.56128	0.56088	0.56031	0.55955
1.8	0.2	0.04717	0.04720	0.04731	0.04749	0.04773	0.04805	0.04845	0.04891	0.04946	0.05008	0.05077
	0.4	0.09434	0.09440	0.09460	0.09492	0.09537	0.09594	0.09665	0.09749	0.09846	0.09955	0.10078
	0.6	0.14151	0.14160	0.14186	0.14229	0.14290	0.14367	0.14462	0.14574	0.14702	0.14845	0.15004
	0.8	0.18868	0.18878	0.18909	0.18961	0.19032	0.19124	0.19235	0.19366	0.19514	0.19680	0.19860
	1.0	0.23585	0.23596	0.23630	0.23687	0.23765	0.23865	0.23986	0.24126	0.24285	0.24460	0.24649
	1.2	0.28302	0.28314	0.28349	0.28407	0.28488	0.28591	0.28713	0.28855	0.29014	0.29188	0.29374
	1.4	0.33019	0.33030	0.33065	0.33122	0.33201	0.33300	0.33419	0.33554	0.33704	0.33866	0.34037
	1.6	0.37736	0.37747	0.37779	0.37832	0.37904	0.37995	0.38102	0.38223	0.38355	0.38495	0.38641
	1.8	0.42453	0.42462	0.42490	0.42536	0.42598	0.42674	0.42764	0.42863	0.42969	0.43078	0.43188
	2.0	0.47170	0.47177	0.47199	0.47234	0.47281	0.47339	0.47404	0.47474	0.47546	0.47616	0.47681
2.0	0.2	0.04000	0.04003	0.04011	0.04025	0.04044	0.04069	0.04100	0.04137	0.04180	0.04229	0.04285
	0.4	0.08000	0.08005	0.08020	0.08046	0.08082	0.08128	0.08185	0.08252	0.08331	0.08421	0.08521
	0.6	0.12000	0.12007	0.12028	0.12063	0.12113	0.12176	0.12254	0.12347	0.12453	0.12574	0.12710
	0.8	0.16000	0.16009	0.16034	0.16077	0.16137	0.16215	0.16309	0.16420	0.16548	0.16692	0.16852
	1.0	0.20000	0.20010	0.20039	0.20088	0.20156	0.20243	0.20348	0.20473	0.20615	0.20774	0.20950
	1.2	0.24000	0.24011	0.24042	0.24094	0.24167	0.24261	0.24373	0.24505	0.24655	0.24822	0.25004
	1.4	0.28000	0.28011	0.28044	0.28098	0.28173	0.28268	0.28384	0.28518	0.28669	0.28836	0.29017
	1.6	0.32000	0.32011	0.32043	0.32097	0.32172	0.32266	0.32380	0.32511	0.32657	0.32818	0.32990
	1.8	0.36000	0.36010	0.36042	0.36093	0.36165	0.36255	0.36362	0.36484	0.36621	0.36768	0.36924
	2.0	0.40000	0.40010	0.40039	0.40086	0.40151	0.40233	0.40330	0.40439	0.40559	0.40688	0.40821

CHAPTER VI

NON-LINEARITY : ANALYSIS IN TERMS OF MIXTURES

6.0 Some causes of non-linearity

As has been pointed out at the end of chapter V, circumstances may arise in which proportionality of absorbance and concentration cannot be expected to hold. The example given there for the system naphthalene/2-methylnaphthalene was rather an artificial one in that $s/\Delta\nu_{\frac{1}{2}}$ could be drastically reduced for our instrument, which effectively restored linearity. However, as one enters the part of the infra-red accessible to quartz instruments (up to $\lambda = 2.5\mu$ for the Zeiss PMQ II), considerable departure from linearity becomes the rule rather than the exception.

The reasons for this undesirable effect are several:

a) While absorption bands tend to be narrower in the infra-red than in the visible and ultra-violet regions, detector sensitivity with present instruments is lower at longer wavelengths (cf. Goddu in Reilley 1960:353-4); hence $s/\Delta\nu_{\frac{1}{2}}$ is adversely affected both in its numerator and denominator.

b) Dispersion of most prism materials in common use is lower in the near infra-red than elsewhere in the usable part of the spectrum: a given mechanical slit width therefore corresponds to a larger optical slit width s, again tending to increase the ratio $s/\Delta\nu_{\frac{1}{2}}$. Lowering s is, moreover, impracticable on account of harmful diffraction effects, since these also effectively impair linearity.

c) For many compounds - there are some notable exceptions - molar absorptivities ε are low in the near infra-red, often being of unit order of magnitude. This compares unfavourably with rather usual values for ε as found in the ultra-violet, such as 10^3 to 10^5. As a consequence, concentrations c must be rather high, say of the order of magnitude of 1 mole/litre, for the absorbances to be comfortably observable at the usual cell lengths of one, or at most several, centimetres. (A matched set of eight 1 cm cells was used throughout the work in the near infra-red region.) At such concentrations, an additional complication sets in, because one of the conditions for the validity of Beer's law is being violated. Among others, equality of refractive index of solution and solvent is necessary for the law to hold. If the refractive index n varies with concentration, not ε, but $\frac{\varepsilon n}{(n^2+2)^2}$ may be shown to be theoretically independent of c (Kortüm 1962:23). With the usual solvents for this region (CS_2, C_2Cl_4 and CCl_4), a simple calculation shows that linearity of a with c cannot be expected to hold up to the required, approximately molar concentrations, if, as often must happen, the refractive indices of the analyte and the solvent differ to any appreciable extent.

These three causes combine to make the near infra-red a little-used region for quantitative spectrophotometry. A notable exception is afforded by the estimation of water and hydroxyl groups (e.g. Crisler and Burrill 1959), where, however, non-linear calibration curves are obtained even when using dilute solutions and 10 cm cells.

6.1 The degree of non-linearity

Some exploratory measurements under our experimental conditions confirmed the reality of the expected deviations from linearity. As an example, we cite the absorbances observed in 1 cm cells on a CCl_4 solution of o-xylene and its dilutions. The slit width was the smallest practicable, viz. 0.022 mm; unit relative concentration corresponded to 1.01 mole l^{-1} ≙ 107 g l^{-1}.

Relative concentration	0.2541	0.4725	0.5877	0.7456	0.8879	1.0000
Wavelength/µ	Absorbances					
2.28	0.274	0.512	0.638	0.808	0.960	1.072
2.30	0.286	0.533	0.662	0.838	0.989	1.112
2.32	0.386	0.711	0.881	1.102	1.303	1.456

If the highest relative concentration were accepted as a reference, the relative concentrations of the dilutions would be estimated as follows:

True relative concentration	0.2541	0.4725	0.5877	0.7456	0.8879
Conc. estimated from absorbance at:					
2.28µ	0.2556	0.4776	0.5951	0.7537	0.8955
2.30µ	0.2572	0.4793	0.5953	0.7536	0.8894
2.32µ	0.2651	0.4883	0.6051	0.7569	0.8949

The systematic positive deviation of estimated concentrations is definitely not entirely due to the strong slopes of the absorption bands at the wavelengths selected, since the same effect is found to be present quite strongly at the maximum of ± 2.315μ and even in the ultra-violet at 0.285μ, using a very similar slit width (0.020 mm) and the identical solutions (cf. Fig. 6.0, where the relevant part of the near infra-red spectrum and some (a,c)-curves are shown):

True relative concentration	0.2541	0.4725	0.5877	0.7456	0.8879	1.000
a at 2.315μ	0.442	0.810	1.001	1.254	1.477	1.645
c estimated from 2.315μ	0.2687	0.4924	0.6085	0.7623	0.8979	-
a at 0.285μ	-	0.795	0.986	1.244	1.466	1.653
c estimated from 0.285μ	-	0.4809	0.5965	0.7526	0.8869	-

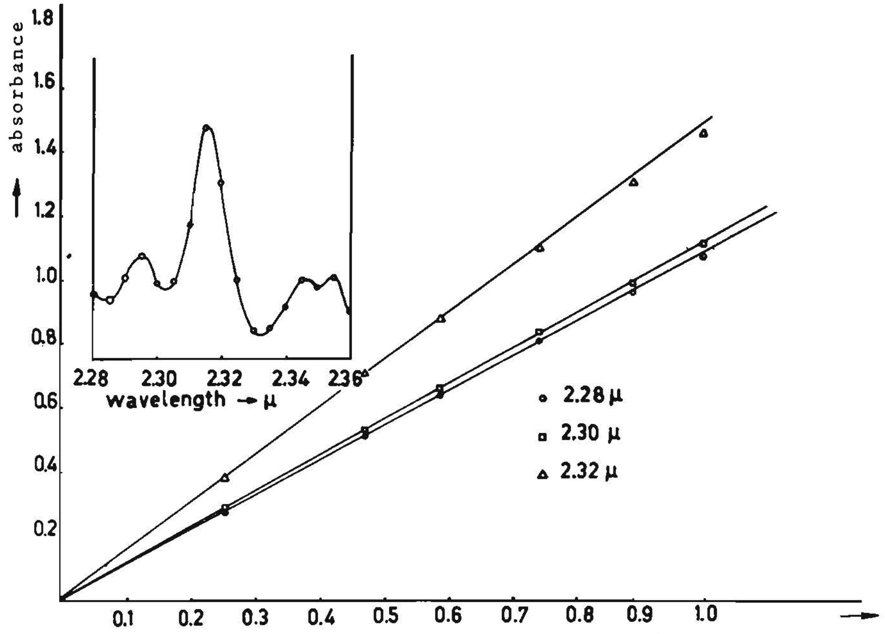

Fig. 6.0 To show the non-linearity in the near infra-red region; data are for a CCl_4 solution of o-xylene.
Inset: Part of the NIR-spectrum.

We suspect that the non-linearity in the ultra-violet may be ascribed, at least in part, to the refractive index effect mentioned under c) above. Most of the results shown in the preceding tables are entirely unacceptable for precision analysis purposes. Since the deviations appear to be quite systematic, no improvement can be gained by subjecting the data to least-squares analysis; such a technique will merely produce some weighted average of systematically deviating estimates, which will itself be systematically deviating from the true result.

6.2 A remedy

It is fortunate, therefore, that a different approach is possible in the near infra-red and in other cases where non-linear (a,c)-relationships engender complications. Physically, this approach may be described as a narrowing of the range in concentrations over which a is assumed to be proportional to c. In other words, the linear interpolation in absorbances is not made to cover the entire range from concentration zero up to the reference concentration, but is restricted to a relatively small part of this range. The methods of experiment and calculation are obvious: if a_1 and a_2 are the absorbances of solutions of concentrations c_1 and c_2 respectively, then a concentration x, $c_1 \leq x \leq c_2$, is estimated from its observed absorbance a_0 by

$$x = c_1 + \frac{a_0 - a_1}{a_2 - a_1} (c_2 - c_1)$$

Viewed alternatively, the concentration is estimated from the absorbances of <u>two</u> reference concentrations and the unknown, it being understood there is still only a single substance to be determined. The reference concentrations c_1 and c_2 correspond formally to 0 and c_{ref} in the more usual case. Indeed, x may be written as a linear combination of c_1 and c_2.

$$x = (1 - \lambda)c_1 + \lambda c_2$$

in which $\lambda = (a_0 - a_1)/(a_2 - a_1)$ varies linearly from 0 to 1 over the range c_1, c_2. (See Fig. 6.1).

That this technique yields some measure of success in cases where the analysis in terms of a single reference will fail, may be read off from the following table, constructed from the same observational data as before.

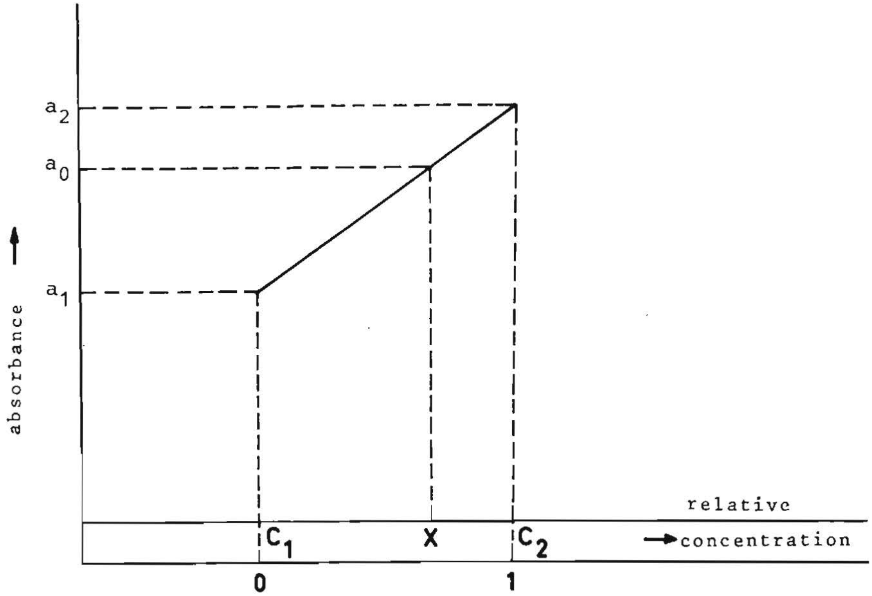

Fig. 6.1 To illustrate linear interpolation between two references in the single-component case.

Relative concentration taken	0.7456			0.5877		
Wavelength/μ	2.28	2.30	2.32	2.28	2.30	2.32
Found in terms of unit reference	0.7537	0.7536	0.7569	0.5951	0.5953	0.6051
Found - Taken	+0.0081	+0.0080	+0.0111	+0.0084	+0.0086	+0.0184
Found in terms of c_1 = 0.5877 c_2 = 0.8879	0.7462	0.7492	0.7447			
Found in terms of c_1 = 0.4725 c_2 = 0.7456				0.5888	0.5888	0.5918
Found - Taken, two references	+0.0006	+0.0036	-0.0009	+0.0011	+0.0011	+0.0041

The distinct improvement is only what might have been expected on physical grounds; narrowing the range of interpolation for x between c_1 and c_2 is equivalent, in the limit as $c_1 \rightarrow c_2$, to concluding to an identity of concentration from an identity of absorbance. In the limiting case, no assumption about the (a,c)-relationship is needed; it need not surprise that the correctness of specific assumptions about this relationship is less relevant to the correctness of the results as the concentrations of the solutions compared become more similar.

In practice,the difference in concentration of the reference solutions c_1 and c_2 definitely need not be very small: for the example shown, a difference of about 0.3 mole l^{-1} could be allowed between reference concentrations; the range 0 to 1 mole l^{-1} would be sufficiently well covered if solutions of 0.25, 0.5,0.75 and 1.0 mole l^{-1} were available for observation of their absorbances along with that of the unknown.

The estimation in terms of more than one reference concentration does not constitute a novel development; it is, in fact, merely a hybrid form between spectrophotometry and colorimetry in Kortüm's classification (Kortüm 1962). However, so far as the author has been able to ascertain, the variation and extension of the technique about to be described has not been reported before. The variation consists in the attempt, well known and widely applied before, to match concentrations of reference and unknown as closely as possible; if the match is close, a single reference will again suffice for a single substance to be estimated with acceptable precision. The extension consists, essentially, in the idea that, if total concentrations match, the interval of interpolation may be narrowed by using mixtures of known composition as references; n mixtures are required if there are n components to be estimated.

As in the case of a single substance determined from two neighbouring reference solutions, the composition of the reference mixtures should bracket that of the unknown. A practical way of accomplishing this bracketing is by making up the reference mixtures (keeping in mind they should have approximately the same total concentration of analytes as the unknown) in such a way that one reference mixture, e.g. the first, contains a higher amount of one component, e.g. the first, than the unknown is expected to contain, while its content of the remaining components should be equal to or somewhat lower than estimated for the unknown. The second, third, ..., n-th reference mixtures should show a slight excess of the second, third, ..., n-th component and a corresponding deficit in at least some of the others.

6.3 An example

To give an example: if the concentration of the unknown is expected to be $\underline{x}$,

$$\begin{cases} x_1 \doteq 0.60 \\ x_2 \doteq 0.30 \\ x_3 \doteq 0.10 \end{cases}$$

in arbitrary units, a possible triplet of mixtures to serve as references is given by the columns of matrix $\underline{C}$ below

$$\underline{C} = \begin{pmatrix} 0.75 & 0.50 & 0.55 \\ 0.25 & 0.50 & 0.25 \\ 0 & 0 & 0.20 \end{pmatrix} = (\underline{c}_1 \ \underline{c}_2 \ \underline{c}_3).$$

That is to say that the first reference mixture, c_1, should have concentrations 0.75, 0.25 and 0 in the first, second and third components respectively, measured in the same units as have been employed to express the components of x. (These units need not, in principle, be identical for the components).

