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NOMOGRAM FOR THE RAPID DETERMINATION OF THE CONTRIBUTION OF SCATTERED LIGHT TO THE ABSORPTION SPECTRA OF BIOPOLYMER SOLUTIONS

E. E. Gussakovskii and A. A. Abramov UDC 616-073.584+57:541.64+57:547

It is known that in the use of the absorption spectra of solutions of biopolymers (proteins, nucleic acids) in the ultraviolet region, in addition to the true absorption D_{true} due to the chromophores an additional optical density D_{sc} due to the Rayleigh scattering of the light is recorded, and

$$D_{true} = D_0 - D_{sc}, \quad (1)$$

where D_0 is the measured optical density.

It must be noted that D_{sc} may amount to 80% of D_0 [1], and therefore the measurement of D_{sc} is of prime importance.

At the present time it is customary to find D_{sc} by means of the relation

$$\lg D_{sc} = \lg a - n \lg \lambda, \quad (2)$$

which follows from the generalized formula of Rayleigh scattering [2]. However, it is extremely laborious and requires the expenditure of much time to find D_{sc} with the aid of formula (2).

The nomogram shown in Fig. 1 permits the process of finding D_{sc} to be considerably shortened and simplified. For this purpose it is sufficient to transfer the absorption spectrum pointwise to a nomogram, to find the region of wavelengths in which the points fall on a straight line (this region will correspond to the absence of true absorption), and to perform linear extrapolation. The value of D_{sc} must be found by recalculation from the extrapolation line with the ordinate corresponding to the selected wavelength. The resolving capacity of the nomogram is 0.2-0.3% of the optical density of the scattered light to be determined. The accuracy of measuring D_{sc} is determined by the accuracy of plotting the experimental points on the nomogram and the accuracy of extrapolation. The time necessary for determining D_{sc} is about 1 minute.

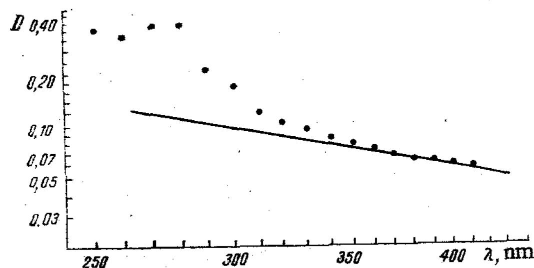

Fig. 1. Nomogram for determining the contribution of the apparent optical density due to the scattering of light in a measured absorption spectrum. The points show the absorption spectrum of an aqueous solution of thyroglobulin. The optical density of light scattering at 280 nm is 0.110.

Institute of Biochemistry, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 740-741, September-October, 1979. Original article submitted April 25, 1979.

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The nomogram is based on the principle expressed in formula (2). Log D and log λ are plotted along the axes of ordinates, but instead of the logarithms each division is assigned the value of the magnitude at which the logarithm is taken (D and λ, respectively). In view of this there is no necessity for any mathematical operations whatever. Of course, if desired, it is not difficult to plot a nomogram with different ranges of D and λ.

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MODIFICATION OF LYSOZYME BY MONOMYCIN A IN THE PRESENCE OF A WATER-SOLUBLE CARBODIIMIDE

I. G. Smirnova, A. V. Skladnov,
G. S. Katrukha, and G. V. Murav'eva

UDC 547.964

The lysozyme of hens' egg protein is a globular protein consisting of a single polypeptide chain including 129 amino acid residues. An excess of 19 positive groups over 11 negative groups (taking the N and C-terminal groups into account) imparts highly basic properties to the protein molecule [1, 2].

Monomycin A (paromomycin) has five free amino groups, the most reactive NH_2 group being that of deoxystreptamine [3, 4].

As the main method of condensing lysozyme with monomycin A we selected the carbodiimide method [5] and, as experiment showed, the most promising substances in this respect proved to be water-soluble carbodiimides [6, 7].

The product of the condensation of lysozyme with monomycin A was obtained with a threefold excess of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide and monomycin A per COOH group of the protein (pH 4.8; 20°C; 24 h). The preparation was purified by dialysis.

The homogeneity of the preparation obtained was checked by paper electrophoresis in the 1 N CH_3COOH and the $HCOOH$—CH_3COOH—H_2O (28:20:52) systems. The formation of a covalent complex of lysozyme and monomycin was confirmed by the composition of an acid hydrolysate of this complex determined with the aid of a Hitachi type KLA-3B amino acid analyzer. As markers we used glucosamine, deoxystreptamine (components of monomycin A), and an acid hydrolysate of lysozyme.

The results of the amino acid analysis showed that condensation had formed a covalent lysozyme—monomycin A complex in which there was one mole of monomycin A to one mole of lysozyme.

The results of physicochemical and biological investigations of the lysozyme—monomycin complex obtained will be published in the near future.

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M. V. Lomonosov Moscow State University.

Translated from Khimiya Prirodnykh Soedinenii, No. 5, p. 741, September-October, 1979. Original article submitted May 3, 1979.

0009-3130/79/1505-0659$07.50

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