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(Ultra-Rapid) Fluorescent Labelling of Proteins

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Chemistry
Title

Name ----- Cynthia Elaine Wilson
Course ----- Honors Special Studies
Title ----- "(Ultra-Rapid) Fluorescent Labelling
of Proteins"
Advisor ----- Dr. H. H. Everett
Date ----- May 30, 1969

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Fluorescent Labelling of Proteins

The goal at the outset of this research project was to prepare fluorescent labelled bovine plasma albumin. A reprint of Ultra-Rapid Fluorescent Labelling of Proteins by Dr. Henry Kinkadee, Director of California Corporation for Biochemical Research, Los Angeles, California, was secured to serve as reference material for the project.

The method described in the reprint involved treatment of a protein solution with an active labelling dye dispersed on diatomaceous earth or other inert materials. The method had been successfully used with fluorescein isothiocyanate, 'Lissamine rhodamine B 200' chloride and dimethylaminonaphthalene sulphonyl chloride on 'Celite' or cellulose powder with rabbit serum as the protein used.

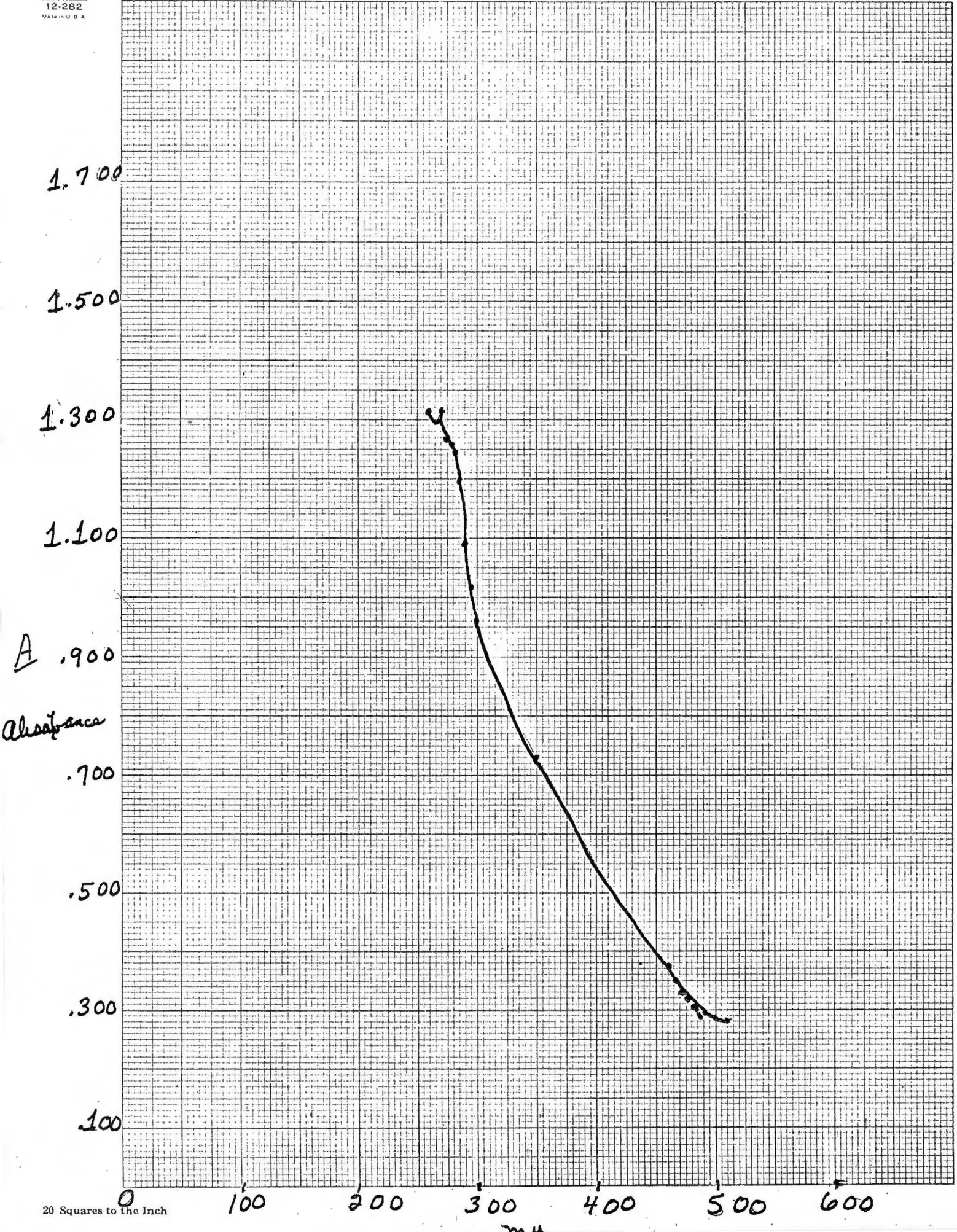
A mixture of 22 ml. of concentrated BPA and 22 ml. of 0.05M sodium carbonate - bicarbonate buffer of pH 8.5 was shaken for three minutes with 10 mg. of 5-Dimethyl Amino - 1 - naphthalene Sulphonyl Chloride on Celite, B grade. The mixture was centrifuged for 3 minutes and the supernatant was allowed to flow into a Sephadex column which had been washed in advance with several column volumes of 0.02M sodium phosphate buffer of pH 6.5. The column was developed with phosphate buffer (0.02M, pH 6.5).

