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The temperature dependence of derivative ultraviolet absorption spectra

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Abstract: The effects of variation of temperature in the range 0°–40°C on the zero and second derivative ultraviolet absorption spectra of ten compounds have been investigated. When the temperature of the solutions was increased, most of the substances showed a linear reduction of maximum absorbance (A_{max}), with temperature coefficients of $-0.07 \pm 0.03\%$ per degree. The second derivative amplitudes of all the substances were reduced, with temperature coefficients (-0.1 to -0.5% per degree) that bore no significant relationship to those of the corresponding A_{max} values. These effects on the extrema of the derivative spectra are explained by the small reduction in curvature at the corresponding wavelengths of the fundamental spectra, that occurs with increasing temperature. The precise and accurate assay of substances by derivative UV spectrophotometry requires that the temperatures of the standard and sample solutions are identical at the time of measurement.

Keywords: *Ultraviolet spectrophotometry; derivative spectrophotometry; temperature effects.*

Introduction

The ability of modern instrumentation to convert absorption spectra to higher order derivative spectra has greatly extended the range of applications of ultraviolet-visible spectrophotometry. Derivative spectrophotometry is of particular benefit in the assay of drugs, impurities or degradation products in pharmaceutical formulations in which the presence of other absorbing substances, such as co-formulated drugs and excipients, may otherwise interfere in a conventional spectrophotometric assay [1].

The measured value in a quantitative derivative spectrophotometric assay is normally the amplitude between a maximum and minimum that is least affected by the other components of the sample. Derivative spectral amplitudes depend on the slope and curvature of the fundamental spectrum around the wavelengths of the amplitude rather than on the absorbance; substances of narrow spectral bandwidth display larger amplitudes than those of broad bandwidth. The discrimination of the technique in favour of narrow bandwidth substances in samples also containing broad band components that interfere in conventional absorbance measurements has markedly enhanced the accuracy and specificity of spectrophotometric assays of many substances [2–5].

During the development of derivative ultraviolet spectrophotometric procedures for benzenoid drugs in formulations [6–8] it was observed that while in the cell compartment

solutions of stable drugs displayed steadily decreasing second and fourth derivative amplitudes; this was attributed to the increase in temperature of the solution. An investigation of the effects of temperature variation on the fine structure of the fundamental spectra in the region 240–270 nm showed that the maxima are barely affected by an increase of temperature whereas the minima between the bands increase significantly, with temperature coefficients (percentage change at 20°C per degree increase) as high as +0.32% [9]; this was explained by the phenomenon of band broadening with increasing temperature [10–13]. The small increase in spectral bandwidths of the fundamental spectra was also found to result in second and fourth derivative amplitudes which decrease linearly with increasing temperature, with temperature coefficients in the range −0.60 to −1.12% for second derivative amplitudes and −0.71 to −1.20% for fourth derivative amplitudes.

The present work was undertaken to extend the knowledge of temperature effects on the derivative amplitudes of drugs and other substances and to assess the importance of temperature control in their derivative spectrophotometric analysis.

Experimental

Substances

Acetazolamide was obtained from Sigma London Chemical Co. Dipipanone hydrochloride was obtained from Burroughs Wellcome and Co. Promethazine hydrochloride was obtained from May & Baker. Adrenaline hydrogen tartrate, caffeine, holmium oxide, phenol (Analar), potassium dichromate (Analar), potassium hydrogen phthalate (Analar) and sodium salicylate were obtained from BDH Chemicals.

Spectra

Zero order spectra of the substances were recorded over the wavelength range indicated in Table 1 using a Perkin–Elmer 552 double-beam ultraviolet-visible recording spectrophotometer. The scan speed was 2 nm s^{-1}, the response (time constant) 0.5 s, the

Table 1
Temperature coefficients of absorbance and second derivative amplitudes

Substance	Concentration ($\mu g\ ml^{-1}$)	Solvent*	Wavelength range (nm)
Caffeine	30	0.5M HCl	310–240
Adrenaline hydrogen tartrate	125	0.01M HCl	310–240
Promethazine hydrochloride	100	0.01M HCl	360–220
Potassium hydrogen phthalate	150	1% (v/v) $HClO_4$	310–250
Sodium salicylate	50	Water	350–240
Acetazolamide	30	0.1M HCl	330–230
Phenol	40	0.1M NaOH	320–240
Phenol	50	Ethanol	310–220
Potassium dichromate	30	0.05M KOH‖	470–220
Dipipanone hydrochloride	800	Water	340–240

*Aqueous solution unless otherwise specified.