If the reference solutions are thus made to bracket the unknown, one has the property that the unknown, when expressed as a linear combination of the columns of $\underline{C}$, represents such a combination with all its coefficients positive and less than unity. This is tantamount to stating that the composition of the unknown may be found by <u>interpolating</u> among the composition of the references. In fact, for the example given, we may write

$$\underline{x} = \underline{c}_1 y_1 + \underline{c}_2 y_2 + \underline{c}_3 y_3$$

$$= \begin{pmatrix} 0.75 \\ 0.25 \\ 0 \end{pmatrix} 0.30 + \begin{pmatrix} 0.50 \\ 0.50 \\ 0 \end{pmatrix} 0.20 + \begin{pmatrix} 0.55 \\ 0.25 \\ 0.20 \end{pmatrix} 0.50$$

$$= \underline{\underline{C}}\underline{y} \qquad = \underline{C} \begin{pmatrix} 0.30 \\ 0.20 \\ 0.50 \end{pmatrix}$$

$$\underline{y} \quad = \underline{C}^{-1} \ \underline{x} \ = \quad \begin{pmatrix} 0.30 \\ 0.20 \\ 0.50 \end{pmatrix}$$

Interpreting the above, we may say that we consider the unknown $\underline{x}$ to be a mixture consisting of 0.30 of the first reference mixture $\underline{c}_1$, 0.20 of the second reference mixture $\underline{c}_2$ and 0.30 of the third reference mixture $\underline{c}_3$. In practice, solving the relevant system of equations gives $\hat{\underline{y}}$; the operation of premultiplying $\hat{\underline{y}}$ by $\underline{C}$ yields the estimate

$$\hat{\underline{x}} = \underline{C}\hat{\underline{y}}$$

where $\hat{\underline{x}}$ is expressed in terms of the solutions that served to make up the reference mixtures: the elements of $\hat{\underline{x}}$ represent fractions of the concentrations of these primary solutions. In order to convert $\hat{\underline{x}}$ into the more generally useful form of absolute concentrations $\hat{c}$ of the primary components, a further premultiplication of $\hat{x}$ by $\underline{D}$ is necessary; $\underline{D}$ is a diagonal matrix ($d_{ij} = 0$ if $i \neq j$), the elements d_{jj} of which represent the concentrations (on some absolute scale, e.g. mole l^{-1}) of the primary solutions:

$$\hat{\underline{c}} = \underline{D}\hat{\underline{x}} = \underline{D}\underline{C}\hat{\underline{y}} = \underline{F}\hat{\underline{y}},$$

say.

If we compare this to the usual procedure of directly obtaining $\hat{x}$ from observations on the mixture and solutions of the pure, primary components (where the operation performed is

$$\hat{\underline{c}} = \underline{D}\hat{\underline{x}}$$

in order to derive the absolute concentration vector $\hat{\underline{c}}$ from the estimate of the relative concentration vector $\hat{\underline{x}}$), we remark that the difference between

$$\hat{\underline{c}} = \underline{F}\hat{\underline{y}}$$

and

$$\hat{\underline{c}} = \underline{D}\hat{\underline{x}}$$

lies merely in the fact that $\underline{D}$ is diagonal, whereas $\underline{F}$ is not.

6.4 Geometrical illustration of interpolation and linear approximation.

We can give a possible illuminating geometrical illustration for the cases where one and two components are estimated and the (a,c) relationship is non-linear.

One component, simple linear approximation

We refer to Fig. 6.2. The reference concentration C is observed to have an absorbance a_C; the curve ODA is unknown. The arc OD is replaced by the chord OD, which is used for the purpose of estimation: the concentration X, giving an absorbance a_X, will be estimated as $\hat{X}$. This approximation will yield acceptable results only if X is not too far from C.

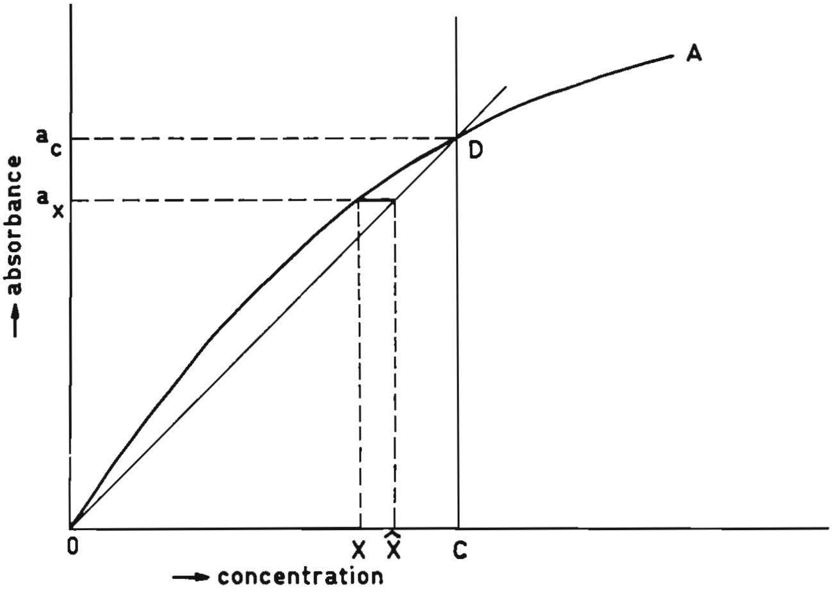

Fig. 6.2 To illustrate linear interpolation with a single reference in the single-component case in the presence of non-linearity.

One component, linear approximation with interpolation

Referring to Fig. 6.3, we demonstrate the alternative procedure, which has been shown to have a beneficial effect on the accuracy in the examples above. We observe the absorbances a_1 and a_2 of the reference concentrations C_1 and C_2. The unknown curve ODA is now approximated by the chord D_1D_2; the chord will not, when produced, pass through the origin O; as against this, the approximation which yields $\hat{X}$ instead of X for an observed absorbance a_X of the unknown, will often be acceptable for $OC_1 \leq OX \leq OC_2$ and even somewhat beyond these boundaries. The systematic deviation within this interval will now be largest at a point roughly halfway between C_1 and C_2.

In general, the second procedure would appear to give the better approximation; also, it does not require the previous knowledge about the concentration to be analyzed to be quite so exact; a rough estimate of OX is sufficient for a reasonable choice of OC_1 and OC_2, that is, of c_1 and c_2.

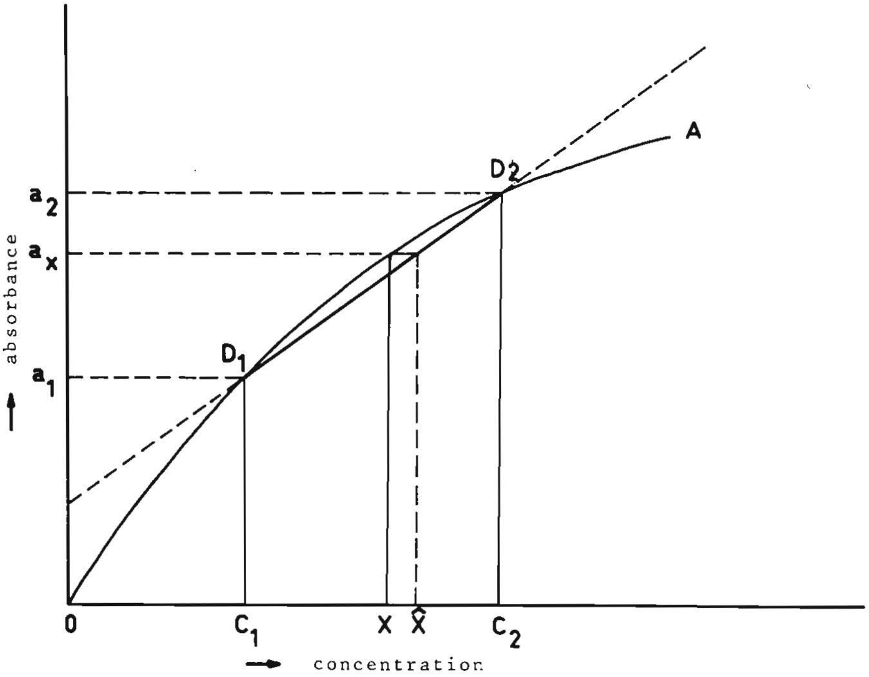

Fig. 6.3 To illustrate linear interpolation with two references in the single-component case in the presence of non-linearity.

Two components, simple linear approximation

For the two-component case, we refer to Fig. 6.4. The simple linear approximation now consists of replacing the arcs QA and OC by the corresponding chords and then applying the familiar graphical procedure, viz. constructing MM'//OA and MM''//OC. The mixture M is then concluded to have the composition

$$\begin{cases} x_1 = OM'/OC \\ x_2 = OM''/OA \end{cases}$$

In other words, the curvilinear net of Fig. 6.4 is being linearized in its entirety, that is, globally, or for

$$\begin{cases} 0 \leq x_1 \leq 1 \\ 0 \leq x_2 \leq 1 \end{cases}$$

This is evidently a poor substitute for what would be the correct procedure: if the curvilinear net shown in the figure

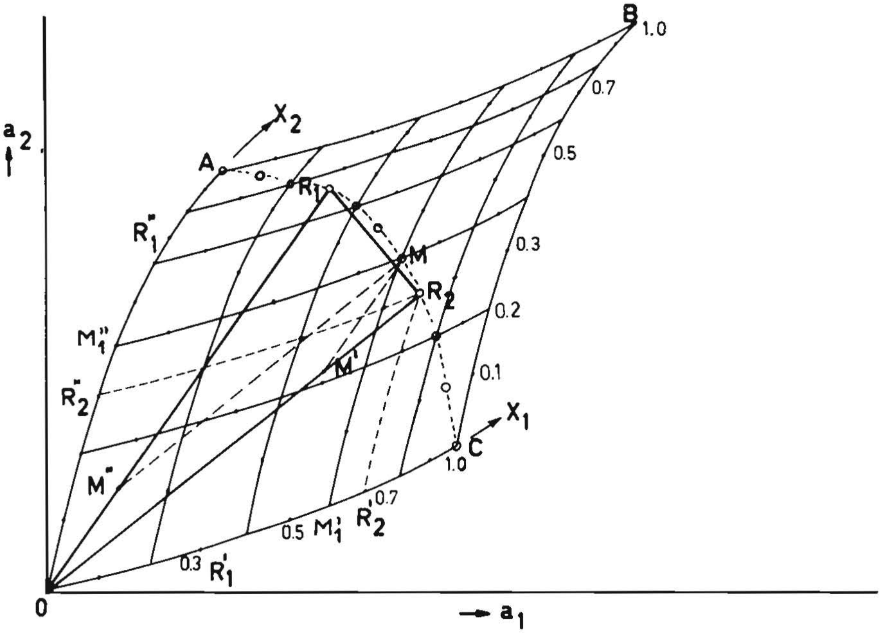

Fig. 6.4 To illustrate linear interpolation in a restricted range, using the mixtures R_1 and R_2 as references, when both components show non-linearity.

could be constructed (for a given pair of analytical wavelengths), one should locate the point M by the absorbance a_{10} and a_{20} of the mixture in the rectangular coordinates (axes Oa_1, Oa_2) and then curvilinearly read off its composition x_1, x_2 by following the curves MM_1' and $MM'{}_1'$ to the points (M_1' and $M'{}_1'$) where these meet the curved coordinate axes (arcs OA and OC), assuming these to have been suitably provided with a scale.

Now the net spanned by the arcs OA and OC is not only curvilinear, but also suffers from the additional disadvantage that its mesh size along OA and OC is variable. The error committed by regarding OA and OC as straight lines and by assuming the mesh size to be constant along each of these axes may therefore be quite considerable.

Two components, linear interpolation in a restricted range

In order to avoid the drastic measure of the above global linearization, we now introduce the reference mixtures R_1 and R_2, bracketing M and having approximately the same total relative concentration (Fig. 6.4; a curve $x_1 + x_2 = 1$ is shown as the arc AR_1MR_2C). We then consider the straight lines OR_1 and OR_2 as defining an oblique rectilinear net, with mesh size constant along each axis. The analytical result is now obtained in the familiar way again, that is, by oblique projection, drawing $MM'//OR_1$ and $MM''//OR_2$. The composition of M is expressed (for M not too far from the line R_1R_2), by local linearization, in terms of the fractions OM''/OR_1 and OM'/OR_2 of the compositions of the reference solutions R_1 and R_2. These latter, in turn, are known correctly in terms of x_1 and x_2.

The further requirements, viz. the approximately constant $x_1 + x_2$ for R_1, R_2 and M and the bracketing requirement:

$$\begin{cases} x_1(R_1) \le x_1(M) \le x_1(R_2) \\ x_2(R_2) \le x_2(M) \le x_2(R_2) \end{cases}$$

are seen to define the extent of local linearization, viz.

$$\begin{cases} R_1' \le x_1 \le R_2' \\ R_1'' \le x_2 \le R_2'' , \end{cases}$$

thus further clarifying the analogy to the linear approximation for a single component with interpolation in a restricted range as treated above.

Since this linearization is equivalent to considering the arc R_1MR_2 as a straight line, one concludes that the procedure will give maximal deviations from the true composition at points M about halfway between R_1 and R_2, again as in the analogous one-component case. The same type of bias of the composition found is also observed experimentally in the case of bias due to too large a slit width.

6.5 Least squares technique using mixtures as references

Using mixtures as references is a procedure which combines very well with a least squares treatment of the observations; the combination of methods is not necessarily confined to the NIR, but may be used to advantage whenever there are significant departures from linearity of the (a,c) relation.

As an example, we cite analyses of two-component mixtures of naphthalene and 2-methylnaphthalene in the ultraviolet, $\lambda = 300(1)$ 325 nm, using a slit width ($s = 0.15$ mm) that was shown before to cause considerable departure from linearity (cf. the results of analyses nos. 240.07 to 240.09, Sec. 5.5).

In all, five solutions of the components in 96% ethanol were employed: the naphthalene reference had a concentration of 5.26 millimole/l, the reference solution for 2-methylnaphthalene was

2.82 millimole/l; the three mixtures of these reference solutions are shown as columns 2,3 and 4 in the matrix $\underline{C}$ below:

$$\underline{C} = \begin{array}{cl} \begin{pmatrix} 1 & 0 & 0.4046 & 0.5025 & 0.5992 \\ 0 & 1 & 0.5954 & 0.4975 & 0.4008 \end{pmatrix} & \begin{array}{l} \text{naphthalene} \\ \text{2-methylnaphthalene} \end{array} \end{array}$$

The complete observational data for this system are given in Table 6.1.

Table 6.1

Absorbances × 1000 as observed in the system of section 6.5 (Naphthalene ($\underline{c}_1$), 2-methylnaphthalene ($\underline{c}_2$) and some mixtures ($\underline{c}_3$, $\underline{c}_4$ and $\underline{c}_5$)).

Wavelength λ/nm	Absorbances × 1000				
	$\underline{c}_1$	$\underline{c}_2$	$\underline{c}_3$	$\underline{c}_4$	$\underline{c}_5$
300	1446	975	1164	1210	1256
301	1520	900	1153	1213	1273
302	1315	871	1057	1101	1142
303	1153	966	1042	1060	1078
304	1133	1235	1201	1191	1180
305	1085	1382	1262	1233	1204
306	1020	1159	1103	1089	1075
307	836	919	882	876	865
308	675	800	749	736	722
309	695	770	745	738	728
310	1031	785	898	920	943
311	1161	849	986	1015	1044
312	768	818	803	798	791
313	478	696	608	584	563
314	383	616	520	496	473
315	343	511	442	424	407
316	252	446	368	348	328
317	147	595	433	390	346
318	95	1064	702	612	516
319	93	1362	857	732	610
320	97	920	613	532	452
321	76	520	350	307	263
322	48	275	184	162	137
323	28	160	104	91	77
324	19	116	74	64	53
325	13	82	52	45	37

An attempt to express the mixtures $\underline{c}_3$, $\underline{c}_4$ and $\underline{c}_5$ in terms of the pure references $\underline{c}_1$ and $\underline{c}_2$ fails completely under the conditions chosen, as shown by the following results.

The concentrations found, with their deviations from the known true compositions, were (Model II):
($\hat{\underline{\sigma}}(\hat{\underline{x}}_j)$ in the following represents the vector of estimated standard deviations of the estimate $\hat{\underline{x}}_j$).

