

PROBLEMS IN THE DETERMINATION OF PHENOLIC pK_A VALUES BY SPECTROPHOTOMETRY

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Precise dissociation constants of some phenolic bibenzyls were required as part of a QSAR investigation. A u.v. spectrophotometric technique was employed, depending on dissociated and undissociated species absorbing at different wavelengths. The procedure is described in detail by Albert & Serjeant (1971). In preliminary experiments with hexoestrol and stilboestrol, 3 buffer combinations were used, borax/KOH, $Na_2CO_3/NaHCO_3$ and butylamine/HCl, providing 7 buffer solutions, covering a range of pH values from 9.4 to 11.4. The pK_a values were reproducible at each individual pH, but appeared to increase progressively with increasing pH. The change was subsequently linked with time. Determinations had always been carried out starting with the lowest pH mixture and working up the pH scale. When the sequence was altered, a different pK_a/pH profile emerged, the pK_a values changing with the time interval between preparation of solution and measurement of absorbance. Autoxidation, which is known to occur in aqueous solutions of phenols, was therefore suspected. The reaction is accompanied by formation of quinones (Kenyhercz & Kissinger, 1978), which are stronger chromophores than phenols. The absorbances of the synthetic oestrogen solutions increased with time, commensurate with this, but the rate of increase decreased with increasing pH, until at pH values in the region of 13 (0.1 M NaOH) the absorbance remained constant. The observed pK_a values must therefore have been dependent on the pH of the medium, as well as the time interval between preparation of solution and measurement of absorbance. Variation in the rate of autoxidation of phenols with pH has been noted before (Beynon & James, 1967) and attributed to the unionised phenol being the reactive species. The above observations fit this suggestion.

The absorbances reached limiting values, assumed to represent exhaustion of the dissolved oxygen. When the measurements were repeated using freshly boiled and cooled water, and in an atmosphere of nitrogen, more stable readings were obtained, but the decline in absorption with time was not completely eliminated. More drastic methods of removing oxygen are therefore necessary.

An alternative approach was attempted, whereby plots of absorbance against time were extrapolated back to zero time. Curve fitting calculations indicated that the two variables were binomially related, so that absorbances at zero time could be obtained from the characteristic quadratic equations. The results yielded pK_a values which were independent of time. There was no drift with pH, and the reproducibility was significantly improved.

The experiences encountered here suggest that some previously reported phenolic pK_a values may not be as precise as is believed.

Albert. A. & Serjeant. E.P. (1971) In The Determination of Ionization Constants, pp 44-64. London: Chapman and Hall.

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