†Wavelength in nm of maximum absorption unless specified as a minimum (min) or shoulder (sh).

‡Temperature coefficient as % of absorbance or derivative amplitude at 20° per degree. The values are uncorrected for the coefficients of cubical expansion of the solvents.

§Wavelength in nm of the minimum in the derivative spectrum measured to its longer (L) or shorter (S) wavelength satellite.

spectral slit width 1 nm, and the ordinate maximum settings were selected to record the A_{max} values at approximately 80% of full-scale deflection. Second derivative spectra, generated using a Hitachi derivative accessory, were recorded simultaneously with the zero order spectra. The mode setting was either 5 or 6, selected to record the largest derivative amplitude at greater than 50% full-scale deflection. Fourth derivative spectra of holmium perchlorate solutions were obtained using the Hitachi derivative accessory operating in the second derivative mode (mode 5) in series with the spectrophotometer operating in the second derivative mode.

Temperature variation

The spectra of the solutions in a 1-cm jacketted quartz cell were recorded at 5° intervals over the temperature range 0°–40°C, as previously described [9].

Calculation of temperature coefficients

Measurements of absorbance in the zero order spectrum and amplitudes in the second and fourth derivative spectra of each solution were made in triplicate at each temperature. Regression analysis was applied to the mean values (y) of amplitude (in mm) or absorbance and temperature, (x) (in °C), using the method of least squares. If a significant linear correlation was found to exist at the 95% probability level between the measured y value and temperature, the linear regression equation was calculated in the form $y = a + bx$. The temperature coefficients, as a percentage per degree at 20°C, were calculated from $100b/y_{20}$ where y_{20} is the amplitude or absorbance at 20°C, calculated using the regression equation.

Results and Discussion

The substances were selected to provide examples of inorganic compounds (potassium chromate, holmium perchlorate) and simple acidic (phenol, salicylic acid), basic (dipipanone) and 'neutral' (caffeine) organic compounds together with several sub-

Table 1 (continued).

Zero order spectra				Second derivative spectra			
λ_1†	Temperature coefficient‡ (column A)**	λ_2†	Temperature coefficient‡	Amplitude§	Temperature coefficient (column B)**	Amplitude§	Temperature coefficient‡
269	−0.071			269_L	−0.12	269_S	−0.14
278	−0.093			278_L	−0.22		
298	−0.100	248	−0.035	298_L	−0.21	248_L	−0.10
275	−0.051	262(min)	+0.054	282_L	−0.48		
295	−0.042			295_L	−0.09	295_S	−0.07
266	−0.080			266_L	−0.26	266_S	−0.23
286	+0.011			286_L	−0.34		
272	−0.238	278(sh)	−0.187	278_L	−0.50		
373	−0.078	274	−0.062	373_L	−0.15	274_L	−0.09
258	NS¶	290	NS¶	258_S	−0.55	290_S	−0.15

‖The spectrum is that of potassium chromate.
¶NS = non-significant linear relationship.
**The values in column A (excluding those of phenol in ethanolic solution and dipipanone) have no significant linear relationship with the corresponding values in column B.

stances of higher molecular weight that exhibit a range of spectral bandwidths. Emphasis was given to the effects of temperature variation on the second derivative spectra; this is the derivative mode most frequently employed in quantitative spectrophotometry since it provides a satisfactory compromise between specificity and precision.

