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Precise spectrophotometry of small quantities of drugs in the presence of irreproducible quantities of impurities

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A variety of methods of spectrophotometric assay of compounds in the presence of one or more irrelevant impurities have been recently described, employing, for example, the use of Legendre polynomials and trigonometric functions. The use of least-squares solutions of overdetermined systems (Scheid 1968) in spectrophotometry is outlined, with conditions under which simplified overdetermined systems have been used for precise spectrophotometric assay of small quantities of drug substances in the presence of largely irreproducible quantities of irrelevant impurities.

Error-free spectrophotometry implies the determinability of the concentrations of m substances in solution by measuring the absorbance of the mixture at m wavelengths, if the specific absorbance of each component at each of the m wavelengths is known. Instrumental error requires that measurements are made at a larger number of wavelengths, n, with application of some mathematical criterion of 'best fit'. Glenn (1963) described the use of 4 to 8 point (wavelength) orthogonal polynomials in the analysis of mixtures of one or two substances with an impurity, making possible the selective use of polynomial 'components' not found in the impurity. Such polynomials are more convenient to use than trigonometric functions, and equally as accurate.

Under certain conditions, for example, in the determination of low amounts of substances desorbing from cast polymer films or in other situations, least-squares simplified overdetermined systems may be as accurate, quicker and simpler to use. The present availability of fast programmable calculators and minicomputers can lead to improved accuracy through the use of a large number of data points, or wavelengths at which absorbance is measured.

Theory

If absorbance could be measured with negligible error over the wavelength range considered, the concentrations of m absorbing substances in solution could be found by determining the total absorbance, B, of the mixture at m different wavelengths, preferably near the peaks of each component, by solving the m simultaneous equations

$$A_{\alpha 1} C_1 + A_{\alpha 2} C_2 + A_{\alpha 3} C_3 \ldots + A_{\alpha m} C_m = B_\alpha$$

$A_{\alpha\beta}$ being the absorbance of the βth component at wavelength α, and C being the concentration of each component.

The existence of random errors superimposed on instrumental D.C. drifts, amplifier errors and photon statistics requires that the measurements of total absorbance B are carried out at a larger number of wavelengths, n, for greater accuracy. Then equations in m unknown concentrations constitute an overdetermined system which can be best solved, for $C_1 \ldots C_m$ in spectrophotometry, by least squares (Scheid 1968).

If the concentration of the measured model drug substance is c and $A_{\alpha 0}$ is its specific absorbance at the αth of the n wavelengths at which it is measured, and if the specific absorbance of the βth of m relevant impurities, at concentration c_β, at the same wavelength is $A_{\alpha\beta}$, then the rigorous overdetermined system from which $c \ldots c_m$ could be obtained may be expressed as

$$A_{10}c + A_{11}c_1 + A_{12}c_2 + A_{13}c_3 \ldots A_{1m}c_m = B_1$$

$$A_{20}c + A_{21}c_1 + A_{22}c_2 + A_{23}c_3 \ldots A_{2m}c_m = B_2$$

$$\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$$

$$A_{n0}c + A_{n1}c_1 + A_{n2}c_2 + A_{n3}c_3 \ldots A_{nm}c_m = B_n \quad (1)$$

where B_α is the total absorbance of the test solution of the αth wavelength, if each $A_{\alpha\beta}$ is known, and if $n > (m + 1)$. The larger n, the greater the precision; the n wavelengths preferably include the main absorbance bands of the substance assayed. The knowledge of every $A_{\alpha\beta}$ is required in the case of absolute irreproducibility in the concentration of each component, a condition not generally fulfilled in practice.

In some preliminary experiments carried out on the desorption of drugs from model polymer long-acting formulations (Vezin & Florence 1977), spectrophotometric absorption by irrelevant impurities was sometimes marked, and, in blank experiments carried out under the same conditions without the drug substances with simultaneous sampling and spectrophotometric assay, a variable absorbance background was obtained, arising from both absorbing and scattering impurities. This background may be regarded as having a total absorbance, or amplitude, E_α at the αth wavelength, where—

$$E_\alpha = \sum_{\beta=1}^{\beta=m} A_{\alpha\beta} \; F(D_\beta t) \quad .. \quad .. \quad (2)$$

at time t, D_β being the diffusion coefficient of the βth component in the polymer. For full descriptions of $F(D_\beta t)$ see Crank (1975) and Crank & Park (1968).

