

Results and Conclusions

We have summarized our analytical results and calculations of the molecular weight of the simplest casein species in Table XII and conclude that the value 98,000 for the molecular weight agrees best with all our different analyses and also with our previous results with the ultracentrifuge.

Summary

1. The protein contained in crude casein, which has been shown to have a molecular weight between 75,000 and 100,000 by the ultracentrifuge, has been subjected to analysis.
2. The sulfur, phosphorus, cystine, tryptophane, tyrosine and histidine contents have been found to be 0.785, 0.856, 0.488, 1.237, 5.55 and 1.776%, respectively.
3. The most probable value for the molecular weight of this protein, consistent between the above analyses and the results obtained with the ultracentrifuge, was shown to be 98,000.

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THE ABSORPTION SPECTRA OF ORTHO-CHLOROPHENOL INDOPHENOL AND OF ORTHO-CRESOL INDOPHENOL

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Spectrophotometric determinations of the absorption spectra of *o*-chlorophenol indophenol and of *o*-cresol indophenol¹ between wave lengths of 480 and 680 $m\mu$ are presented in this paper. Two samples of each dye were used: a very pure sample² obtained from Dr. W. Mansfield Clark, to whom the writer is indebted for its presentation, and a commercial sample from the LaMotte Chemical Products Co.

The dyes were dissolved in various buffer solutions in concentrations of 0.00005, 0.0001 and 0.0002 *M*. These concentration values were based upon the assumption that the samples used consisted of the designated dyestuffs exclusively. Since in the case of one sample, this was apparently not true, the actual concentrations in this case were probably lower than

¹ Cohen, Gibbs and Clark, *Public Health Rpts.*, 39, 381 (1924).

² Dr. Clark writes: "Each of the dyes was sufficiently free from components of an oxidation-reduction system other than the dye system to permit fairly accurate characterizations of the oxidation-reduction potential of the true dye system, but no claim is made that the material consists of dye alone. Indeed, it was found preferable to salt-out the sodium salts of the indophenols rather than to attempt methods of purification which were found to produce appreciable decomposition of the dye with the introduction of other oxidation-reduction systems. In consequence, many of the preparations in this group of dyes contain large quantities of sodium chloride."

those given. At P_H 8.0, Sørensen's phosphate buffers were used; at P_H 's 8.6 and 9.6, Clark's boric acid buffers in the concentrations indicated. These buffers differ in salt concentration but neither adding sodium chloride nor changing the concentration of the buffer produced any change in the absorption curve.

The analyses were made with a 1928 model Bausch and Lomb spectrophotometer.

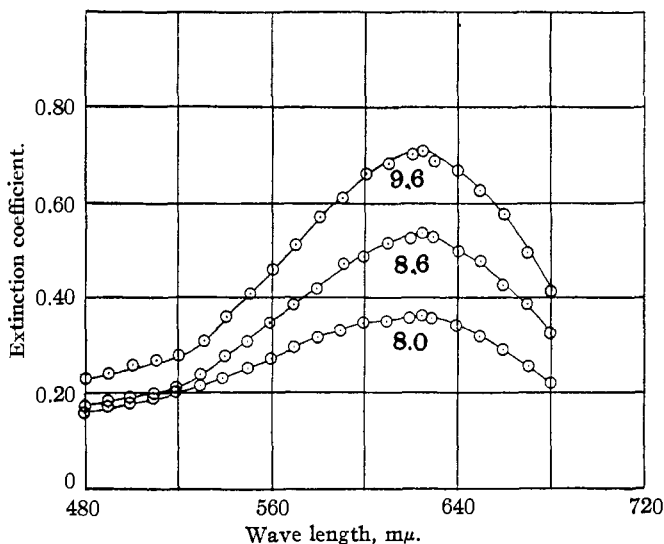


Fig. 1.—*o*-Chlorophenol indophenol: 0.0001 M ; Clark's sample.