Table 1 records the temperature dependence of the A_{max} (absorbance at the wavelength of maximum absorption) of a number of substances in the range 0°–40°C. All those examined, except dipipanone and phenol in alkaline solution, showed a small linear reduction of A_{max} with increasing temperature, with temperature coefficients in the range −0.04 to −0.10% per degree at 20°C. The values of −0.078% and −0.062% for potassium chromate solution at its maxima 373 and 274 nm, respectively, and −0.051% and +0.054% for potassium hydrogen phthalate at its maximum and minimum 275.5 and 262 nm are in very good agreement with those previously reported (−0.09%, −0.06%, −0.05% and +0.05%, respectively) for the temperature range 17°–37°C [14]. Table 1 also shows the temperature coefficients of the amplitudes of one or more second derivative bands of each solution. Every amplitude is reduced as a result of the increase of temperature, the amplitude and temperature bearing a linear correlation at the 99% level of confidence. There was, however, no significant linear correlation between the temperature coefficients of absorbance and those for the reduction of second derivative amplitudes, indicating that the temperature dependence of derivative amplitudes is not simply a direct consequence of the change in A_{max} values.

To investigate if a relationship exists between the temperature coefficients of absorbance and derivative amplitudes for the different absorption bands of the same substance, zero, second and fourth derivative ultraviolet-visible spectra (500–300 nm) of holmium (III) perchlorate solution (5% holmium oxide in 17.5% (v/v) perchloric acid) were recorded at temperatures in the range 0°–40°C, increasing with 5° increments. The results in Table 2 show that at seven wavelengths of maximum absorption, the absorbance decreases with increasing temperature, with temperature coefficients in the range −0.06 to −0.27%; these changes are accompanied by reductions in the second derivative amplitudes and, in most instances, by larger reductions of fourth derivative amplitudes. Linear regression analysis of the data shows that, although there is a linear correlation that is significant at the 99% confidence level between the temperature coefficients of the second and fourth derivative amplitudes, there is no significant linear correlation between the temperature coefficients for the second or fourth derivative amplitudes and those for the reduction of absorbance.

As a second derivative spectrum is a plot of the curvature of the fundamental spectrum against wavelength, in which the derivative extrema correspond with the wavelengths of maximum curvature [2], the reduction of derivative amplitudes indicates that a reduction of curvature of the fundamental spectrum occurs with increasing temperature. To investigate the relationship between temperature, spectral curvature and derivative amplitudes, the zero order spectra of the substances in Tables 1 and 2 were recorded, each superimposed at 0°, 20° and 40°C, with a wavelength scale expansion of 1 nm cm^{-1}. Other than the reduction of A_{max}, there were no consistent changes in the spectral characteristics. Thermochromism, that is, a shift of λ_{max} to longer wavelengths with increasing temperatures, by up to 2 nm in the 0° to 40°C range, is exhibited by some substances, whereas others give identical λ_{max} values throughout the temperature range. Some compounds display significantly broader bands, particularly around the long wavelength base of the absorption band, with increasing temperature, whereas others show either no measurable change of spectral bandwidth or a reduced absorbance

Table 2
Temperature coefficients of absorbance and of second and fourth derivative amplitudes of holmium (III) perchlorate solution

Wavelength (nm)	D^0 Column 1*	D^2_L Column 2†	D^2_S Column 3†	D^4_L Column 4‡	D^4_S Column 5‡
486	−0.27	−0.57	−0.51	−0.70	−0.88
451	−0.06	−0.53	−0.64	—	−0.65
416	−0.22	—	−0.51	−0.70	−0.67
361	−0.13	−0.51	−0.41	−0.61	−0.72
287	−0.13	−0.30	−0.23	−0.36	−0.27
279	−0.14	−0.17	−0.20	−0.21	−0.23
241	−0.12	−0.13	−0.13	−0.13	−0.13
Linear correlation with values in column 1?		No§	No§	No§	No§
Linear correlation with values in column 2?				Yes‖	
Linear correlation with values in column 3?					Yes‖