$\underline{c}_3$ in terms of $\underline{c}_1$, $\underline{c}_2$:

$\hat{\underline{x}}_3 = \begin{pmatrix}0.3953\\0.6104\end{pmatrix}$ with $\hat{\underline{\sigma}}(\hat{\underline{x}}_3) = \begin{pmatrix}0.0037\\0.0054\end{pmatrix}$ and $\hat{s} = 7.81 \times 10^{-3}$, differing from the true composition $\underline{c}_3$ by $\Delta\underline{x}_3 = \hat{\underline{x}}_3 - \underline{c}_3 = \begin{pmatrix}-0.0093\\+0.0150\end{pmatrix}$

Similarly, for $\underline{c}_4$ in terms of $\underline{c}_1$ and $\underline{c}_2$:

$\hat{\underline{x}}_4 = \begin{pmatrix}0.4921\\0.5139\end{pmatrix}$; $\hat{\underline{\sigma}}(\hat{\underline{x}}_4) = \begin{pmatrix}0.0040\\0.0057\end{pmatrix}$; $\hat{s} = 8.33 \times 10^{-3}$; $\Delta\underline{x}_4 = \begin{pmatrix}-0.0104\\+0.0165\end{pmatrix}$

and for $\underline{c}_5$ in terms of $\underline{c}_1$ and $\underline{c}_2$:

$\hat{\underline{x}}_5 = \begin{pmatrix}0.5889\\0.4182\end{pmatrix}$; $\hat{\underline{\sigma}}(\hat{\underline{x}}_5) = \begin{pmatrix}0.0039\\0.0056\end{pmatrix}$; $\hat{s} = 8.16 \times 10^{-3}$; $\Delta\underline{x}_5 = \begin{pmatrix}-0.0103\\+0.0174\end{pmatrix}$

The failure of the above analyses is quite apparent:

a) the deviations $\Delta\underline{x}_j$ are a decimal order of magnitude larger than what might have been expected from the excellent distinguishability of the spectra and the results obtained for the quite comparable analyses nos. 240.01 to 240.03 at the smaller slit width of 0.022 mm (Sec. 5.5).

b) in each case the elements of $\Delta\underline{x}$ are large when compared with the corresponding elements of the estimated standard deviations, the extreme ratios being

$$\text{minimally} \quad 0.0093/0.0037 \doteq 2.3$$
$$\text{maximally} \quad 0.0174/0.0056 \doteq 3.1$$

Since, under the usual assumptions, these ratios are distributed as t (there are 26 - 3 = 23 degrees of freedom), we may consult the tables for t, which give

$$P\ (t \geq 2.069) = 0.05$$
$$P\ (t \geq 2.500) = 0.02$$
$$P\ (t \geq 2.807) = 0.01$$

We should therefore conclude to a rejection of the hypotheses $(\hat{\underline{x}}_j)_i = (\underline{c}_j)_i$, $j = 3,4,5$, $i = 1,2$ with most of the usual levels α.

c) the standard deviations of the residuals are excessive in each case. From the average value of ε, say 1.2×10^{-3}, we expect $\hat{s}$ to be $(1 + \hat{\underline{x}}^{\tau}\hat{\underline{x}})^{\frac{1}{2}}\varepsilon$ or about 1.5×10^{-3}; values five times as large have a probability of being exceeded of the order of magnitude of 5×10^{-7}. The residuals, when plotted, of course strongly show the slit width bias patterns described in Sec. 5.5.

d) a multivariate test of the hypotheses $\hat{\underline{x}}_j = \underline{c}_j$ likewise fails completely. The calculated F-ratios

$$F_j = \frac{(\underline{x}_j - \underline{c}_j)^{\tau}\ \underline{\Sigma}^{-1}\ (\hat{\underline{x}}_j - \underline{c}_j)}{r_j^{\tau} r_j} \cdot \frac{(k - n)}{n - 1}$$

($k = 26$, $n = 3$, $\underline{\Sigma}^{-1}$ derived from $(\underline{A}^{\tau}\underline{A})^{-1}$ by striking out the border consisting of column sums etc. and inverting the remaining $(n-1) \times (n-1)$ matrix) were 4.47, 4.78 and 5.33($j = 3,4,5$). These values would cause the hypotheses $\hat{\underline{x}}_j = \underline{c}_j$ to be rejected at the 5% level ($P(F(2,23) \geq 3.42) = 0.05$), though the hypotheses are acceptable at the 1% level. ($P(F(2,23) \geq 5.66) = 0.01$).

It should be noted that both the F and t tests give very wide confidence intervals for the $\underline{x}_j$ and their elements respectively, the size of these intervals being large by reason of the excessive values of $\underline{r}^{\tau}\underline{r}$. In spite of being therefore much wider than usual, they fail to include the $\underline{c}_j$ (or their elements),which again stresses the magnitude of the deviations $\Delta\underline{x}_j$.

However, regarding $\underline{c}_4$ as the mixture to be analyzed and $\underline{c}_3$ and $\underline{c}_5$ as the references, the results are much improved:

One has the expected composition $\underline{y}_4$ in terms of $\underline{c}_3$, $\underline{c}_5$:

$$\underline{y}_4 = \underline{C}^{-1}\underline{c}_4 = (\underline{c}_3\ \underline{c}_5)^{-1}\ \underline{c}_4 = \begin{pmatrix} 0.4046 & 0.5992 \\ 0.5954 & 0.4008 \end{pmatrix}^{-1} \begin{pmatrix} 0.5025 \\ 0.4975 \end{pmatrix}$$

$$\underline{y}_4 = \begin{pmatrix} 0.4969 \\ 0.5031 \end{pmatrix},$$

whereas in fact we find $\hat{\underline{y}}_4 = \begin{pmatrix} 0.4988 \\ 0.5009 \end{pmatrix}$, $\hat{\underline{\sigma}}(\hat{\underline{y}}_4) = \begin{pmatrix} 0.0030 \\ 0.0027 \end{pmatrix}$

$$\Delta\underline{y}_4 = \hat{\underline{y}}_4 - \underline{y}_4 = \begin{pmatrix} +0.0019 \\ -0.0022 \end{pmatrix}$$

The value found in terms of $\underline{c}_3$, $\underline{c}_5$ is, in terms of $\underline{c}_1$, $\underline{c}_2$:

$$\hat{\underline{x}}_4 = \underline{C}\hat{\underline{y}}_4 = \begin{pmatrix} 0.4046 & 0.5992 \\ 0.5954 & 0.4008 \end{pmatrix} \begin{pmatrix} 0.4988 \\ 0.5008 \end{pmatrix} = \begin{pmatrix} 0.5019 \\ 0.4978 \end{pmatrix};$$

$$\Delta\underline{x}_4 = \underline{C}\hat{\underline{y}}_4 - \underline{c}_4 = \begin{pmatrix} -0.0006 \\ +0.0003 \end{pmatrix}$$

The value of $\hat{s}$ is 1.07×10^{-3}, whereas the test of the hypothesis $\hat{\underline{y}}_4 = \underline{y}_4$ leads to the entirely satisfactory F-ratio of 0.52. The improvement over the analysis in terms of $\underline{c}_1$, $\underline{c}_2$ is striking.

Analyses of comparable accuracy are obtained even when one analyses $\underline{c}_3$ in terms of $\underline{c}_4$ and $\underline{c}_5$ or $\underline{c}_5$ in terms of $\underline{c}_3$ and $\underline{c}_4$. Analysing $\underline{c}_3$ in terms of $\underline{c}_4$, $\underline{c}_5$ (<u>extra</u>polating), one finds:

$$\underline{y}_3 = \underline{C}^{-1}\ \underline{c}_3 = (\underline{c}_4\ \underline{c}_5)^{-1}\ \ \underline{c}_3 = \begin{pmatrix} 2.0124 \\ -1.0124 \end{pmatrix}$$

$$\hat{\underline{y}}_3 = \begin{pmatrix} 2.0030 \\ -1.0026 \end{pmatrix}\ ;\ \ \hat{\underline{\sigma}}\ (\hat{\underline{y}}_3) = \begin{pmatrix} 0.0121 \\ 0.0116 \end{pmatrix}\ ;\ \ \Delta\underline{y}_3 = \begin{pmatrix} -0.0094 \\ +0.0102 \end{pmatrix}$$

$$\Delta\underline{x}_3 = (\underline{c}_4\ \underline{c}_5)\ \ \Delta\underline{y}_3 = \begin{pmatrix} +0.0014 \\ -0.0006 \end{pmatrix}$$

F-ratio for the hypothesis $\underline{y}_3 = \hat{\underline{y}}_3$: 0.61 ; $\hat{s} = 2.15 \times 10^{-3}$

Likewise analysing $\underline{c}_5$ in terms of $\underline{c}_3$, $\underline{c}_4$, one finds:

$$\underline{y}_5 = \underline{C}^{-1}\underline{c}_5 = (\underline{c}_3\underline{c}_4)^{-1}\underline{c}_5 = \begin{pmatrix} -0.9877 \\ +1.9877 \end{pmatrix}$$

$$\hat{\underline{y}}_5 = \begin{pmatrix} -0.9944 \\ +1.9949 \end{pmatrix}\ ;\ \ \hat{\underline{\sigma}}\ (\hat{\underline{y}}_5) = \begin{pmatrix} 0.0115 \\ 0.0110 \end{pmatrix}\ ;\ \ \Delta\underline{y}_5 = \begin{pmatrix} -0.0067 \\ +0.0072 \end{pmatrix}$$

$$\Delta\underline{x}_5 = (\underline{c}_3\ \underline{c}_4)\ \Delta\underline{y}_5 = \begin{pmatrix} +0.0009 \\ -0.0004 \end{pmatrix}$$

F-ratio for the hypothesis $\hat{\underline{y}}_5 = \underline{y}_5$: 0.42 ; $\hat{s} = 2.14 \times 10^{-3}$

From these results, it would appear that some extrapolation from the references to the unknown is permissible, that is, that bracketing is not strictly necessary. In the light of the examples to follow, one does well, however, to remember that the situation here is exceptionally favourable, the total span $|\underline{s}| = |\underline{c}_3 - \underline{c}_5|$ being just $\pm\begin{pmatrix} 0.2 \\ 0.2 \end{pmatrix}$; as the span increases, the extrapolation becomes less advisable.

It should be noted that the three estimates of s above are strictly dependent, all three being based on the same set of three absorbance vectors; in fact, since $\hat{s}^2 = \hat{\underline{\xi}}^\tau\hat{\underline{\xi}}\ .\hat{\varepsilon}^2$, we expect,

$$\frac{\hat{s}^2(\underline{y}_3)}{\hat{s}^2(\underline{y}_5)} = \frac{1 + 0.4988^2 + 0.5009^2}{1 + 2.0030^2 + 1.0026^2} \doteq \frac{1}{4.01}\text{, whereas we find}$$

$$\frac{1.07^2}{2.15^2} \doteq \frac{1}{4.04}\text{, in excellent agreement with expectation.}$$

The estimate $\hat{\varepsilon}$ for the standard deviation of a single absorbance for this experiment is

$$\hat{\varepsilon} = (\hat{s}^2/\hat{\underline{\xi}}^\tau\hat{\underline{\xi}})^{\frac{1}{2}} \doteq 10^{-3} \times (1.14/1.50)^2 \doteq 0.0009$$

in absorbance.

6.6 Some further examples

In the near infra-red, several sets of two-component analyses were successful: in the examples that follow, the region 2.20 - 2.45μ was chosen as the basis for the analyses because it was desired to show the applicability of the method of using mixtures as references. The examples lack somewhat in realism because it would be possible to obtain better results with less effort in the ultra-violet for the components cited.

Example 1.

System: ortho-xylene/meta-xylene

Solvent: CCl_4

Stock solutions: ortho-xylene, 0.89 mole/l (A)
para -xylene, 0.89 mole/l (B)

Observations: 25 wavelenghts, 2.25 (0.005) 2.37μ

Reference and analyte solutions:

$$\underline{c} = \begin{pmatrix} 0.2525 & 0.3192 & 0.7436 & 0.8752 \\ 0.7475 & 0.6008 & 0.2564 & 0.1248 \end{pmatrix} \begin{matrix} (A) \\ (B) \end{matrix} = (\underline{c}_1\ \underline{c}_2\ \underline{c}_3\ \underline{c}_4)$$

Of these, the mixtures $\underline{c}_2$ and $\underline{c}_3$ were expressed in terms of $\underline{c}_1$ and $\underline{c}_4$, the span being thus about $\begin{pmatrix} 0.65 \\ 0.65 \end{pmatrix}$; the maximal absorbances observed just touched 1.5. The spectra of $\underline{c}_1$ and $\underline{c}_4$ are shown in Fig. 6.5.

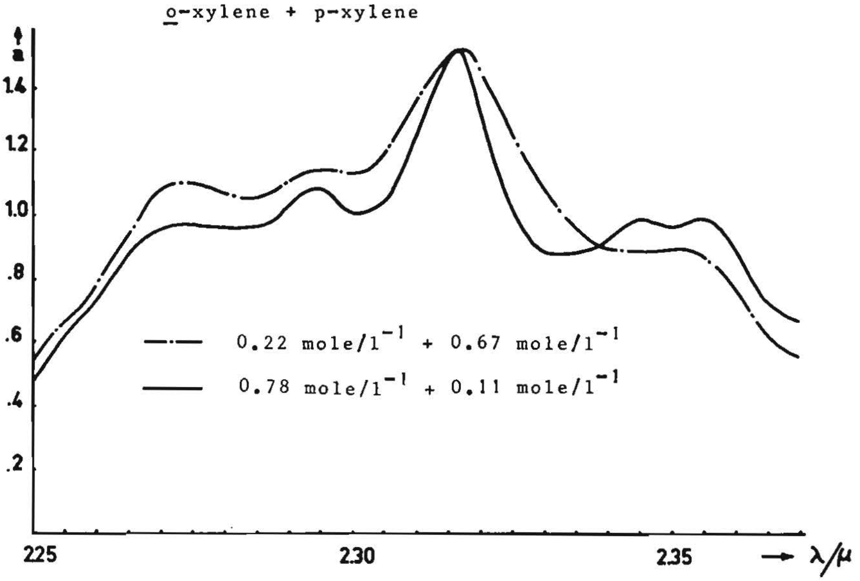

Fig. 6.5 Mixture reference spectra for Example 1, Sec. 6.6

The results were (after the rejection of an obviously outlying observation), by Model II:

A) $\underline{c}_2$ in terms of $\underline{c}_1$ and $\underline{c}_4$

$$\underline{y}_2 = (\underline{c}_1\ \underline{c}_4)^{-1}\ \underline{c}_2 = \begin{pmatrix}0.8928\\0.1071\end{pmatrix}$$

$$\hat{\underline{y}}_2 = \begin{pmatrix}0.8873\\0.1109\end{pmatrix}\ ;\quad \hat{\underline{\sigma}}(\hat{\underline{y}}_2) = \begin{pmatrix}0.0027\\0.0033\end{pmatrix}\ ;\quad \Delta\underline{y}_2 = \begin{pmatrix}-0.0056\\+0.0038\end{pmatrix}$$

$$\Delta\underline{x}_2 = \hat{\underline{x}}_2 - \underline{c}_2 = \underline{C}\Delta\underline{y}_2 = \begin{pmatrix}+0.0020\\-0.0037\end{pmatrix}$$

F-ratio for the hypothesis $\underline{y}_2 = \hat{\underline{y}}_2$: 3.92

$\hat{s} = 1.30 \times 10^{-3}$, corresponding to $\hat{\varepsilon} = 0.97 \times 10^{-3}$

B) $\underline{c}_3$ in terms of $\underline{c}_1$ and $\underline{c}_4$

$$\underline{y}_3 = (\underline{c}_1\ \underline{c}_4)^{-1}\ \underline{c}_3 = \begin{pmatrix}0.2113\\0.7887\end{pmatrix}$$

$$\hat{\underline{y}}_3 = \begin{pmatrix}0.2135\\0.7883\end{pmatrix}\ ;\quad \hat{\underline{\sigma}}(\hat{\underline{y}}_3) = \begin{pmatrix}0.0022\\0.0027\end{pmatrix}\ ;\quad \Delta\underline{y}_3 = \begin{pmatrix}+0.0022\\-0.0004\end{pmatrix}$$

$$\Delta\underline{x}_3 = \hat{\underline{x}}_3 - \underline{c}_3 = \underline{C}\Delta\underline{y}_3 = \begin{pmatrix}+0.0002\\+0.0016\end{pmatrix}$$

F-ratio for the hypothesis $\underline{y}_3 = \hat{\underline{y}}_3$: 2.46

$\hat{s} = 1.06 \times 10^{-3}$, corresponding to $\hat{\varepsilon} = 0.82 \times 10^{-3}$

Although this example is satisfactory chemically, in case A the hypothesis $\underline{x}_2 = \hat{\underline{x}}_2$ (equivalent to $\underline{y}_2 = \hat{\underline{y}}_2$) would be rejected at the 5% level: for 2 and 21 degrees of freedom, the 5 and 1% levels of F are 3.47 and 5.48 respectively.