Figures 1 and 2 represent the absorption curves of Clark's sample and of the commercial sample of *o*-chlorophenol indophenol, respectively. The letters on the curves refer to P_H of the solution. In both cases the absorption maximum is at 625 $m\mu$. Since the two samples give extinction coefficients that are almost identical, they would appear to have essentially the same purity with respect to dye content. Since this dye is almost completely dissociated at P_H 9.6 (pK , 7.0) the absorption curve at that P_H is that of the blue ion. The curves at P_H 8.0 and 8.6, which represent predominantly also the blue ion, differ from that at P_H 9.6 only in the height of the peak, decreasing as the P_H decreases. In Fig. 2 9.6 (2) represents a concentration of 0.0002 M or twice that of the remaining curves of both figures. The height of the maximum in this case is about twice that of the corresponding curve at half the concentration, as demanded by the Lambert-Beers Law.

Figures 3 and 4 are absorption curves of *o*-cresol indophenol, based upon Clark's and the commercial sample, respectively. Since the pK in this case is at 8.4, the dye at 9.6 is about 95% dissociated, and the absorption

maximum of the blue ion is at $610\text{ m}\mu$. As the P_{H} and the concentration of the dye decrease, there is a decrease in the height of the peak. In these curves the symbol (1) represents a M concentration of 0.00005 ; (2), 0.0001 M and (3), 0.0002 M .

At P_{H} 8.0 the red acid dye predominates and the absorption maximum shifts to $500\text{ m}\mu$ with a slight secondary maximum at 610 , indicating the presence of some of the blue ion (28%).

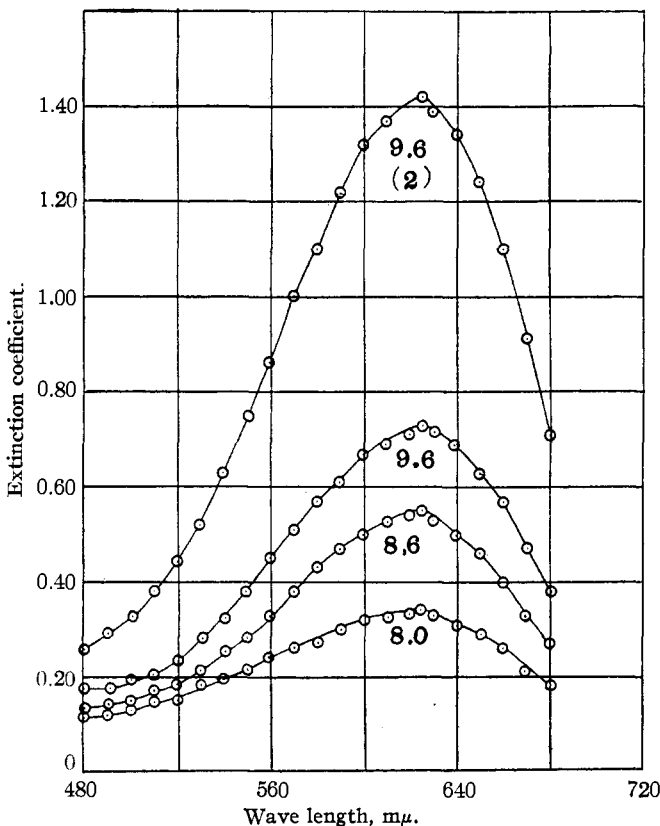


Fig. 2.—*o*-Chlorophenol indophenol: 0.0001 M ; 0.0002 M ; commercial sample.

These curves show the rise in the absorption maximum with increase in P_{H} and in concentration of the dye solution. Alkalinities higher than P_{H} 9.6 have no further effect.

Corresponding to rather rough colorimetric studies indicating that the color of the commercial sample was about 50% of that of Clark's sample, it will be seen that the extinction coefficients of the commercial sample at the wave length of maximum absorption at all P_{H} values are between 51 and

54% of those for Clark's sample. It therefore appears that uncolored impurities are present in the commercial sample of *o*-cresol indophenol to the extent of about 47.5% by weight.

When the depth of the absorbing layer was increased to 2 or 3 cm. (as compared with 1 cm. used in the figures), the absorption curves coincided with those observed when a 1 cm. layer of dye solution of twice or three times the concentration was used. Lambert and Beer's Law is therefore valid over this range of concentrations.