*Temperature coefficients of absorbance at seven wavelengths of maximum absorbance (λ_{max}) in the fundamental (D^0) spectrum.
†Temperature coefficients of the second derivative amplitudes measured from the minima, corresponding to the λ_{max}, to the longer (L) or shorter (S) wavelength satellites.
‡Temperature coefficients of the fourth derivative amplitudes measured from the minima, corresponding to the λ_{max}, to the longer (L) or shorter (S) wavelength satellites.
§$p = 0.95$.
‖$p = 0.99$.

at all wavelengths under the absorption envelope. Notwithstanding these dissimilarities, a small but discernible reduction of curvature occurred in all spectra around the wavelengths of maximum negative curvature at the λ_{max} or shoulders, corresponding to the minima in the second derivative spectra, and around the wavelengths of maximum positive curvature at the minima and near the long wavelength base of the absorption band, corresponding to the short and long wavelength satellite maxima respectively in the second derivative spectra. These observations confirm that the reduction of derivative amplitudes is due to the slight reduction of curvature that occurs in the fundamental spectrum around the wavelengths of the derivative extrema. An example of these temperature effects is given in Fig. 1 which shows the zero and second derivative absorption spectra of potassium hydrogen phthalate at 0° and 40°C. The absorbance at the maximum 275.5 nm is 2.1% lower at 40°C than at 0°C. The reduction of curvature around the shoulder at 283 nm and around the long wavelength base of the absorption band results in a reduction of the largest second derivative amplitude, measured from the minimum at 283 nm to its long wavelength satellite, of 19.5% over the 0°–40°C temperature range.

The temperature coefficients of the second derivative amplitudes of the non-benzenoid compounds (Table 1) are generally smaller in magnitude than those of the fine structure of benzenoid compounds already reported [9]. This difference is also demonstrated by dipipanone which has both fine structure at 250–270 nm, typical of benzenoid compounds, and also a broad maximum at 290 nm due to the $n \rightarrow \pi^*$ transition of the β-ketone group. The temperature coefficients of the largest second derivative amplitude in the fine structure, measured from 258 nm to its short wavelength satellite, and of the amplitude corresponding to the broad band maximum at 290 nm measured to its short wavelength satellite, are −0.55% and −0.15%,

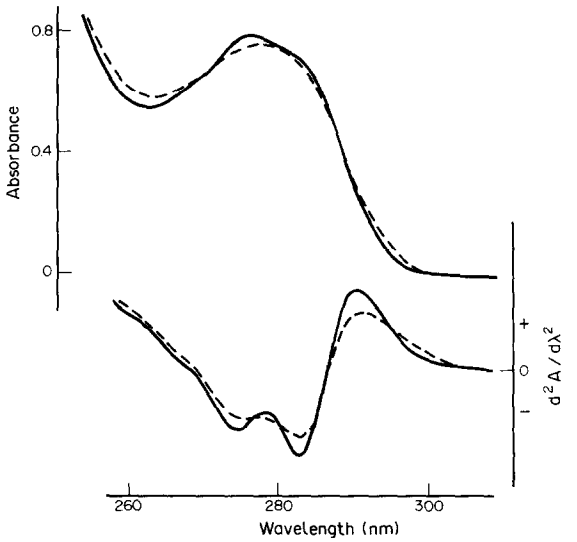

Figure 1
The zero order (upper) and second derivative (lower) absorption spectra of potassium hydrogen phthalate (125 μg ml^{-1}) in 1% (v/v) aqueous perchloric acid at 0°C (solid line) and at 40°C (broken line).

respectively. These differences are probably due to a smaller reduction of curvature of broad, single bands occurring at elevated temperatures than of the fused narrow bands of the fine structure of the benzenoid compounds, particularly around the minima of the latter which are affected by the broadening of two adjacent bands [9].

For eight of the ten samples in Table 1, the temperature coefficients of at least one of their second derivative amplitudes are in excess of −0.2%. Values of this order are sufficiently high to justify a special requirement of derivative spectrophotometric assays that all standard and sample solutions have an identical temperature during the recording of derivative spectra; this requirement would enable results of the highest accuracy and precision to be obtained.

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