Example 2.

System: ethylbenzene/*n*-propylbenzene

Solvent: CCl_4

Stock solutions: ethylbenzene, 1.01 mole/l (A)

n-propylbenzene, 1.03 mole/l (B)

Observations: 21 wavelengths, 2.22(0.01)2.42µ

References and sample solutions:

$$\underline{C} = \begin{array}{l} \begin{pmatrix}0.1993 & 0.2987 & 0.3997 & 0.6057 & 0.7064 & 0.8007\\0.8007 & 0.7013 & 0.6003 & 0.3943 & 0.2936 & 0.1993\end{pmatrix} \end{array} \begin{array}{l}(A)\\(B)\end{array}$$

$$= (\underline{c}_1\ \underline{c}_2\ \underline{c}_3\ \underline{c}_4\ \underline{c}_5\ \underline{c}_6)$$

Of these, $\underline{c}_2$ to $\underline{c}_5$ were analysed in terms of their nearest neighbours ($\underline{c}_2$ in terms of $\underline{c}_1$ and $\underline{c}_3$ etc.), the maximal span thus being about $\begin{pmatrix}0.3\\0.3\end{pmatrix}$; the maximal absorbances observed were just below 1.5. The spectra of $\underline{c}_1$ and $\underline{c}_6$ are shown in Fig. 6.6.

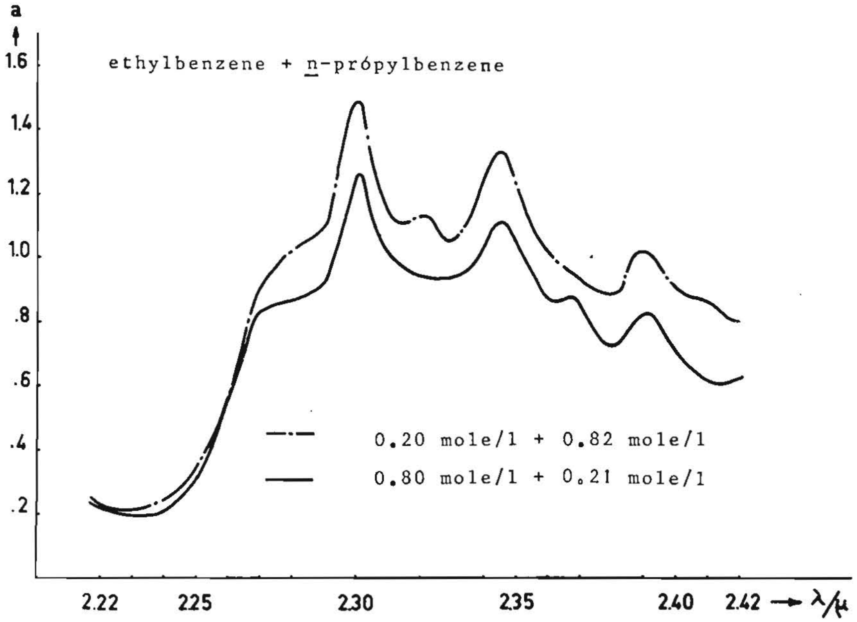

Fig. 6.6 Mixture reference spectra for Example 2, Sec. 6.6

The results of these analyses, expressed in terms of (A) and (B), are as follows:

$$\hat{\underline{X}} = (\hat{\underline{x}}_2\ \hat{\underline{x}}_3\ \hat{\underline{x}}_4\ \hat{\underline{x}}_5) = \begin{vmatrix} 0.2926 & 0.4014 & 0.6064 & 0.7059 \\ 0.7046 & 0.5999 & 0.3924 & 0.2568 \end{vmatrix}$$

$$\Delta\underline{X} = (\Delta\underline{x}_2\Delta\underline{x}_3\Delta\underline{x}_4\Delta\underline{x}_5) = (\hat{\underline{x}}_2 - \underline{c}_2\ \cdots\ ) = \begin{vmatrix} -59 & +17 & +7 & -5 \\ +33 & -4 & -19 & +12 \end{vmatrix} \times 10^{-4}.$$

The standard deviation of the residual vector was

$$(1.63\quad 1.75\quad 1.60\quad 1.01) \times 10^{-3}$$

for the respective analyses, while the test of the hypotheses

$\hat{\underline{y}}_j = \underline{y}_j$, $j = 2,3,4,5$, $\underline{y}_j = (\underline{c}_{j-1}\underline{c}_{j+1})^{-1}$

yielded the F-ratios

$$(1.17\quad 1.63\quad 1.16\quad 1.09)$$

for 2 and 18 degrees of freedom respectively.

This example is therefore completely satisfactory, both from the point of view of the practical chemist and from that of the statistician.

Example 3.

System: n-propylbenzene/i-propylbenzene

Solvent: CCl_4

Stock solutions: n-propylbenzene, 1.03 mole/l (A)
i-propylbenzene, 1.03 mole/l (B)

Observations: 21 wavelengths, 2.22(0.01)2.42μ

References and sample solutions:

$$\underline{c} = \begin{pmatrix} 0.1989 & 0.2966 & 0.3964 & 0.4989 & 0.6030 & 0.7027 & 0.8031 \\ 0.8011 & 0.7034 & 0.6036 & 0.5011 & 0.3970 & 0.2973 & 0.1969 \end{pmatrix} \begin{matrix} (A) \\ (B) \end{matrix}$$

$$= (\underline{c}_1\ \underline{c}_2\ \underline{c}_3\ \underline{c}_4\ \underline{c}_5\ \underline{c}_6\ \underline{c}_7)$$

The spectra of $\underline{c}_1$ and $\underline{c}_7$ are shown in Fig. 6.7. A fairly complete table of results obtained is exhibited below, in order to show the dangers of extrapolation. For each solution, two analyses will be given, the first one in terms of near neighbours (that is, interpolating, except for $\underline{c}_1$ and $\underline{c}_7$, where slight

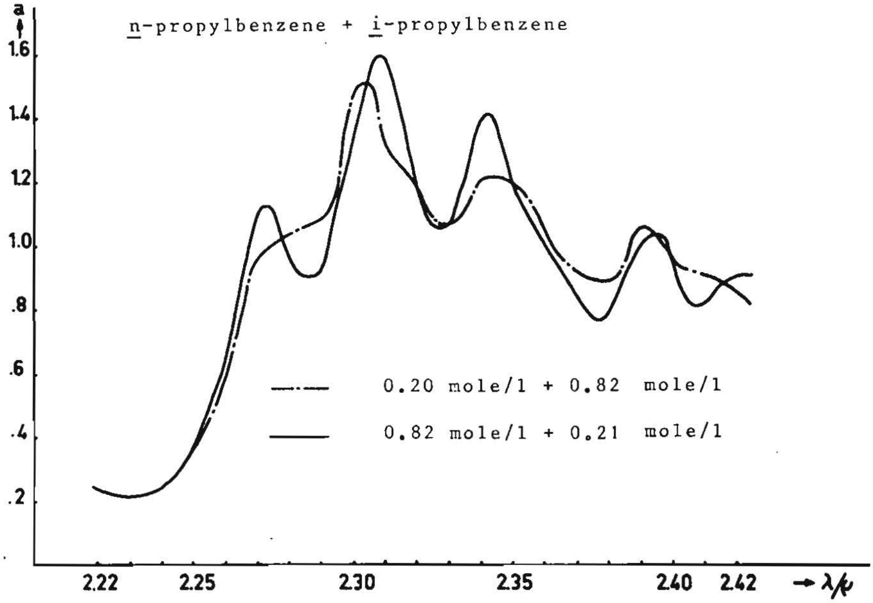

Fig. 6.7 Mixture reference spectra for Example 3, Sec. 6.6

extrapolation is unavoidable), the second one involving an extrapolation.

Sample	References	$\Delta\underline{x}/10^{-4}$	F-ratio	$\hat{\varepsilon}/10^{-3}$
$\underline{c}_1$	$\underline{c}_2, \underline{c}_3$	$\begin{pmatrix} +15 \\ -15 \end{pmatrix}$	0.38	0.7
$\underline{c}_1$	$\underline{c}_4, \underline{c}_7$	$\begin{pmatrix} -244 \\ +166 \end{pmatrix}$	16.1	1.3
$\underline{c}_2$	$\underline{c}_1, \underline{c}_4$	$\begin{pmatrix} -14 \\ +14 \end{pmatrix}$	0.44	0.9
$\underline{c}_2$	$\underline{c}_4, \underline{c}_7$	$\begin{pmatrix} -168 \\ +129 \end{pmatrix}$	38.3	0.7
$\underline{c}_3$	$\underline{c}_1, \underline{c}_4$	$\begin{pmatrix} -18 \\ +13 \end{pmatrix}$	1.86	0.7
$\underline{c}_3$	$\underline{c}_4, \underline{c}_7$	$\begin{pmatrix} -98 \\ +72 \end{pmatrix}$	41.1	0.5
$\underline{c}_5$	$\underline{c}_4, \underline{c}_7$	$\begin{pmatrix} +18 \\ -20 \end{pmatrix}$	0.82	0.9
$\underline{c}_5$	$\underline{c}_1, \underline{c}_4$	$\begin{pmatrix} -59 \\ +37 \end{pmatrix}$	13.6	0.6
$\underline{c}_6$	$\underline{c}_4, \underline{c}_7$	$\begin{pmatrix} +5 \\ -10 \end{pmatrix}$	1.09	0.5
$\underline{c}_6$	$\underline{c}_1, \underline{c}_4$	$\begin{pmatrix} -145 \\ +103 \end{pmatrix}$	21.0	0.9
$\underline{c}_7$	$\underline{c}_5, \underline{c}_6$	$\begin{pmatrix} +4 \\ +3 \end{pmatrix}$	0.72	0.4
$\underline{c}_7$	$\underline{c}_1, \underline{c}_4$	$\begin{pmatrix} -225 \\ +171 \end{pmatrix}$	19.7	1.2

In this table, the entry under F-ratio stands for the quantity obtained when testing the hypothesis $\hat{\underline{y}}_j = \underline{y}_j$, where $\underline{y}_j$, is the expected result $(\underline{c}_{ref1}\underline{c}_{ref2})^{-1}\underline{c}_{sample}$ and $\hat{\underline{y}}_j$ is the actual vector of regression coefficients. It should be compared with F for 2 and 18 degrees of freedom for numerator and denominator respectively. Since the elements of $\hat{\underline{y}}$ are not proper fractions (as is the case when pure compounds, not mixtures are used as references), but may reach about two units in magnitude, $\hat{\varepsilon}$ has been tabulated rather than $\hat{s}$, since the former is independent of the magnitude of the elements of $\hat{\underline{y}}$.

Again, it should be pointed out that the results above are not independent; however, they are most strongly indicative of a very clear trend: results obtained by any but slight extrapolation in the near infra-red are very unsatisfactory, both chemically and statistically. Against this, interpolation in a moderate span yields results that are unexceptionable from either point of view.

Example 4.

System: ethylbenzene/$\underline{i}$-propylbenzene/$\underline{n}$-propylbenzene

Solvent: CCl_4

Stock solutions: ethylbenzene, 1.01 mole/l (A)
$\underline{i}$-propylbenzene, 1.03 mole/l (B)
$\underline{n}$-propylbenzene, 1.03 mole/l (C)

Observations: 21 wavelengths, 2.22(0.01)2.42μ

References and sample solutions:

$$\underline{c} = \left(\begin{array}{ccccccc} 0.6012 & 0.2013 & 0.2011 & 0.3011 & 0.4515 & 0.2011 & 0.7047 \\ 0.1990 & 0.5985 & 0.1991 & 0.3000 & 0.2493 & 0.3991 & 0.1473 \\ 0.1998 & 0.2002 & 0.5998 & 0.3989 & 0.2992 & 0.3998 & 0.1480 \end{array}\right) \begin{array}{c} (A) \\ (B) \\ (C) \end{array}$$

$$= (\underline{c}_1\ \underline{c}_2\ \underline{c}_3\ \underline{c}_4\ \underline{c}_5\ \underline{c}_6\ \underline{c}_7)$$

The spectra of the reference solutions $\underline{c}_1$, $\underline{c}_2$ and $\underline{c}_3$ are shown in Fig. 6.8. The results, summarized in the table below, are typically those of a borderline case: statistically, they do not indicate any significant departure from the known composition yet some of the analyses, especially that of $\underline{c}_6$, are less than gratifying from an analytical chemist's point of view; also, there is no obvious correlation between the F-ratios and the vectors $\Delta\underline{x}$. We conclude that the distinguishability of the spectra of the components in the wavelength region considered is too

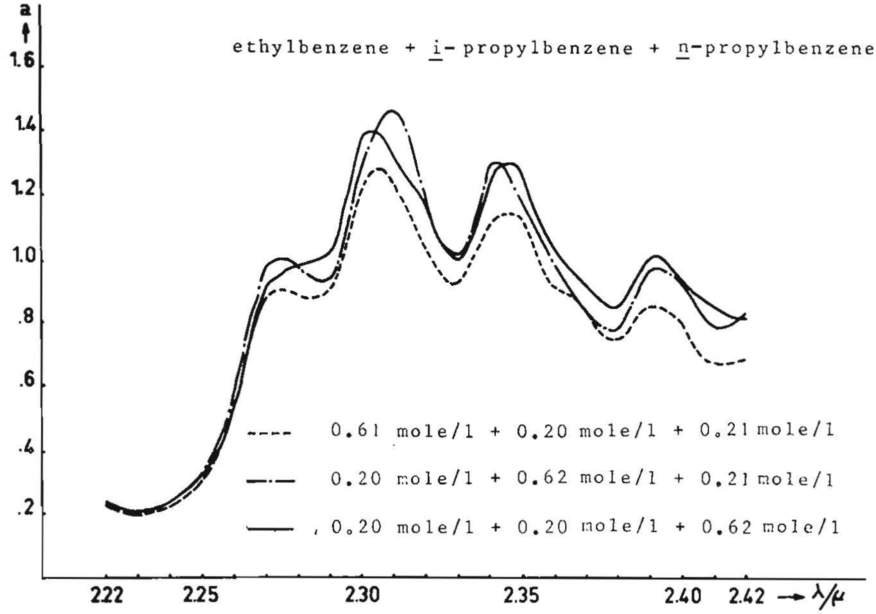

Fig. 6.8 Mixture reference spectra for Example 4, Sec. 6.6

small to afford sufficient resolving power at the given level of instrumental precision: in this sense, the limit of applicability of the method has been reached in this example.

Sample	Deviation	$\hat{s}/10^{-3}$	F-ratio**
$\underline{c}_j$	$\Delta\underline{x}_j = (\hat{\underline{x}}_j - \underline{c}_j)/10^{-4}$		
$\underline{c}_4$	$\begin{pmatrix} +11 \\ +61 \\ -53 \end{pmatrix}$	1.3	3.93
$\underline{c}_5$	$\begin{pmatrix} +94 \\ -34 \\ -34 \end{pmatrix}$	1.9	0.60
$\underline{c}_6$	$\begin{pmatrix} +104 \\ +65 \\ -145 \end{pmatrix}$	2.7	2.10
$\underline{c}_7^{*}$	$\begin{pmatrix} +7 \\ -37 \\ +8 \end{pmatrix}$	1.8	2.21

* slight extrapolation: $\hat{\underline{y}} \doteq \begin{pmatrix} 1.26 \\ -0.14 \\ -0.13 \end{pmatrix}$

** The F-ratio is the one obtained for the test of the hypothesis $\hat{\underline{y}}_j = \underline{y}_j$; because of an obviously necessary rejection, the results are based on 20 observations only. F therefore has 3 and 16 d.f. for numerator and denominator respectively: $P(F > 3.24) = 0.05$, $P(F > 5.29) = 0.01$.

