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SHORT COMMUNICATION

A NOTE ON THE ESTIMATION OF CHLOROPHYLL *c*

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Abstract—Chlorophyll *c* estimations using the method of Parsons[1] has given conflicting results in the hands of different authors. The present note shows that this difference was due to the concentrations used. The method as it stands is only applicable for use at very low chlorophyll *c* concentrations. Means of improving the method so that it could be used at higher chlorophyl *c* concentrations are suggested. Pheophytin *c* shows a reduced extinction coefficient in the assay system used (at the wavelength of maximum absorption) at higher concentrations.

INTRODUCTION

PARSONS[1] has described a micromethod for the determination of chlorophyll *c* in sea water, which has been applied by the present author[2] for use with phytoflagellate cultures. The method involves the extraction of the pigments, other than chlorophyll *c*, from an aqueous acetone solution with hexane. The drop in absorbance at the 450 nm absorption maximum on conversion to pheophytin *c* by the addition of acid was then determined. Parsons[1] found no differences in the magnitudes of the 450 nm chlorophyll *c* and the 430 nm pheophytin *c* absorption maxima, whereas Ricketts[2] found the latter to be 65–82 per cent of the magnitude of the former, working with a variety of phytoflagellate cultures. The present note explains these differences.

RESULTS AND DISCUSSION

Re-examination of earlier results[2] indicated that there was a tendency for the differences between the magnitudes of the major chlorophyll *c* and pheophytin *c* maxima to increase as the chlorophyll *c* concentration of the solution (obtained as in Ref. 1) increased. Consequently there was an increasingly larger percentage drop in the absorption at 450 nm on addition of acid as the chlorophyll *c* concentration (judged by initial absorbance at 450 nm) increased. Hence increasingly larger over-estimates of the chlorophyll *c* value occurred as the concentration of the test solution increased.

Further examination of very dilute solutions of chlorophyll *c* (absorbances of less than 0·1 using 1 cm cuvettes), obtained by dilution with 62 per cent v/v aqueous acetone (which is the approximate acetone concentration in the final chlorophyll *c* extract[1]), led to the finding that there were differences of less than 5 per cent between the magnitudes of the chlorophyll *c* (450 nm) and pheophytin *c* (430 nm) maxima.

The results might be explained in three ways:

(1) That in acid 62 per cent aqueous acetone the solubility of pheophytin *c* is much less than that of chlorophyll *c*.

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[1] T. R. PARSONS, *J. Marine Res.* **21**, 164 (1963).
[2] T. R. RICKETTS, *Phytochem.* **4**, 725 (1965).

(2) That in 62 per cent aqueous acetone the degree of adsorption to glassware is increased at higher pigment concentrations. Pheophytin *c* is readily adsorbed on glassware in some organic solvents.[3]

(3) That at higher pheophytin *c* concentrations all the pigment is in solution but the absorbance per unit of pheophytin *c* is decreased at the wavelength of maximum absorption. The instability in the magnitude of the chlorophyll *c* band in this spectral region has been noted before.[4]

The first explanation was excluded because the more concentrated chlorophyll *c* solutions remained clear on conversion to the pheophytin and showed no change in absorbance at 750 nm.[5]

The second possibility was excluded because cuvettes, containing concentrated solutions of chlorophyll *c* which had then been converted to the pheophytin and shown the expected drop in magnitude of maxima, showed no significant amount of pheophytin *c* after decanting the solution and the addition of 100 per cent acetone.

When the acetone concentration of the chlorophyll *c* solutions was increased to 90 per cent v/v by the addition of 100 per cent acetone the decrease in the magnitude of the pheophytin *c* peak formed on adding acid was much lessened, being within 90 per cent of the 450 nm chlorophyll *c* value even at high concentrations. At absorbances of less than about 0·25 (using 1 cm cuvettes), it was within 95 per cent of this value.

It was found that concentrated chlorophyll *c* solutions showed no change in absorbance (at the wavelength of maximum absorption) on dilution of a 62 per cent v/v aqueous acetone solution to 90 per cent v/v with acetone (allowing for the dilution factor) whereas acidified or non-acidified pheophytin *c* solutions showed an increase in absorbance when similarly treated and also a slight shift in position of maxima. When acidified pheophytin *c* solutions were diluted to *low* concentrations with 62 per cent v/v aqueous acetone there was a similar increase in relative absorbance. It would therefore appear that the third explanation of the decrease in the magnitude of the pheophytin *c* maximum compared with that of the chlorophyll *c* maximum is correct, and that at higher pheophytin *c* concentrations the actual absorbance per unit of pheophytin *c* is diminished.

The results show that the method of Parsons[1] for the estimation of chlorophyll *c* gives reliable values only at low chlorophyll *c* concentrations (reading at final absorbance values of less than about $0{\cdot}1^2$ or $1{\cdot}0^1$ using 1-cm or 10-cm cuvettes respectively).

It also appears that by increasing the final acetone concentration to 90 per cent v/v, by the addition of more acetone, the method of Parsons[1] could be used for higher chlorophyll *c* concentrations, thus obviating possible large turbidity errors due to readings at low absorbances.

EXPERIMENTAL

Axenic cultures of *Prymnesium parvum* Carter (Israeli strain), grown as described by Ricketts,[6] were used as the source of the chlorophyll *c*-containing pigment solutions. The latter were obtained by the method of Parsons.[1] All reagents used were of analytical grade where available. Operations were carried out in a manner which reduced exposure to light and air to a minimum.

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[3] J. H. C. SMITH and A. BENITEZ, In *Modern Methods of Plant Analysis* (Edited by K. PAECH and M. V. TRACEY), Vol. 4, p. 166. Springer Berlin (1955).
[4] S. W. JEFFREY, *Biochem. J.* **86**, 313 (1963).
[5] T. R. PARSONS and J. D. H. STRICKLAND, *J. Marine Res.* **21**, 155 (1963).
[6] T. R. RICKETTS, *J. Roy. Microscop. Soc.* **83**, 459 (1964).