In samples from blank desorption experiments taken at equal times, it was found that although their absorbances, E_α were not generally reproducible, but varied between experiments, the shapes of the absorbing backgrounds in all cases could be approximated by $c_1 f_2(\lambda)$ where $f_2(\lambda)$ has the value $A_{\alpha 1}$ at wavelength α, different values of c_1 being assignable to each blank. The absorbance of the background, E_α, $(=A_{\alpha 1}c_1)$ plus the additional contribution to absorbance by drug alone, $A_{\alpha 0}c$, leads to total absorbance B_α, where

$$A_{\alpha 0}c + A_{\alpha 1}c_1 = B_\alpha \quad .. \quad .. \quad .. \quad (3)$$

leading to a simplification of the overdetermined system (eqn 1) by incorporation of columns 1 to m, to

$$\begin{aligned} A_{10}c + A_{11}c_1 &= B_1 \\ A_{20}c + A_{21}c_1 &= B_2 \\ &\cdots\cdots\cdots\cdots \\ A_{n0}c + A_{n1}c_1 &= B_n \end{aligned} \quad .. \quad .. \quad .. \quad (4)$$

from which c (and c_1) are obtained by least squares solution using n data points $A_{\alpha 1}$ obtained from a blank in which total background absorbance appears similar.

Application and method

Samples of the test solutions were taken, with simultaneous sampling from six to twelve blanks, and a few blanks selected with absorbance similar to the test solution in question ($c_1 = 1$). The mean absorbances of these solutions, A_{11} to A_{n1} were measured at each of n wavelengths, λ_α, and also the mean absorbances of a standard solution of the drug substance at the same wavelengths (A_{10} to A_{n0}) and those of the test sample (B_1 to B_n). Measurements were made directly from scans obtained with a Perkin-Elmer 124 scanning spectrophotometer, plus chart recorder with 5 cm cells (Fig. 1).

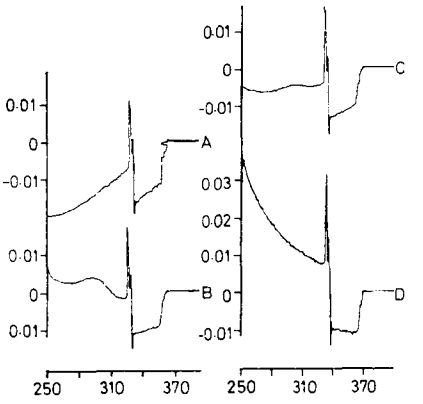

FIG. 1. Instrumental (double beam) scans: A, an instrumental base line, obtained with identical solutions of buffer in each beam. B, blank buffer with impurity, with c = 36·6 ng ml^{-1}(σ = 2·9 ng ml^{-1}) salicylic acid. C, c = 17·6 ng ml^{-1} (σ = 2·9 ng ml^{-1}) salicylic acid. D, a typical blank. All borate buffer, pH 8·0. Ordinate: absorbance. Abscissa: wavelength (nm).

The sample wavelengths, λ_α, and the values of n chosen generally depended upon the shapes of the total absorbance curves B_α obtained and the relative contribution due to the drug, and the wavelength of its absorbing maximum. With salicylic acid, experiments were confined to total maximum background absorbances of about 0·02–0·04, when sufficiently accurate values of c were obtained with values of n = 6 or 7, where the additional peak absorbance of the drug (A_{max}) = 0·02, increasing to n = 20 where A_{max} = 0·002~. The wavelengths were confined to the waveband in which at least 90% of the integrated absorbance of the drug occurs (where drug absorption is 'significant', i.e. 267–324 nm for salicylic acid). Inclusion of absorbance data outside these areas supplies no information, and may contribute slightly to random errors arising from instrumental base-line drifts. Since the main absorption band of salicylic acid is nearly symmetrical with respect to wavelength, as was true of nearly all the compounds studied (Vezin & Florence 1977) the wavelengths λ_α were selected symmetrically, equispaced, about the wavelength of absorbance maximum. The absorbance band of trinitrophenol is 'asymmetric', however, having a very steep cut off in the short-wave visual

region. This method was not used with this compound, there being little interfering absorbance in the desorption experiments described, in the visual region. However, its application may suggest that, with an equal number of wavelengths, λ_α, on each side of the absorbance maximum, the ratio of the spacing of the wavelengths on each side should be in the approximate inverse ratio of the slopes of each side of the absorbance band.

Solutions of equation 4 were carried out with a programmable TI 59 calculator.

RESULTS

Fig. 1 shows single representative scans obtained from experiments involving the desorption of salicylic acid from solid solution in poly-(methylmethacrylate) generally at lower concentrations than were used in the original experiments. Scan A was obtained with identical solutions of pure borate buffer, pH 8·0, in each beam. Scans B and C are comparisons of pure buffer with buffer samples obtained during desorption experiments, containing salicylic acid plus impurities. The two concentrations indicated were the two means of four solutions each of equation 4 (two scans of each sample, only one of which is illustrated, against two different blanks) using 20 equispaced wavelengths. σ is the standard deviation of the four single values of c. The peaks at 330 nm are instrumental artifacts (grating changes) affecting both the base line A and the scans B, C, and D equally, contributing no net error. Fig. 2 shows the total true absorbances, B_α and E_α corrected for the baseline. Fig. 3 shows C–A and its two absorbing components; salicylic acid and impurities.