6.7 Influence on precision

Example 4 of the preceding section clearly shows that even systems of as few as three components may have such badly distinguishable spectra as to prevent useful application of the method. At first glance, the technique of using mixtures as references might be thought reponsible; however, it is not difficult to demonstrate that using mixtures, while constituting a remedy against non-linearity, does not, in principle, lead to a loss of precision.

Consider an n-component mixture; let its true relative concentration vector be $\underline{y}$ and let $\hat{\underline{x}}$ be found from an analysis by least squares. Let the matrix of reference absorbances (of the pure components) be $\underline{A}$. Then a confidence region for $\underline{y}$ is given by

$$\frac{k-n}{n} \cdot \frac{(\hat{\underline{x}} - \underline{y})^{\tau} (\underline{A}^{\tau}\underline{A}) (\hat{\underline{x}} - \underline{y})}{\underline{r}^{\tau}\underline{r}} \leq F(n, k-n; \alpha) \qquad (6.7:1)$$

for Model I (cf. Section 4.6.2).

Now let the same mixture $\underline{y}$ be expressed in terms of a matrix of reference mixtures $\underline{C}$; then the true composition $\underline{y}$ in terms of $\underline{C}$ is $\underline{w}$, such that

$$\underline{Cw} = \underline{y}$$

where $\underline{C}$ is known without error and, of course, is not singular. Then the matrix of reference absorbances is $\underline{AC}$ and a confidence region for $\underline{w}$ is given by

$$\frac{k-n}{n}\cdot\frac{(\hat{\underline{v}}-\underline{w})^{\tau}((\underline{AC})^{\tau}\underline{AC})(\hat{\underline{v}}-\underline{w})}{\underline{r}^{\tau}\underline{r}} \le F(n,k-n;\alpha) \qquad (6.7:2)$$

when $\hat{\underline{v}}$ has been found by least squares. But finding $\hat{\underline{v}}$ is equivalent with finding $\underline{C}\hat{\underline{v}} = \hat{\underline{y}}$, say, in terms of the pure components; hence, using $\underline{w} = \underline{C}^{-1}\underline{y}$, we have

$$\begin{aligned}
&(\hat{\underline{v}}-\underline{w})^{\tau}((\underline{AC})^{\tau}\underline{AC})(\hat{\underline{v}}-\underline{w}) &=\\
&(\underline{C}^{-1}(\hat{\underline{y}}-\underline{y}))^{\tau}((\underline{AC})^{\tau}\underline{AC})\underline{C}^{-1}(\hat{\underline{y}}-\underline{y}) &=\\
&(\hat{\underline{y}}-\underline{y})^{\tau}(\underline{C}^{-1})^{\tau}\underline{C}^{\tau}\underline{A}^{\tau}\underline{A}\ \underline{CC}^{-1}(\hat{\underline{y}}-\underline{y}) &=\\
&(\hat{\underline{y}}-\underline{y})^{\tau}(\underline{A}^{\tau}\underline{A})(\hat{\underline{y}}-\underline{y}),
\end{aligned}$$

so that the confidence region of (6.7:2) is equal to that of (6.7:1), provided $\underline{r}^{\tau}\underline{r}$ is equal in both cases.

Now the expected value of $\underline{r}^{\tau}\underline{r}$ is the same in both cases when we arrange, as we can always do, that

$$\hat{\underline{x}}^{\tau}\hat{\underline{x}} = \hat{\underline{v}}^{\tau}\hat{\underline{v}} ,$$

$E(\underline{r}^{\tau}\underline{r})$ being proportional to $(1+\hat{\underline{x}}^{\tau}\hat{\underline{x}})$ and $(1+\hat{\underline{v}}^{\tau}\hat{\underline{v}})$ respectively. (In practice, $\hat{\underline{x}}^{\tau}\hat{\underline{x}}$ will be nearly equal to $\hat{\underline{v}}^{\tau}\hat{\underline{v}}$ whenever we do not extrapolate), Modification of the proof for Model II is obvious.

We draw the conclusion:

In principle, the use of exactly known mixtures as references does not influence the precision of an analysis.

CHAPTER VII

SOME APPLICATIONS

7.0 Scope

This chapter will briefly survey some recent applications of the method to a few practical problems. It is gratifying to record that the results were satisfactory, especially since the systems (which were not of our own choosing) seemed, at first sight, tractable with difficulty only by other available methods.

7.1 Cortisone and prednisone acetates

7.1.1 Two-wavelength analysis

At the suggestion of van Dijck and van Gorp (*), an investigation was undertaken of the applicability of the method to the determination of cortisone acetate in the presence of a large excess of prednisone acetate. The structural formulae of these very similar compounds and their ultraviolet absorption spectra are shown in Fig. 7.1.

It is clear that the difficulty of analysis here resides in the rather pronounced similarity of the featureless spectra.Their maxima very nearly coincide at 238.5 nm; their molar absorptivities at maximum in methanol are both within 2% of 15000 mole^{-1}. l. cm^{-1}; the only difference consists in a slower falling off of ε with λ for prednisone acetate, which, given its additional double bond, is in accordance with expectation.

A preliminary attempt at two-wavelength analysis gave an estimated standard deviation of 0.6% in cortisone acetate relative concentration (judging from 5 analyses): the wavelengths selected were 239 and 260 nm; as a precaution, the values of the molar absorptivities of the pure compounds were estimated simultaneously with the absorbances of the mixture, making the analysis inherently a relative one. A representative set of observations was:

	Absorbance of prednisone acetate	Absorbance of cortisone acetate	Absorbance of mixture (2% of c)
λ = 239	1.285	1.417	1.290
λ = 260	0.754	0.357	0.751

(*) N.V, Organon, Oss.

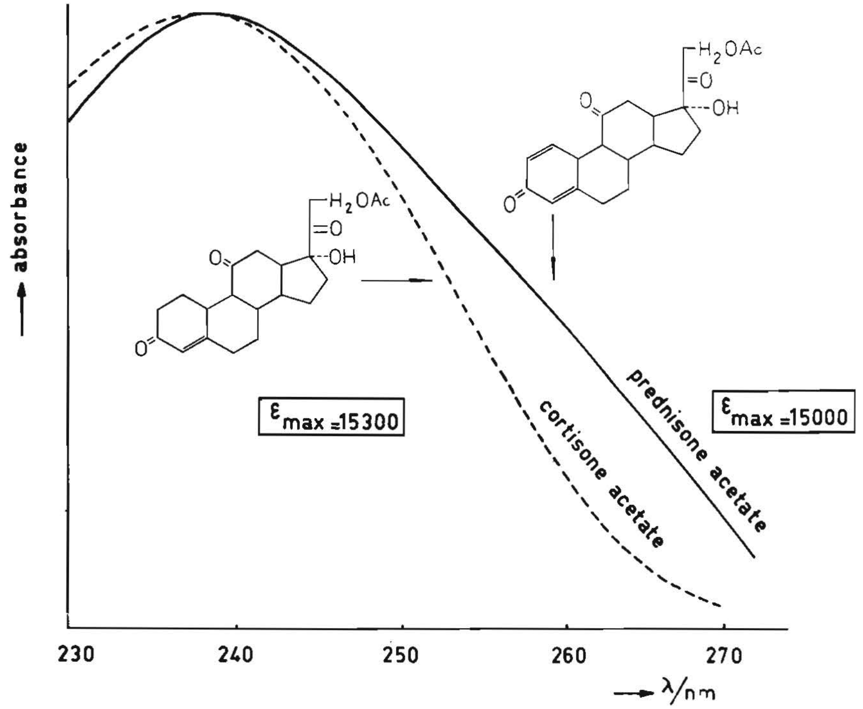

Fig. 7.1 To show the spectral similarity between cortisone and prednisone acetates.

yielding 1.25% of cortisone-acetate and 99.0% of prednisone acetate as against the known composition of 2.0%, 98.0% respectively. While not completely unacceptable, the result seems poor; other two-wavelength analyses in this system gave comparable results. In extenuation, it should be mentioned that these experiments were performed with a set of not very well-matched cells.

7.1.2 Error analysis for the two-wavelength case

It may be instructive to analyse this system for the (order of magnitude of the) expected errors. With

$$\underline{A} = \begin{pmatrix} 1.285 & 1.417 \\ 0.754 & 0.357 \end{pmatrix}, \quad \underline{A}^{-1} \doteq \begin{pmatrix} -0.586 & +2.324 \\ 1.237 & -2.108 \end{pmatrix}$$

$$(\underline{A}^{\tau}\underline{A})^{-1} = \underline{A}^{-1}(\underline{A}^{\tau})^{-1} \doteq \begin{pmatrix} 5.745 & -5.623 \\ -5.623 & 5.972 \end{pmatrix}$$

we calculate the expected value of the standard deviation of the estimated cortisone acetate relative concentration $\hat{x}_2$ as

$$E(\sigma(\hat{x}_2)) = \{(1 + \hat{x}_1^2 + \hat{x}_2^2) . ((\underline{A}^{\tau}\underline{A})^{-1})_{22}\}^{\frac{1}{2}} . \varepsilon$$

$$\doteq (2 \times 5.972)^{\frac{1}{2}} \times 1.2 \times 10^{-3} \doteq 4 \times 10^{-3},$$

where $\varepsilon = 1.2 \times 10^{-3}$ is the mean standard deviation of the instrumental reading of a single absorbance value. From the above calculation, we should expect a standard deviation of 0.4 of a per cent. in relative concentration of cortisone acetate and, since $((\underline{A}^{\tau}\underline{A})^{-1})_{11} \doteq ((\underline{A}^{\tau}\underline{A})^{-1})_{22}$, a closely similar standard deviation is predicted in the prednisone acetate relative concentration.

Now this error analysis is misleading for several reasons; that its results are not borne out in practice should not cause surprise. First and chief among these reasons is the fact that the absorbance of the absorption cells themselves in this particular case was so large (up to 0.04) as to make a correction necessary. This correction itself, being experimental, is also subject to measuring errors with $\varepsilon \doteq 1.2 \times 10^{-3}$, thus doubling the experimental variance of the elements of $\underline{A}$ and $\underline{a}$; secondly, there is no guarantee that these corrections, having been determined prior to the observations of $\underline{A}$ and $\underline{a}$, were indeed valid at the time of the latter observations: experience seems to indicate that the absorbances of the cells themselves are less reproducible when they are (comparatively) large; small cell absorbances (say $a<0.01$) seem definitely more constant in time. Thirdly, one of the wavelengths (λ=239 nm) is on the verge of the usable region, which is taken to be $\lambda \geq 240$ nm troughout the present work; again, it is physically plausible that the large cell absorbances are less well reproducible in the region around 240 nm, where scattering tends to distort absorbance values anyhow.

For these reasons this error analysis, which just takes the stochasticity of the elements of $(\underline{A},\underline{a})$ into account and ignores all other disturbing causes, is naive to a degree.

7.1.3 Least-squares analysis

By setting up an overdetermined system of equations for the prednisone acetate/cortisone acetate analysis, we may eliminate the effects of non-reproducible differences among the absorbance cells, at least in so far as they are independent of λ. Moreover, if the experimental variance of an absorbance value is unduly high, this should at least be reflected in unusually high values of the elements of the residual vector $\underline{r}$ and in correspondingly high values of the estimated standard deviations of the elements of $\underline{\hat{x}}$.

In order to verify these expectations, absorbance readings were taken on solutions of the pure compounds and on a mixture containing 1.99% by weight of the cortisone acetate solution, the remainder being prednisone acetate solution. The results are reproduced in Table 7.1.

The observations yield the following system of normal equations

$$\underline{A}^{\tau}\underline{A}\underline{\hat{x}} = \underline{A}^{\tau}\underline{a} \quad \text{(Model II)}$$

$$\underline{A}^{\tau}\underline{A} = \begin{pmatrix} 37 & 30.686 & 23.247 \\ 30.686 & 29.414\ 792 & 25.193\ 127 \\ 23.247 & 25.193\ 127 & 23.902\ 833 \end{pmatrix}$$

$$\underline{A}^{\tau}\underline{a} = \begin{pmatrix} 30.626 \\ 29.414\ 534 \\ 25.238\ 182 \end{pmatrix}$$

from which we find:

$\hat{x}_o$ (cell correction) $= -0.00063$

$\hat{\sigma}(\hat{x}_o) = 0.0015$

$\hat{x}_1$ (cortisone acetate, relative concentration)

$= 0.0187$ or 1.87%

$\hat{\sigma}(\hat{x}_1) = 0.0022$ or 0.22%

$\hat{x}_2$ (prednisone acetate, relative concentration)

$= 0.9847$ or 98.47%

$\hat{\sigma}(\hat{x}_2) = 0.0034$ or 0.34%

The sum of squares of the residual vector was 0.80×10^{-4}, yielding an estimated standard deviation per equation of 0.15×10^{-2} or 0.0015 in absorbance, corresponding to a standard deviation of $0.0015/(1 + \hat{x}_1^2 + \hat{x}_2^2)^{\frac{1}{2}} \doteq 0.0011$ per observation, and, incidentally, giving the rather satisfactory estimated multiple correlation coefficient $\hat{R} = 0.999\ 990$, which is to say that only about $1 - \hat{R}^2$ or two thousandths of a per cent of the variance of $\underline{a}$ is left unaccounted for. The absolutely greatest element of $\underline{r}$ occurs at $\lambda = 255$ nm and equals 0.003_2 in absorbance.

The cortisone acetate relative concentration is almost exactly equal to the known value; for prednisone acetate, 98.47 % ($\hat{\sigma} = 0.34\%$) is well within 1.5 estimated standard deviations.

Table 7.1 Observed absorbances (× 1000) in the prednisone acetate/cortisone acetate system.

p: prednisone acetate solution

c: cortisone acetate solution

m: a mixture containing 1.99 % by weight of the c solution, the remainder being p.

The readings have been corrected for cell absorbances.

λ	p	c	m
239	1282	1417	1290
240	1283	1409	1290
241*	1276	1394	1283
242	1264	1371	1268
243	1247	1340	1254
244*	1230	1306	1234
245	1210	1267	1212
246	1185	1222	1188
247*	1160	1172	1162
248	1130	1117	1131
249	1100	1059	1102
250*	1071	999	1072
251	1037	932	1039
252	1007	870	1010
253*	972	802	974
254	945	735	945
255	911	667	912
256*	885	603	882
257	850	536	847
258	819	472	815
259*	788	414	783
260	754	357	751
261	723	306	715
262*	691	260	683
263	655	219	649
264	623	185	614
265*	588	154	581
266	553	128	544
267	519	106	512
268*	481	80	475
269	449	75	443
270	414	63	407
271*	382	54	376
272	347	46	342
273	314	41	309
274*	284	35	280
275	254	34	251

A joint test of the hypothesis

$$\begin{cases} x_2 = 0.0199 \\ x_3 = 0.9801, \end{cases}$$

however, yields F = 6.33 with 2 and 34 d.f., which is most significant, $P(F(2,34) > 6.2) = 0.005$.

Now observing a 37 wavelengths-analysis appears to be a rather formidable undertaking for a two-component system; results close to the above are intuitively felt to be achievable with fewer wavelengths. This is borne out by the results obtained by omitting from Table 7.1 every third observation (i.e all those marked*), giving

$$\begin{cases} \hat{x}_1 = 0.0174, \hat{\sigma}(\hat{x}_1) = 0.0030 \\ \hat{x}_2 = 0.9867, \hat{\sigma}(\hat{x}_2) = 0.0046 \end{cases}$$

and F = 4.24 with 2 and 22 d.f.; $P(F(2,22) > 3.44) = 0.05$;
$P(F(2,22) > 5.72) = 0.01$.

We conclude that in this particular, spectrally featureless system, acceptable standard deviations of ∿ 0.3 % in relative concentration may be reached with about 25 roughly equally spaced observations. This estimated standard deviation is approximately equal to that calculated by the simple theory for 2 observations only. The inherently better precision of the least-squares method here evidently only serves the better to allow for unpredictable corrections applicable to the measuring cells: in other words, merely making allowance for a third unknown (the resultant cell correction) so impairs the quality of the systems of 2 equations in the concentrations that we must have recourse to a rather heavily overdetermined system in 3 unknowns in order to obtain the precision suggested (but not, in practice, afforded) by a good system of equations for the unknown concentrations.

The results exhibited above are confirmed by those achieved for the same system with observations at 239(1.5)275 nm, in another experiment which yielded

$$\begin{cases} \hat{x}_1 = 0.74 \% \quad \hat{\sigma}(\hat{x}_1) = 0.28 \% \\ \hat{x}_2 = 99.55 \% \quad \hat{\sigma}(\hat{x}_2) = 0.32 \% \end{cases}$$

for a known composition of $x_1 = 0.98$ %, $x_2 = 99.02$ %.