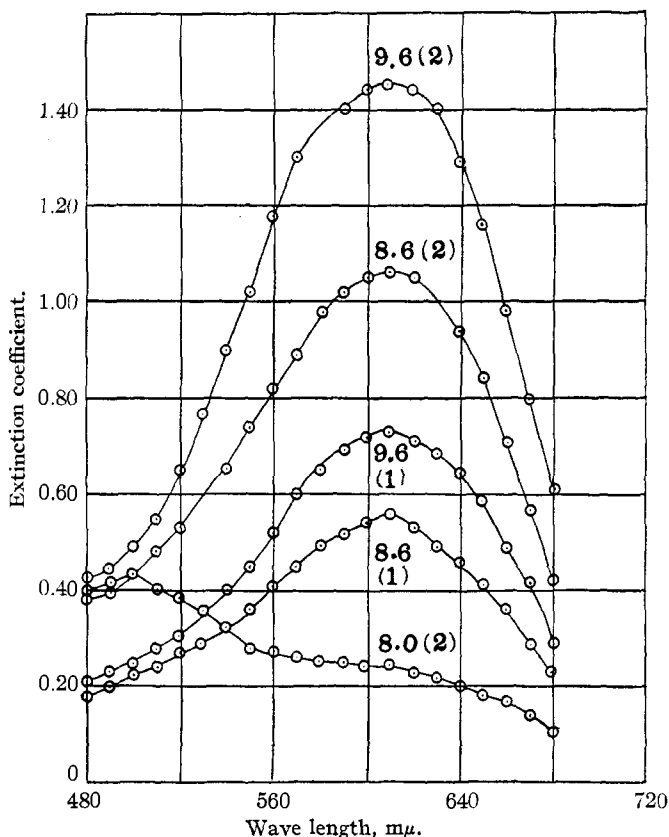


Fig. 3.—*o*-Cresol indophenol: (1), 0.00005 *M*; (2), 0.0011 *M*; Clark's sample.

The writer wishes to thank the Board of Research of the University of California for purchasing the spectrophotometer with which these readings were made.

Summary

1. Spectrophotometric studies of *o*-chlorophenol indophenol and of *o*-cresol indophenol have been made using two samples of each dye; one a

commercial sample and the other a very pure one obtained from Dr. W. Mansfield Clark.

2. The absorption maximum of *o*-chlorophenol indophenol at P_H 's 8.0 to 9.6 is at 625 $m\mu$. At these P_H values the blue ion predominates. The absorption maximum of the dissociated form of *o*-cresol indophenol is at 610 $m\mu$ and that of the red acid form is at 500 $m\mu$.

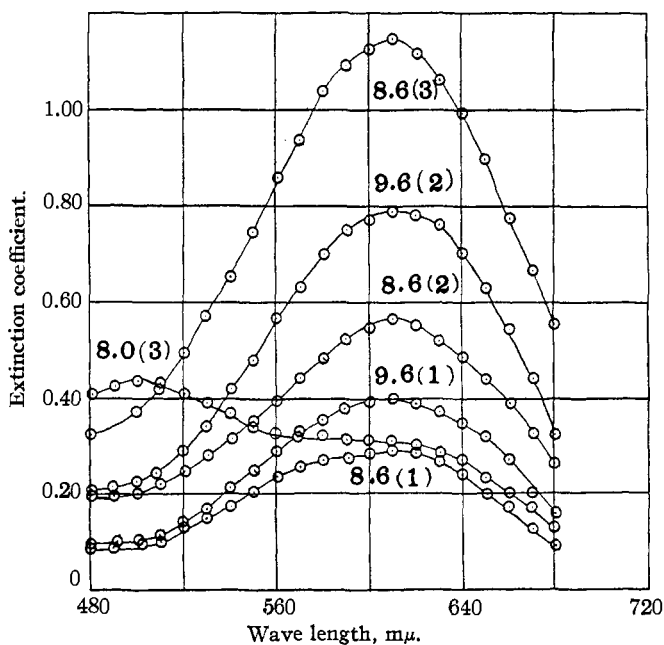


Fig. 4.—*o*-Cresol indophenol: (1), 0.00005 M ; (2), 0.0001 M ; (3), 0.0002 M ; commercial sample.

3. The two samples of *o*-chlorophenol indophenol are about of equal purity, whereas in the case of *o*-cresol indophenol the commercial sample appears to be only between 51 and 54% as pure as that of Clark.

4. The height of the maxima varies with P_H and concentration of the dye.

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