Accuracy of the method

An additional set of experiments was carried out, in which known quantities of salicylic acid were added to samples from a single blank, and the same procedure followed through. The values of c are plotted (Fig. 4) with standard errors of the means as error bars, superimposed upon the zero-error line. The points obtained are means of six solutions each of equation 4 for concentrations 40 and 50 ng ml^{-1} (a single scan against single scans fom six different blanks), twelve each at concentrations 20 and 30 ng ml^{-1} (two scans each against the same six blanks) and eighteen at concentration 10 ng ml^{-1}.

Comments

At very low values of c ($A_{max} = 0{\cdot}002\sim$) the largest contributions to error are instrumental D.C. drifts in the shape of the base line, possibly arising from power supply variations (which are usually slow enough to be followed by taking occasional repeated scans of the base line and interpolating to the times of the scans of the test samples), and background noise. The examples shown were calculated without additional data smoothing, though small drifts in the base line were allowed for. There is no reason

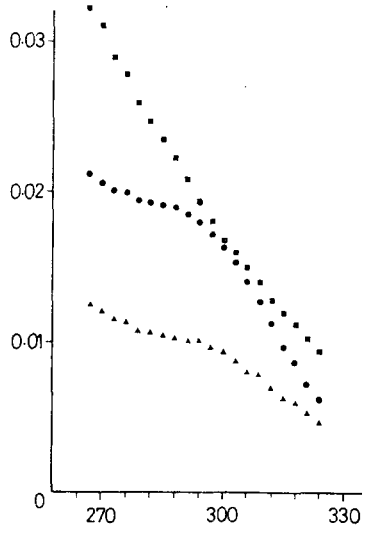

FIG. 2. Total absorbance of scans (Fig. 1) corrected for base line, plotted at wavelengths λ_α used in the calculations: total absorbances are B_α (●B-A and ▲C-A) and E_α (■ = (D-A)/2). Ordinate: absorbance. Abscissa: wavelength (nm).

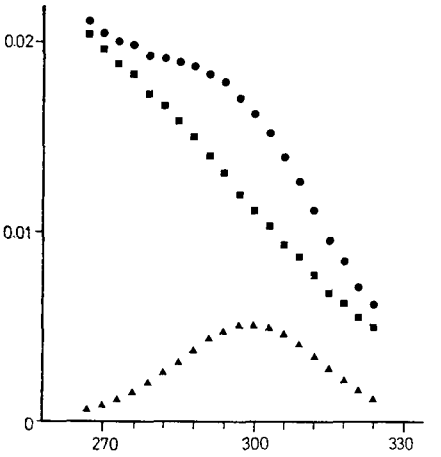

FIG. 3. Components of C-A arising from two components: C-A (●) = salicylic acid (▲) + impurity (■). Ordinate: absorbance. Abscissa: wavelength (nm).

why, with increased numbers of data points and using stabilized power supplies with data smoothing, higher accuracy (~1 ng) cannot be obtained.

The calculations should be carried out with the same number of different absorbing blanks as of scans of the test solutions (say, four of each), as, if only one or two test scans are calculated against a large number of blanks, or vice versa, small sample errors may occur which may be larger than the precision of the method (see point at 20 ng, Fig. 4).

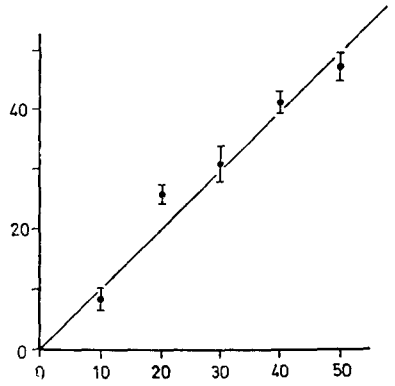

FIG. 4. Accuracy of the method: a plot of known vs calculated concentration (ordinate: ng ml^{-1}) of salicylic acid in a single blank (see text). Abscissa: salicylic acid concentration (ng ml^{-1}).

The applicability of the method, i.e. of the simplified overdetermined system (eqn 4) is shown by the invariance of the mean values of c obtained, from a number of scans of the test solution, with the values of c_1 of a range of blanks; a significant increase or decrease in c with increasing c_1 would suggest either a significant change in the shape of the absorbance curves with time, or an absorption curve shape $f_2(\lambda)$ which depends upon total absorbance, at a fixed time. Such a state of affairs would require recalculation of c with blanks of absorbance c_1 chosen closer to that of the test. This was not observed in these experiments, however; for example, the absorbances of the six blanks used to calculate the data points of Fig. 4 were in the range 0·5–2·5 of that of the test samples, without any systematic effect on c being observable.

If in some cases the interfering absorbance can be described by a larger overdetermined system ($m \geqslant 2$ m, eqn 1) a minicomputer, rather than a calculator is required. But even with the simplified system described, a direct five-figure absorbance readout, with smoothing and direct minicomputer interfacing, would be a distinct advance in time and accuracy.

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