7.2 The C_9 aromatic hydrocarbons in a mineral turpentine

7.2.1 Introductory

At the suggestion of Professor A.I.M. Keulemans, an experiment was devised which, if successful, might convincingly demonstrate the applicability of least-squares analysis to practical cases. Martin and Winters (1963) reported a fairly complete analysis of crude mineral oils in which relative errors (presumably standard deviations) of 6% per component were achieved. While it was clear at the outset that spectrophotometry would be unable to allow comparably precise results for minor constituent aromatics, because its error is inherently of an absolute nature, it was thought that for aromatic components present in major amounts a much better precision would result.

A sample of an aromatic mineral turpentine (Shell Nederland N.V., batch IP 2164/64), having a boiling point range of 140 to 190° and an approximate aromatic content of 20 %, was analyzed. This approximate composition was confirmed by fluorescent indicator analysis, which resulted in about 19% aromatics, the remainder being (essentially) saturated non-aromatic hydrocarbons. The aromatic and non-aromatic fractions were collected separately and subjected to gas chromatography, which revealed, by a count of peaks, the presence in this particular turpentine of at least 40 alkanes and 45 aromatics; a considerable number of the latter could be identified by their retention indices, A.P.I. samples serving as reference compounds. In order to reduce the number of aromatic compounds to be determined simultaneously, the turpentine was fractionated on a spinning band column of about 25 theoretical plates at atmospheric pressure. In all, 12 fractions were collected, numbered SB-1 to SB-12 (Table 7.2).

Table 7.2 Fractions collected on distillation of the turpentine

Fraction name	Boiling point range/°C	Amount/cc
SB-1	125 - 138	10
SB-2	138 - 148	7.5
SB-3	148 - 154.5	8
SB-4	154.5 - 158	6.5
SB-5	158 - 159	5
SB-6	159 - 164.3	7
SB-7	164.3 - 167.5	8
SB-8	167.5 - 168.5	4.5
SB-9	168.5 - 171.5	5
SB-10	171.5 - 173.5	8.5
SB-11	173.2 - 175.5	6.5
SB-12	> 175.5*)	11.5

*) residue

7.2.2 Analysis by gas-liquid chromatography

A sample of each SB fraction was analyzed by gas chromatography on a cupro-nickel capillary column, 50 m long and of 0.25 millimetre internal diameter coated with polyethylene glycol (PEG 400), at 100°C and a 1 atm nitrogen pressure. The stationary phase was chosen because it gave a clean separation between alkanes and aromatics. The detector sensitivity was assumed to be equal for all C_9 aromatics; the surfaces under each of the aromatic peaks were determined by planimeter in the chromatograms and divided by the sum total of these surfaces; the figures so obtained will be referred to as gas-liquid chromatography percentages (%GLC) and are reproduced here as Table 7.3 for the fractions SB-4 to SB-8 (see Fig. 7.2).

The alkanes would not be expected to impair the spectrophotometric analysis to be described, since they do not absorb in the ultra-violet at $\lambda > 240$ nm. Matrix effects, too, can be presumed absent since the turpentine's alkanes form a negligible amount relative to the iso-octane used as a solvent.

Table 7.3

Percentage composition of the aromatics of some spinning band column fractions as estimated by planimetry of GLC peaks.

Compound	Short	SB-4	SB-5	SB-6	SB-7	SB-8	B.p/°C
i-propylbenzene	iPr	2.3	0.4	-	-	-	152.39
o-xylene	oXy	0.2	0.02	-	-	-	144.41
n-propylbenzene	nPr	17.7	8.2	2.4	0.1	-	159.22
1-methyl-3-ethylbenzene	1M3E*)	63.3	58.5	37.6	9.0	1.6	161.31
1,3,5-trimethylbenzene	135tM**)	3.9	8.4	13.8	13.4	6.3	164.72
1-methyl-2-ethylbenzene	1M2E***)	11.4	21.2	31.6	21.5	9.7	165.15
1,2,4-trimethylbenzene	124tM	1.2	3.3	14.3	54.0	73.0	169.35
1,2,3-trimethylbenzene	123tM	-	-	0.3	2.0	7.2	176.08
sec-butylbenzene	sBu	-	-	-	-	2.3	173.31

*) Peak coincident with that of 1-methyl-4-ethylbenzene (1M4E)

**) Peak coincident with those of i-butylbenzene (iBu) and tert-butylbenzene (tBu)

***) Peak coincident with that of 1-methyl-3-i-propylbenzene (1M3iP)

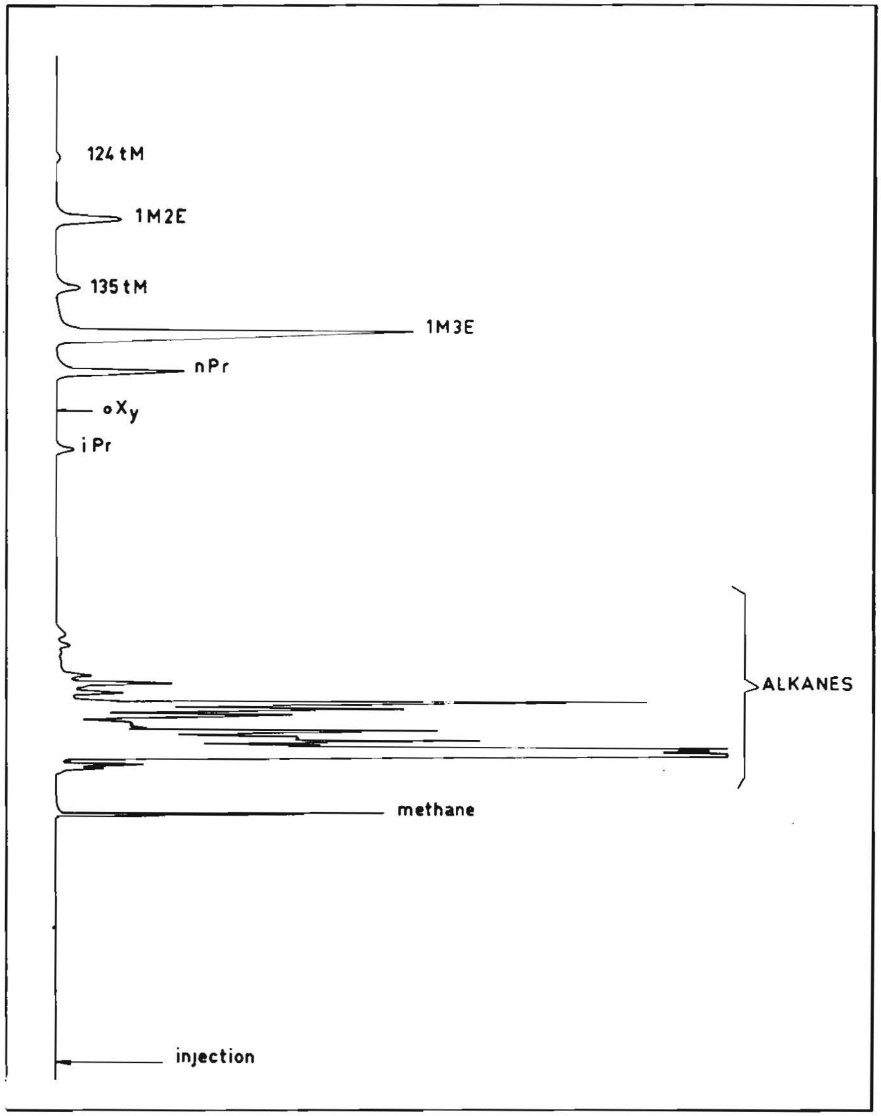

Fig. 7.2 A chromatogram of fraction SB-4, to show the good separation of the aromatics from one another and from the alkanes. (For the abbreviations, see Table 7.3).

It will be seen from Table 7.3 that 1-methyl-3-ethylbenzene cannot, in this GLC analysis, be distinguished from its 1,4-isomer. Also, three C_{10} aromatics could be hidden under the peaks of 1,3,5-trimethylbenzene and 1-methyl-2-ethylbenzene. The coincidence of the 1M3E and 1M4E peaks is particularly unfortunate, the boiling points of these C_9 compounds differing very little (161°.305 and 161°.989 respectively, according to the A.P.I. Tables). The interference from the C_{10} compounds would not be expected to be especially harmful in fractions collected by boiling point*. We can therefore expect the results in the Table to be meaningful as they stand for all compounds shown with the exception of the 1M3E percentages which should be interpreted as percentage contents of 1M3E + 1M4E relative to total aromatics.

7.2.3 Analysis by spectrophotometry

7.2.3.1 A first attempt - detection of a contaminant

Some attempts to prepare suitable samples for multicomponent spectrophotometric analysis by preparative gas-liquid chromatography failed because of persistent contamination of the fractions obtained (as shown by their UV spectra); in addition, recovery was far from quantitative. It was therefore decided to subject the fractions described in 7.2.1 to the spectrophotometric method without further purification. We confined our attention to the fractions richest in C_9 hydrocarbons, i.e. SB-4 to SB-8, SB-6 being omitted because it was not expected to present new problems or to yield new information.

The procedure adopted was as follows: standard solutions in <u>iso</u>-octane were made up of each of the hydrocarbons mentioned in Table 7.3; Each of these solutions had a maximum absorbance in the range 1.2-1.6. The compounds referred to were A.P.I. samples. For completeness's sake, similar solutions of ethyl-benzene, <u>m</u>- and <u>p</u>-xylene were also prepared. In addition to these, three accurately known mixtures ('synthetic mixtures') of these solutions were also prepared as a check, together with solutions having a maximal absorbance in the desired range of each of the fractions SB-4, SB-5, SB-6 and SB-7. Each of these 23 solutions then had its absorbances observed at the 37 wavelengths 245(1)280 and 285 nm. The three synthetic mixtures (S1, S2 and S3) and the four fractions were then analyzed in terms of the standard solutions by the least-squares procedure.

Against all expectation, the analysis seemed to fail completely. Standard deviations in the residual vector for the S and SB solutions were of the order of magnitude of 10 to 50×10^{-3},

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*) 135tM (b.p. 164°.72) coincides with iBu (172°.76) and tBu (169°.12); 1M2E (b.p. 165°.15) coincides with 1M3iPr (175°.05)

whereas we have been led to expect a value of 1 to 2×10^{-3}. Apparently, there was some source of gross error. Fortunately, it turned out that this source of error could be identified and eliminated. The procedure adopted was as follows: the compositions $\underline{y}_1$ of S1 and $\underline{y}_2$ of S2 were accurately known; so was (within the measuring precision) the matrix $\underline{A}$ of the absorbances of the standard solutions. We could therefore reconstruct the calculated spectra $\underline{b}_j = \underline{A}\underline{y}_j$ $(j = 1,2)$ and compare them with the mixture spectra $\underline{a}_j$ as actually observed. It was very striking that, for all wavelengths, the elements of the $\underline{b}_j$ were in excess of those of the $\underline{a}_j$. Moreover, these differences were roughly parallel for S-1 and S-2 (Cf. Fig.7.3). A contamination having a spectrum proportional to that of $\underline{b}_j - \underline{a}_j$ was therefore suspected.

Even though the nature of this contaminant was not known at the time, its presence could be allowed for by a simple device. The reconstructed contamination spectrum $\underline{c} = \sum_j (\underline{b}_j - \underline{a}_j)$ was considered as an additional reference spectrum and the remaining unknowns, S-3 and the SB's, were then analyzed in terms of the standard solutions plus spectrum $\underline{c}$.

For the known mixture S-3, the results were quite satisfactory:

Compound	% Taken	% Found	St. deviation (%)
nPr	0.25	0.34	0.26
1M3E	12.00	12.13	0.51
135tM	9.30	8.99	0.64
sBu	1.35	1.25	0.27
1M2E	13.76	13.44	0.38
124tM	62.22	62.38	0.17
123tM	1.09	1.50	0.32
contaminant	?	21.32 *)	0.5 *)

*) In terms of the concentration corresponding to spectrum $\underline{c}$; all others in terms of the reference solution concentrations. A test of the hypothesis "S-3 Taken = S-3 Found" yielded an F-ratio of 1.13 (with 7 and 28 degrees of freedom).

The standard deviation of the residual vector was now in accordance with expectation too; by allowing for the contaminant, it had fallen from 8.3×10^{-3} to 1.4×10^{-3}.

Further inquiry afforded a clue as to the identity of the contaminant. Contrary to the previous practice, the glassware employed had been rinsed with acetone. That acetone was indeed the contaminant was easily confirmed by observation of its spectrum in <u>iso</u>-octane. A solution of 0.94 g/litre gave the spectrum recorded as curve (3) in Fig.7.3.It can easily be seen that there is a good general correspondence between the shapes of the three curves; also, the very slight dip in the spectrum at ∿ 276 nm (amounting to perhaps 2% of the absorbance at neighbouring wavelengths) can be well distinguished in the reconstructed spectra $\underline{b}_j - \underline{a}_j$.

Allowing for the presence of acetone (spectrum $\underline{c}$). provisional results were obtained for SB-4, SB-7 and SB-8. SB-5 on closer observation proved to have absorbances up to 2.05 and was rejected for that reason. While no final significance is attached to them, they are reproduced here as Table 7.4 , chiefly because of the inferences to be drawn from them as to the qualitative composition of the SB fractions. For SB-4 and SB-7 we could conclude that the (1M3E + 1M4E) coincident chromatographic peak must indeed be attributed to a mixture of both compounds. We could not

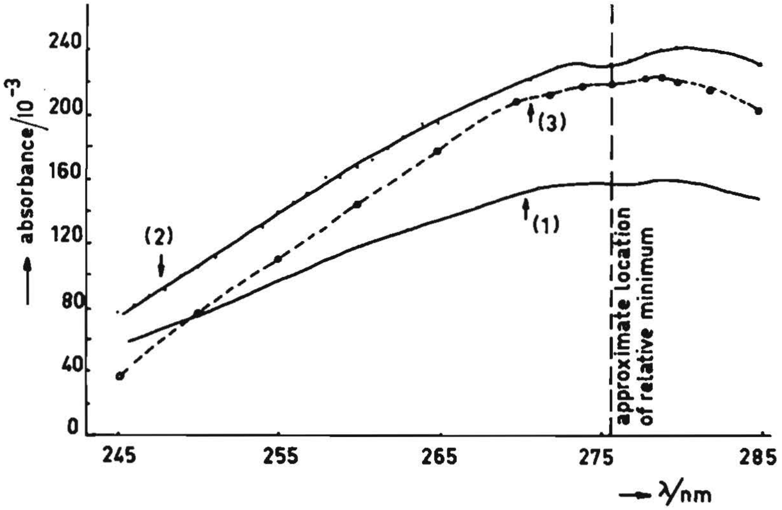

Fig. 7.3 To show the difference spectra attributable to the acetone contaminant.
Curve (1): Difference spectrum $\underline{b}_1 - \underline{a}_1$
Curve (2): Difference spectrum $\underline{b}_2 - \underline{a}_2$
Curve (3): Spectrum of aceton in iso-octane (0.94 g/l).

Table 7.4

Spectrophotometric results *) for mineral turpentine fractions

Compound	SB-4 +)					SB-7					SB-8					
	a)	b)	c)	d)	e)	a)	b)	c)	d)	e)	a)	b)	c)	d)	e)	f)
iPr	1.2	0.4	0.0101	1.7	2.3	-	-		-	-	-	-		-	-	0.8383
oXy	-	-			0.2	-	-		-	-	-	-		-	-	0.6227
nPr	13.8	0.4	0.1115	18.4	17.7	-	-		-	0.1	-	-		-	-	0.8087
1M3E	36.2	1.0	0.2607	42.9	-	3.6	0.9	0.0259	4.8	-	-	-		-	-	0.7202
1M4E	33.7	0.3	0.1187	19.5	-	7.5	0.3	0.0264	4.9	-	3.2	0.3	0.0113	2.2	-	0.3522
1M3E + 1M4E	-		0.3794	62.4	63.3			0.0523	9.7	9.0			0.0113	2.2	1.6	
135tM	4.3	0.2	0.0296	4.9	3.9	11.6	1.4	0.0798	14.9	13.4	7.2	0.8	0.0496	9.8	6.3	0.6884
1M2E	9.7	0.6	0.0711	11.7	11.4	13.6	0.7	0.0997	18.7	21.5	6.2	0.7	0.0455	9.0	9.7	0.7333
124tM	1.5	0.3	0.0059	1.0	1.2	69.9	0.3	0.2748	51.4	54.0	91.2	0.3	0.3586	70.9	73.0	0.3932
123tM	-	-		-	-	2.9	0.5	0.0279	5.2	2.0	3.0	0.6	0.0289	5.7	7.2	0.9630
sBu	-	-		-	-	-	-		-	-	1.4	0.3	0.0118	2.3	2.3	0.8411
			0.6076	100.1	100.0			0.5345	99.9	100.0			0.5057	99.9	100.1	
Standard deviation of residual vector			2.7×10^{-3}					2.8×10^{-3}					3.0×10^{-3}			

*) From samples suffering from contamination by acetone.

a) Analytical result, expressed as a percentage of the concentration of the corresponding standard solution.

b) Estimated standard deviation in a), in the same unit as a).

c) Concentration of aromatic in milligrams per millilitre of solvent ; c) = a) × f) / 100.

d) Percentage of compound relative to total aromatics estimated : % UV.

e) For comparison with d): the same percentage as estimated by GLC, from table 7.3.

f) Concentration of standard (reference) solutions in milligrams per millilitre of solvent.

+) After rejection of a single observation.

find detectable amounts of 1M3E in SB-8, though it is admitted that the detection limit is quite high. With a standard deviation of ∿ 1% of the standard concentration (∿0.72 milligram/ml of solvent), 1M3E could be present in amounts of say ∿ 0.02 milligram/ml or more. We shall assume 1M3E to be absent from SB-8. Likewise, the C_{10} compounds having peaks coinciding with those of C_9 (see Table 7.3) are presumed absent. The low values of the standard deviation of the residual vector indicate that the SB observed spectra are expressed quite well as linear combinations of the spectra of the compounds assumed to be present.

The agreement between the percentages of the aromatics as found by least squares (% UV) and the comparable percentages as found by gas-liquid chromatography (% GLC) is reasonaly satisfactory.

7.2.3.2 The experiment repeated

Since the results reported in the previous section could be described as encouraging, it was decided to repeat the experiment under exclusion of all contamination. Solutions of the reference compounds as well as the SB factions were freshly made up;

the data are recorded in Table 7.5. As a check, three synthetic mixtures (S-4, S-5 and S-6) of the standard solutions were also prepared and were analysed with the SB samples. The observations are presented in Table 7.6; the absorbances recorded there have not been corrected for cell differences, since the cell absorbances were uniformly small and reasonably constant in λ . In all, 36 wavelengths, 245(1)280 nm, were chosen for observation. As a precaution against read-out errors, the transmittances as well as the absorbances of all observations were recorded, these being checked for consistency before being accepted. The readings took about six hours, during which time three observers cooperated.

The uncorrected absorbances of Table 7.6 have been treated by the least-squares procedure; it is thought that applying cell

Table 7.5

Reference and sample concentrations

Substance *)	Concentration: mg of substance per g of solvent	a_{max} (approx.)	λ_{max} (approx.)
ethylbenzene (E)	1.0422	1.47	262
m-Xylene (mXy)	0.8199	1.40	265
p-Xylene (pXy)	0.3690	1.44	274
iPr	1.2192	1.47	261
oXy	0.8737	1.50	263
nPr	1.1863	1.44	262
1M3E	0.9521	1.47	265
1M4E	0.3708	1.16	274
iBu	1.4122	1.48	259
tBu	1.5424	1.50	257
135tM	1.2639	1.54	265
sBu	1.3035	1.36	261
1M2E	1.1016	1.62	263
1M3iP	1.1553	1.58	264
124tM	0.5478	1.52	276
123tM	1.3366	1.43	261
SB-4	5.4891	1.42	265
SB-5	3.2325	1.39	265
SB-7	2.6828	1.49	267
SB-8	2.6418	1.41	267

*) For the abbreviations, cf. Table 7.3

Table 7.6 Absorbances (× 1000) observed for hydrocarbons and samples.
(For the abbreviations used, compare the previous Tables and the text)

λ/nm	SB-5	SB-7	124tM	S-5	E	nPr	1M4E	pXy	123tM	S-6	mXy	S-4	SB-4	1M2E	iPr	sBu	1M3iPr	SB-8	oXy	1M3E	tBu	iBu	135tM
245	364	301	218	414	543	540	178	198	503	291	333	432	390	509	574	561	444	255	462	405	678	556	348
246	398	338	249	454	621	614	193	214	555	325	366	475	438	557	654	650	479	287	508	437	759	629	390
247	443	376	277	502	724	720	221	238	600	360	406	531	481	610	789	764	523	319	554	480	840	731	432
248	483	412	304	547	801	816	214	271	654	394	443	578	524	664	801	782	568	348	603	518	822	847	483
249	522	455	340	593	799	807	258	285	721	438	482	617	559	729	771	752	626	385	671	564	774	836	523
250	562	504	380	645	775	793	270	312	775	485	519	661	594	808	781	757	705	427	740	618	895	822	578
251	621	549	414	718	833	845	304	314	819	528	600	737	661	875	965	920	774	465	802	702	1147	865	605
252	694	600	450	772	1012	1026	376	396	895	572	652	802	743	933	1103	1072	805	507	847	740	1215	1059	665
253	736	655	505	820	1132	1159	390	432	1002	624	683	858	792	993	1167	1132	845	560	901	777	1153	1202	710
254	779	718	563	865	1112	1152	417	440	1076	686	723	895	829	1073	1140	1086	919	617	990	816	1124	1203	785
255	841	778	608	960	1158	1161	421	501	1092	747	785	976	884	1171	1079	1046	1041	661	1085	918	980	1171	880
256	896	832	650	1028	1044	1080	450	472	1131	800	875	1027	930	1250	973	942	1123	706	1135	1002	1045	1110	966
257	958	897	721	1069	1045	1057	534	556	1206	856	957	1072	998	1258	1232	1164	1154	772	1158	1061	1495	1073	948
258	1005	971	799	1118	1313	1320	550	595	1235	922	971	1142	1059	1278	1386	1354	1178	848	1200	1084	1498	1362	1050
259	1086	1035	850	1144	1370	1425	675	616	1200	974	995	1167	1140	1346	1395	1354	1156	898	1255	1083	1347	1482	1106
260	1107	1079	902	1148	1304	1351	712	868	1233	1014	1004	1158	1150	1420	1316	1238	1176	944	1294	1079	1293	1433	1037
261	1114	1148	982	1224	1471	1437	618	730	1372	1090	990	1236	1156	1464	1471	1355	1341	1013	1384	1156	1109	1358	1135
262	1178	1212	1036	1333	1294	1340	620	650	1428	1172	1151	1318	1212	1548	1004	986	1461	1066	1454	1308	790	1371	1227
263	1181	1219	1037	1353	936	972	613	707	1372	1172	1224	1302	1189	1616	864	817	1472	1068	1501	1366	1021	1017	1218
264	1255	1237	1045	1373	1047	1051	751	684	1223	1173	1261	1339	1275	1567	1111	1077	1577	1080	1407	1398	1109	1035	1258
265	1390	1346	1162	1415	1042	1066	1001	963	1295	1253	1400	1379	1416	1397	939	932	1459	1183	1288	1472	941	1132	1535
266	1213	1449	1377	1215	773	839	873	1087	1178	1347	1222	1154	1200	1291	723	710	1145	1341	1250	1198	708	925	1394
267	1136	1490	1467	1073	742	728	878	855	966	1372	977	1009	1103	1271	1053	941	1042	1405	1198	975	935	713	1258
268	1228	1432	1386	1142	1285	1262	982	1140	835	1312	945	1134	1250	1168	1014	995	1140	1332	1018	1076	644	1141	1287
269	1036	1317	1320	1037	915	960	738	1055	982	1228	1031	1011	1043	1022	588	588	1002	1252	927	1020	358	1001	1084
270	834	1267	1308	864	480	513	535	717	1078	1195	762	790	793	1082	294	304	900	1224	1128	790	184	543	862
271	880	1262	1239	1032	233	252	420	504	867	1212	682	914	819	1338	158	164	1383	1177	1219	1007	117	264	1019
272	997	1170	1086	1237	140	152	446	414	603	1129	1302	1128	960	1136	113	124	1293	1055	993	1391	94	146	1393
273	1041	1030	942	895	97	108	1006	568	400	918	1186	818	1017	708	81	93	785	925	637	1015	60	108	1132
274	892	1059	1098	541	64	77	1162	1438	272	907	712	482	857	385	50	61	406	1015	355	557	36	70	810
275	581	1230	1468	358	43	53	702	1164	197	1100	361	294	519	211	35	51	218	1283	197	287	23	44	658
276	362	1204	1523	258	28	36	363	649	162	1102	193	200	296	130	23	36	135	1301	120	162	14	26	498
277	217	916	1187	178	19	26	174	306	134	848	111	136	166	82	18	34	87	1006	74	103	10	16	314
278	133	594	774	115	15	22	96	157	99	555	74	90	101	52	15	30	55	658	48	67	8	10	203
279	83	330	427	71	12	18	62	82	62	312	43	56	64	32	13	30	34	365	31	41	6	6	171
280	54	177	225	42	10	16	48	60	39	169	25	36	45	22	12	29	21	195	21	26	6	5	116

corrections would not materially affect the results. For the synthetic mixtures, the composition estimated has been set out in Table 7.7. The results exhibited there are uniformly satisfactory from a chemist's point of view, the maximum difference between percentages taken and found being 1.54 (S-6, 1M3E). In such a case the user would be forewarned by a correspondingly high value (1.08) of the estimated standard deviation for that compound.

Table 7.7

Results for the synthetic mixtures S-4, S-5 and S-6

	S-4			S-5			S-6		
Compound:	Taken	Found	$\hat{\sigma}$	Taken	Found	$\hat{\sigma}$	Taken	Found	$\hat{\sigma}$
iPr	3.32	3.13	0.15	0.55	0.46	0.21	-	a)	-
oXy	0.48	0.50	0.60	0.26	0.59	0.87	-	a)	-
nPr	15.08	15.01	0.14	6.59	6.65	0.20	0.34	0.37	0.30
1M3E	63.73	63.49	0.39	60.30	60.80	0.55	7.65	9.19	1.08
1M2E	10.63	10.52	0.67	18.81	18.30	0.95	15.33	14.76	0.69
123tM	-	a)	-	-	a)	-	1.86	1.85	0.67
124tM	3.15	3.78	0.11	6.40	6.71	0.15	67.21	68.06	0.32
135tM	3.60	3.72	0.33	7.08	6.95	0.47	7.53	6.21	1.01
$\hat{s}$)	1.0×10^{-3}			1.4×10^{-3}			3.0×10^{-3}		

All the above have been expressed as percentages of the reference concentrations, cf. Table 7.5

a) Not analysed for

$\hat{s}$) Standard deviation of the residual vector.

Statistically speaking, there is less reason to be satisfied with the estimated compositions. A test of the hypothesis 'Taken= Found' for S-4, for instance, yielded an F-ratio of no less than 61.9 (7,28 d.f.); this improved slightly upon omitting the obviously offending result for 124tM, giving 29.8(6,28 d.f.) for the alternative hypothesis 'S-4 Taken = S-4 Found with the exception of 124tM'. Other experiments gave less extreme F-ratios:

Hypothesis	F-ratio	d.f.
S-5 Taken = S-5 Found	7,25	7,28
do., with the exception of 124tM	1,24	6,28
S-6 Taken = S-6 Found	3,26	6,29
do., with the exception of 124tM	0,69	5,29

It is obvious that at least the results for 124tM are badly in error and that S-4 as a whole is statistically unsatisfactory. These discrepancies stand unexplained, the more so because some other experiments on the same range of compounds (not reported here) yielded more nearly expected results.

Turning to the composition estimated for the turpentine distillation fractions SB, collected in Table 7.8, we note that the results of multicomponent spectrophotometry and those obtained by GLC (columns c) and d) respectively) confirm each

Table 7.8

Spectrophotometric results for mineral turpentine fractions

	Fraction:															
Compound:	SB-4				SB-5				SB-7				SB-8			
	a)	b)	c)	d)	a)	b)	c)	d)	a)	b)	c)	d)	a)	b)	c)	d)
iPr	1.7	0.4	2.3	2.3	0.6	$0.3^{h)}$	0.9	0.4	-	-	-	-	-	-	-	-
oXy	e)		0.0		f)		0.0	0.02	-	-	-	-	-	-	-	-
nPr	13.0	0.4	17.5	17.7	6.1	0.3	8.5	8.2	0.8	0.2	1.2	0.1	-	-	-	-
1M3E	41.0	1.0	44.2	-	33.6	0.7	38.6	-	4.5	0.9	5.8	-	g)	-	0.0	-
1M4E	46.8	0.3	19.7	-	45.9	0.2	20.1	-	10.7	0.3	5.3	-	4.3	0.3	2.7	-
1M3E + 1M4E	-	-	63.9	63.3	-	-	58.7	58.5	-	-	11.3	9.0	-	-	2.7	1.6
135tM	2.3	1.0	3.3	3.9	5.8	0.7	8.6	8.4	7.8	0.9	13.1	13.4	3.2	0.9	6.6	6.3
1M2E	9.5	1.7	11.9	11.4	15.0	1.2	19.5	21.2	14.5	0.5	21.1	21.5	5.3	0.5	9.5	9.7
124tM	1.8	0.3	1.1	1.2	6.0	0.2	3.9	3.3	72.0	0.3	52.2	54.0	82.1	0.3	73.7	73.0
123tM	-	-	-	-	-	-	-	-	0.7	0.5h)	1.3	2.0	2.0	0.5	4.3	7.2
sBu	-	-	-	-	-	-	-	-	-	-	-	i)	1.5	0.2	3.2	2.3
$\hat{s}$)	2.4×10^{-3}				1.7×10^{-3}				2.3×10^{-3}				2.1×10^{-3}			

a) Analytical result, expressed as a percentage of the concentration of the corresponding standard solution, for which see Table 7.5.

b) Estimated standard deviation in a), in the same units as a).

c) Percentage of this compound relative to total aromatics estimated: % UV (from a) and Table 7.5).

d) For comparison with c): the same percentage as estimated by GLC, from Table 7.3.

e) Set equal to zero: a) = -0.22, b) = 0.16.

f) Set equal to zero: a) = +0.07, b) = 1.07.

g) Set equal to zero: a) = +1.53, b) = 0.82.

h) Definitely known to be present, hence not set equal to zero, though a) is very imprecise.

i) Detectable in trace amounts by GLC.

$\hat{s}$) Estimated standard deviation of residual vector.

other about as well as could be expected. The standard deviations in the spectrophotometric results seem realistic when c) and d) are compared. The results also suggest that the GLC estimates are somewhat more precise than those obtained by Martin and Winters 1963.

It is clear, we think, that minor constituents are indeed estimated more precisely by GLC than by spectrophotometry. There is at least some indication that components present in major amounts should be preferably determined by spectrophotometry.

7.2.4 Some conclusions

We venture to submit that the work reported on the turpentine fractions allows the following conclusions to be drawn:

1. Spectrophotometric estimation of aromatics leads to acceptable analytical results with up to at least eight compounds present simultaneously.
2. Standard deviations per component are in the range of 0.2-2.0% of the reference concentration. There are indications that the lower estimated standard deviations (say $\hat{\sigma} \sim 0.2\%$ or less) may represent underestimates.
3. The resolving power of multicomponent spectrophotometry is dependent on the shapes of the spectra in the set. It is predictable from these spectra and, in favorable cases, superior to the GLC resolving power.
4. For the determination of minor constituents, GLC is the method to be preferred.

In conclusion, we append a few miscellaneous remarks.

Re conclusion 1: That the simultaneous determination of some eight components should prove feasible is quite gratifying when one considers the degree to which the spectra are overlapping. For a demonstration, we refer to Fig. 7.4, where, in the upper part of the figure, the recorded absorbances of solutions of seven of the C_9 compounds (nPr, 1M2E, 1M4E, 123tM, 124tM, 135tM) are shown. The lower part of the figure shows the (likewise recorded) spectrum of a mixture consisting of equal parts of the solutions above.The mixture spectrum has maxima too; however, these fail to coincide clearly with any of the constituent's maxima.

Re conclusion 2: The lower values of $\hat{\sigma}$ are invalidated by systematic errors such as slight wavelength-dependent cell differences, non-linearities, scattered light, diffraction effects and a host of other imperfections.

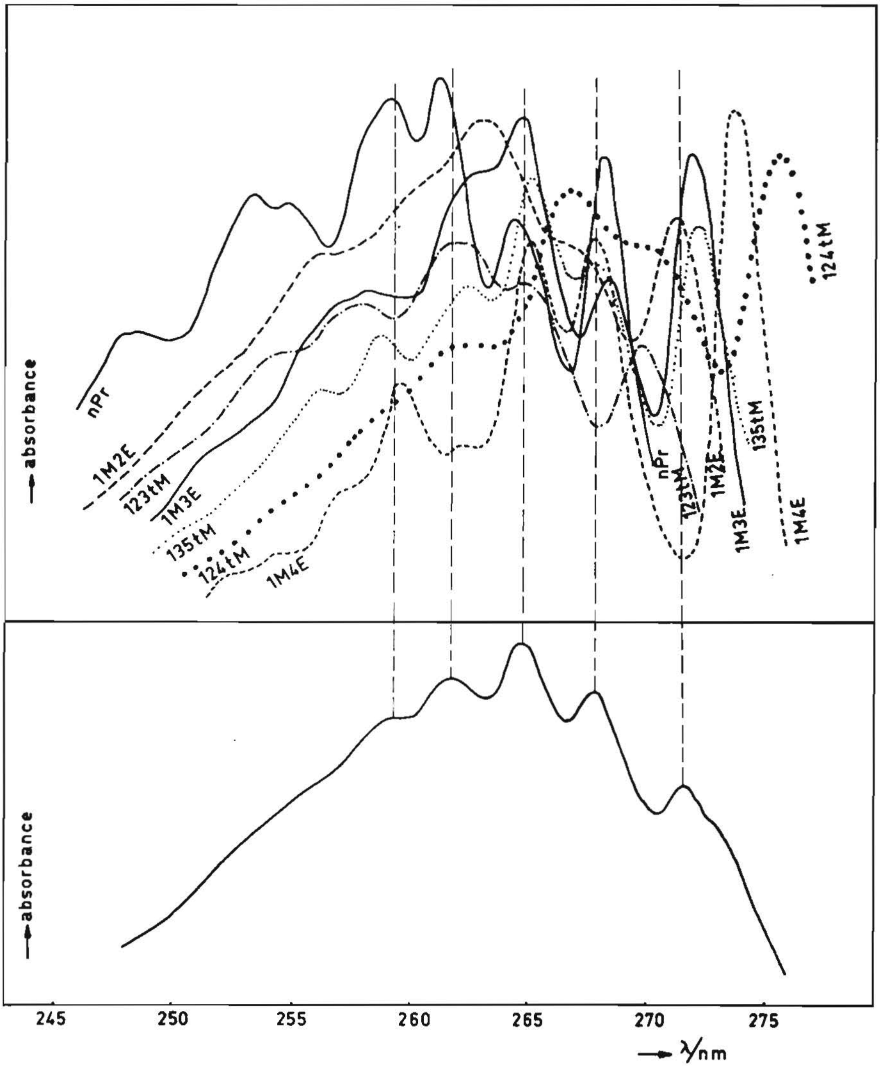

Fig. 7.4 Top: The absorption spectra of nine C_9 aromatics in *iso*-octane.
Bottom: The spectrum of a mixture consisting of equal parts of the absorbers shown above.

Re conclusion 3: The better resolving power of spectrophotometry was convincingly demonstrated in the case of 1M3E and 1M4E, which have only moderately correlated spectra, but could not be separated by GLC under our experimental conditions. However, in fairness it should be added that such a separation is possible in principle on PEG columns and has indeed been achieved by Martin and Winters.

7.3 Other applications

So far, other applications of the method presented have been reported only from the laboratory for Organic Chemistry of the University of Amsterdam. From the publications (Cerfontain c.s. 1963, Arends c.s. 1964, Vollbracht 1962, Wanders 1964) and from a personal communication from Dr. Cerfontain, we learn that the applications to mixtures of various mononuclear arylmono- and disulfonic acids have been completely successful.The routine use of multicomponent spectrophotometry by overdetermined systems has led,in the hands of the authors cited,to many hundreds of successful analyses, chiefly with aqueous and fuming sulfuric acids as the solvents. It is expected that this routine use will be continued for some time to come, since their work is in full progress and the alternative analytical method available, inverse isotope dilution, is at present far more laborious and time-consuming.

Acknowledgements

The work described in the first two sections of this chapter would have been impossible but for the generous cooperation kindly given at Eindhoven by Ir. J.J. Walraven, who not only most ably coordinated the experiments but also volunteered to take an active part in them, and by Mr. H. Groenendijk, who was chiefly responsible for the gas chromatography. To them, the author wishes to express his deep gratitude. He also gladly acknowledges his indebtedness to Dr. Cerfontain for the readiness with which the method presented was accepted as a routine analytical procedure by him and his Amsterdam group. A stipend granted by Dutch State Mines to Mr. Groenendijk is also gratefully recorded as a material contribution to the progress of this work.

SAMENVATTING

Dit proefschrift behelst een onderzoek, gericht op het verbeteren van de nauwkeurigheid in de spectrofotometrie door het gebruik van overbepaalde stelsels lineaire vergelijkingen. Meer in het bijzonder wordt de toepassing op multicomponent-analyses in het gebied toegankelijk voor kwarts-prisma instrumenten beschreven.

In Hoofdstuk I wordt de probleemstelling en ontwikkeling van het onderzoek in grove trekken geschetst; een samenvatting van de inhoud wordt gegeven en de, overigens schaarse, literatuur wordt besproken, terwijl tenslotte de mogelijkheid van volledige automatisering aangestipt wordt.

Hoofdstuk II geeft een beschrijving van de gevolgde werkwijze en behandelt het verloop in de tijd van waargenomen extincties; de non-reproduceerbaarheid van deze waarnemingen dwingt ertoe, zo concluderen wij,de extincties van standaarden (referenties) vrijwel gelijktijdig met die van het te analyseren mengsel te meten. De theoretische wenselijkheid van het waarnemen van tot dusver ongebruikelijk hoog geachte extincties wordt besproken; de mogelijkheid van het gebruik van extincties tot 1,6 (zonder wezenlijk verlies aan meetnauwkeurigheid) wordt aangetoond. De zin van het gebruik van overbepaalde stelsels vergelijkingen komt aan de orde; tot de voordelen hiervan behoren vergrote nauwkeurigheid,een, zij het beperkte mate van zekerheid ten aanzien van de juistheid, de mogelijkheid tot het uitsluiten van schrijffouten en tot het vaststellen van het gebruik van te grote spleetbreedten. Ook kan de aanwezigheid van een vreemde component worden geconstateerd, terwijl voorts de gevoeligheid voor cuvet-verschillen kan worden verminderd. Ook het kunnen beschikken over een maat voor de nauwkeurigheid, die uit de waarnemingen van een analyse zelf wordt verkregen, moet als een zeer aanmerkelijk voordeel beschouwd worden.

Hoofdstuk III geeft een wiskundig model, waarin o.a. enige redelijke eigenschappen van de klassieke kleinste kwadratenschatter $\hat{\underline{x}}$ ter sprake komen. Deze puntschatter verdient -mits de meetfouten klein zijn ten opzichte van de meetwaarde- de voorkeur boven een andere theoretisch juistere schatter, $\hat{\underline{z}}$. De betrekking tussen $\hat{\underline{x}}$ en $\hat{\underline{z}}$ wordt besproken. Bovengrenzen voor enige funkties van $\hat{\underline{x}} - \hat{\underline{z}}$ worden afgeleid, waaruit de voldoende juistheid van de benadering van $\hat{\underline{z}}$ door $\hat{\underline{x}}$ volgt; dit levert tevens een efficiënt rekenvoorschrift voor $\hat{\underline{z}}$. Een variant (Model II) van het model wordt ingevoerd en de equivalentie van twee verschillende rekenwijzen wordt aangetoond.

Hoofdstuk IV behandelt de gebieds-schatting. De covariantiematrix van $\hat{\underline{x}}$ wordt verkregen en gebruikt voor enige eenvoudige toetsen en betrouwbaarheidsgebieden. Verfijndere toepassingen stuiten op de moeilijkheid dat de coëfficiëntenmatrix zelf met fouten behept is. Onder de aanname dat de fouten normaal verdeeld zijn leidt een analogie met het "klassieke" geval tot het opstellen van drie hypothesen over de verdeling van bepaalde grootheden. Toetsen van deze hypothesen met een Monte Carlo methode voert tot de conclusie, dat deze verdelingen voor praktisch gebruik voldoende overeenstemmen met χ^2- en F-verdelingen. Hieruit volgt dat toetsen en betrouwbaarheidsgebieden, analoog aan die geldend voor de klassieke multivariate regressie, ook voor ons geval beschikbaar zijn; het gebruik hiervan wordt besproken.

Hoofdstuk V is gewijd aan een nadere analyse van het als lineair aangenomen verband tussen extinctie en concentratie. Bij van nul verschillende spleetbreedte wordt de lineariteit, naar bekend is, verstoord. Dit effect wordt aan het model van een Lorentzkromme voor de extinctie en een driehoekige spleetfunktie numeriek nagegaan, waaruit maximaal toelaatbare verhoudingen $s/\Delta\nu_{\frac{1}{2}}$ voor het geval van één component volgen. De invloed van $s/\Delta\nu_{\frac{1}{2}}$ op multicomponent systemen wordt uiteengezet. Wij tonen aan dat bij kleinste kwadratenanalyse de residuën bij dit type non-lineariteit een karakteristiek patroon vertonen, waardoor dit type afwijking als zodanig herkend kan worden.

In Hoofdstuk VI wordt het gebruik van bekende mengsels van componenten als referenties besproken. Na een theoretische behandeling van dit onderwerp passen wij deze techniek o.a. toe op het nabije infra-rood, waar notoire afwijkingen van de lineariteit optreden. Wij tonen aan dat deze variant in een aantal vrij sterk non-lineaire twee-componenten systemen tot het gewenste doel voert. Bij een bepaald drie-componenten systeem faalt deze opzet echter; de analyse-uitkomsten zijn, chemisch gezien, onbevredigend. Dit moet echter aan de geringe onderscheidbaarheid van de spectra en niet aan het gebruik van mengsels als referenties geweten worden; van dit laatste wordt namelijk aangetoond dat het in principe geen invloed op de nauwkeurigheid heeft.

Hoofdstuk VII behandelt tenslotte enige toepassingen, en wel eerst het systeem cortison acetaat/prednison acetaat, met sterk op elkaar gelijkende spectra. Vervolgens worden de aromatische C_9 koolwaterstoffen in een terpentina-fractie geanalyseerd, waarbij minstens acht componenten in één mengsel bevredigend naast elkaar bepaald blijken te kunnen worden. De verkregen resultaten zijn in overeenstemming met die van de gas-vloeistof chromatografie en, naar zich laat aanzien, voor de hoofdcomponenten van de fractie zelfs nauwkeuriger. Enige andere praktische toepassingen worden aangestipt.

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LEVENSBERICHT

Op aanraden van de Senaat is hier een kort levensbericht van de schrijver opgenomen.

Hij werd op 18 maart 1928 te Rotterdam geboren. Na de lagere school bezocht hij het Gymnasium Erasmianum aldaar zolang de rassenwetten hem dat toestonden, en daarna achtereenvolgens de Joodse Lycea te Rotterdam en Amsterdam. Op last van de Duitse bezetter werd hij in april 1944 gearresteerd en via Westerbork naar Bergen - Belsen overgebracht. Na zijn bevrijding en terugkeer in Nederland werd in 1946 aan het Gymnasium Erasmianum het einddiploma verkregen.

Daarna studeerde hij scheikunde aan de Universiteit van Amsterdam, waar het doctoraal examen (met als hoofdrichting theoretische organische chemie) afgelegd werd in 1958. Hij trad vervolgens in dienst bij de Staatsmijnen in Limburg, doch bleef tot 1961 gedetacheerd bij het Laboratorium voor Organische Scheikunde der Universiteit van Amsterdam. Sinds januari 1966 is hij werkzaam bij de N.V. Electrologica te Rijswijk (Z.H.).

STELLINGEN

1.

Een gestandaardiseerde wijze van codering van organische structuurformules is voor de toegang tot gegevensverwerkende apparatuur ongewenst en overbodig.

Lit.: Rules for IUPAC Notation for Organic Compounds,
London 1961.

2.

Bij het beoordelen van door Röntgendiffractie verkregen structuren dient men rekening te houden met de vaak hoge correlaties van de geschatte toleranties in de parameters.

3.

Indien voldoend grote lichtstromen beschikbaar zijn biedt het gebruik van Raman-spectroscopie voor analytische doeleinden essentiële voordelen boven het gebruik van spectroscopie in het infra-rood.

4.

De methode van Zscheile c.s. ter schatting van de standaarddeviaties in spectrofotometrische multicomponent-analyses leidt niet tot het gestelde doel; hun werkwijze kan dienen als voorbeeld van het onbezonnen gebruik van een computer.

Lit.: Zscheile, Murray, Baker, Peddicord,
Anal. Chem., <u>34</u>, 1776 (1961).

5.

Het is aantrekkelijk de rijen $\underline{v}_i^{\tau}$ van de links-inverse $\underline{V}^{\tau} \equiv (\underline{A}^{\tau}\underline{A})^{-1}\underline{A}^{\tau}$ van een kleinste - kwadraten coëfficiënten-matrix $\underline{A}$ te berekenen uit $\underline{v}_i^{\tau} = \underline{r}_i^{\tau}/(\underline{r}_i^{\tau}\underline{r}_i)$. Hierin is $\underline{r}_i$ de residu-vector bij ontbinding in de zin van de kleinste kwadraten van de i-de kolom van $\underline{A}$ op de overige kolommen; de normeringsfactor $(\underline{r}_i^{\tau}\underline{r}_i)^{-1}$ is gelijk aan het i,i-de element van $(\underline{A}^{\tau}\underline{A})^{-1}$.

6.

Het gebruik van vergrote rekenprecisie tijdens de herinversie bij lineaire programmering kan dikwijls vermeden worden door de spil-elementen op passende wijze te kiezen; dit kan met een zeer geringe uitbreiding van het gebruikelijke iteratie-algoritme verwezenlijkt worden.

7.

Het oplossen en opstellen van kruiswoordpuzzels - 'cryptogrammen' inbegrepen - is programmeerbaar voor rekenautomaten.

8.

Het is een eis van eenvoudige eerlijkheid de mate van zekerheid van de vertaling van bijbelteksten in de vertaalde tekst te doen blijken.

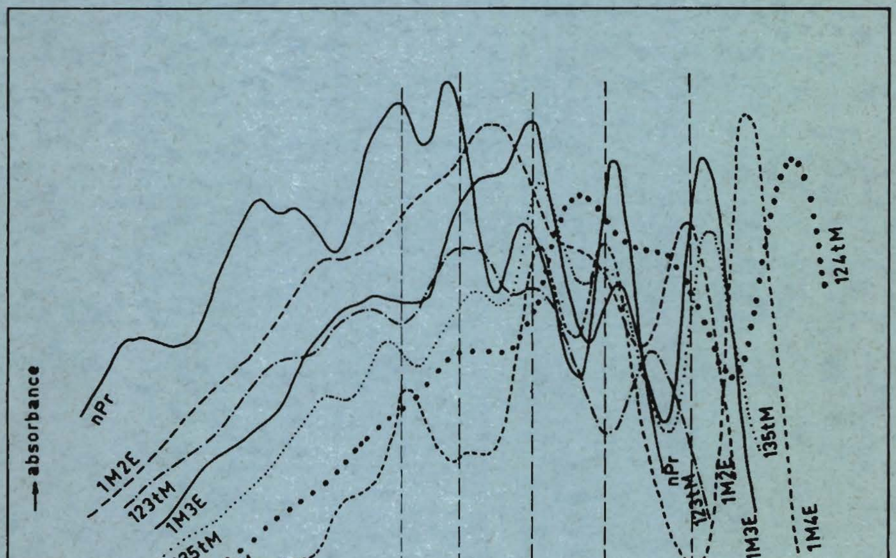
9.

Een commissie van de Akademie van Wetenschappen belaste zich met de redactie van de omschrijvingen van natuurwetenschappelijke termen in de Nederlandse woordenboeken.

10.

Het is in humaan zowel als in economisch opzicht wenselijk dat het onderwijs aan sterk begaafden tenminste evenzeer gesteund wordt als dat aan zwak begaafden.

Eindhoven, 5 juli 1966 I.S. Herschberg


→ absorbance
nPr
1M2E
123tM
1M3E
135tM
124tM
1M4E
→ absorbance
245
250
255
260
265
270
275
→ λ/nm