In contrast to fluorescein isothiocyanate which produces a strong yellow protein band, the naphthalene sulphonyl chloride produced a faint protein band which had to be followed as it progressed down the column with an ultraviolet lamp. The labelled protein was collected in approximately 8 ml. of effluent.

The effluent was tested by subjecting it to ultraviolet radiation. It was discovered that there were test-tubes with protein fluorescing green and test-tubes with protein fluorescing blue. There was no apparent explanation as to why there were fluorescent samples of two distinct colors. It was hoped that the elution curves of sample 1 (green) against phosphate buffer blank, sample 2 (blue) against phosphate buffer blank, and protein-phosphate sample against phosphate buffer blank would shed light onto the matter. However, the only datum obtained was already known information - that BPA has a maximum absorbance at 279 m μ .

It was supposed that the labelling dye might have had some effects upon the obtained results. Therefore, it was attempted to run the spectrum of 2 mg. of Dimethylaminonaphthalene sulphonyl chloride in "elite" - O grade in 5 ml. of phosphate buffer on the DU $\frac{1}{2}$ phosphate buffer. However, 1:1000 fold dilution

Sample # 1 (Green) ⁻³⁻ Against Phosphate Buffer Blank



Sample # 2 (Blue) Against Phosphate Buffer Blank

Absorbance

1.700

1.500

1.300

1.100

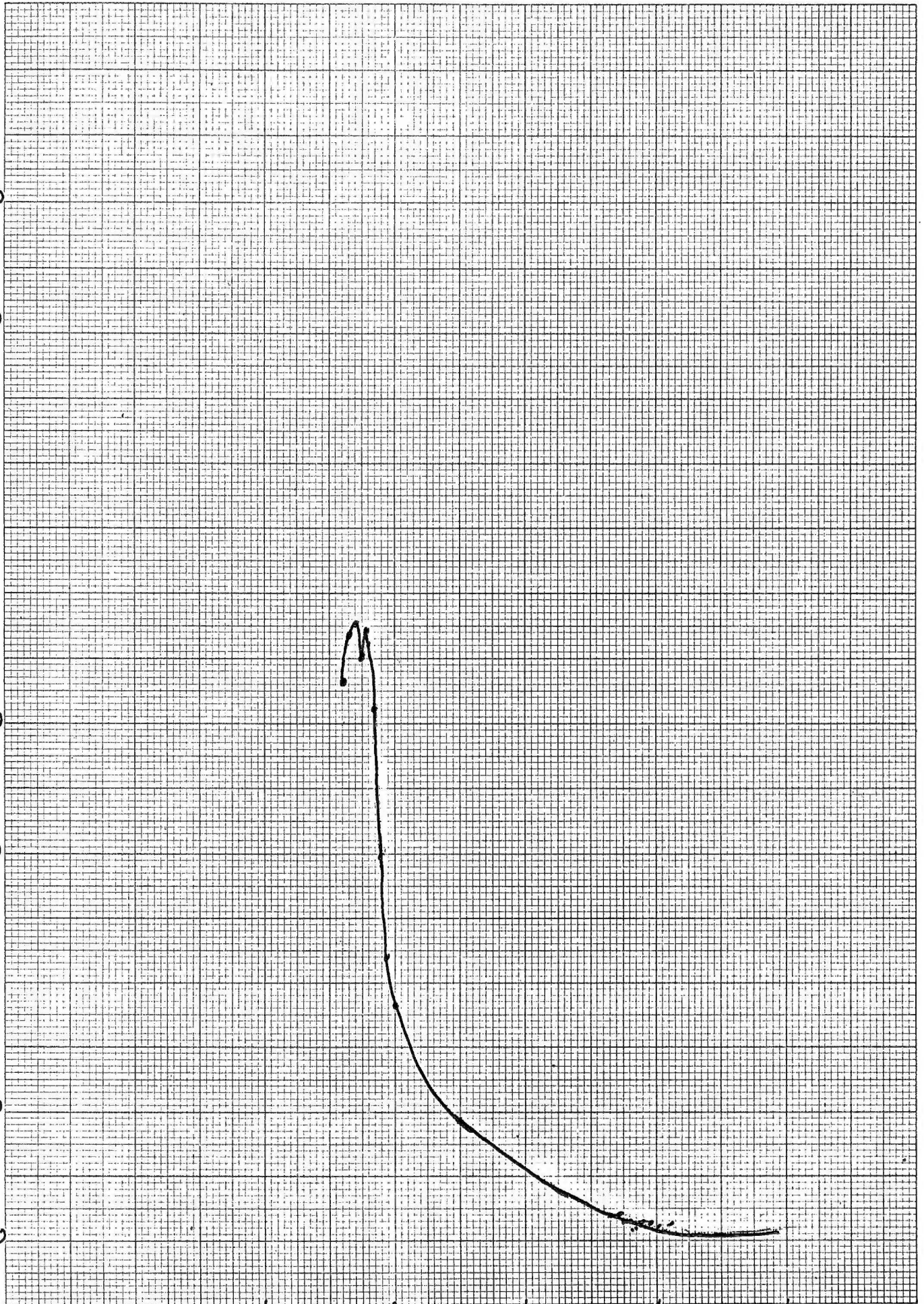
.900

.700

.500

.300

.100



Sample #3

Protein-Phosphate Sample ⁻⁵⁻ against Phosphate Buffer Bl

absorbance

A

1.700

1.500

1.300

1.100

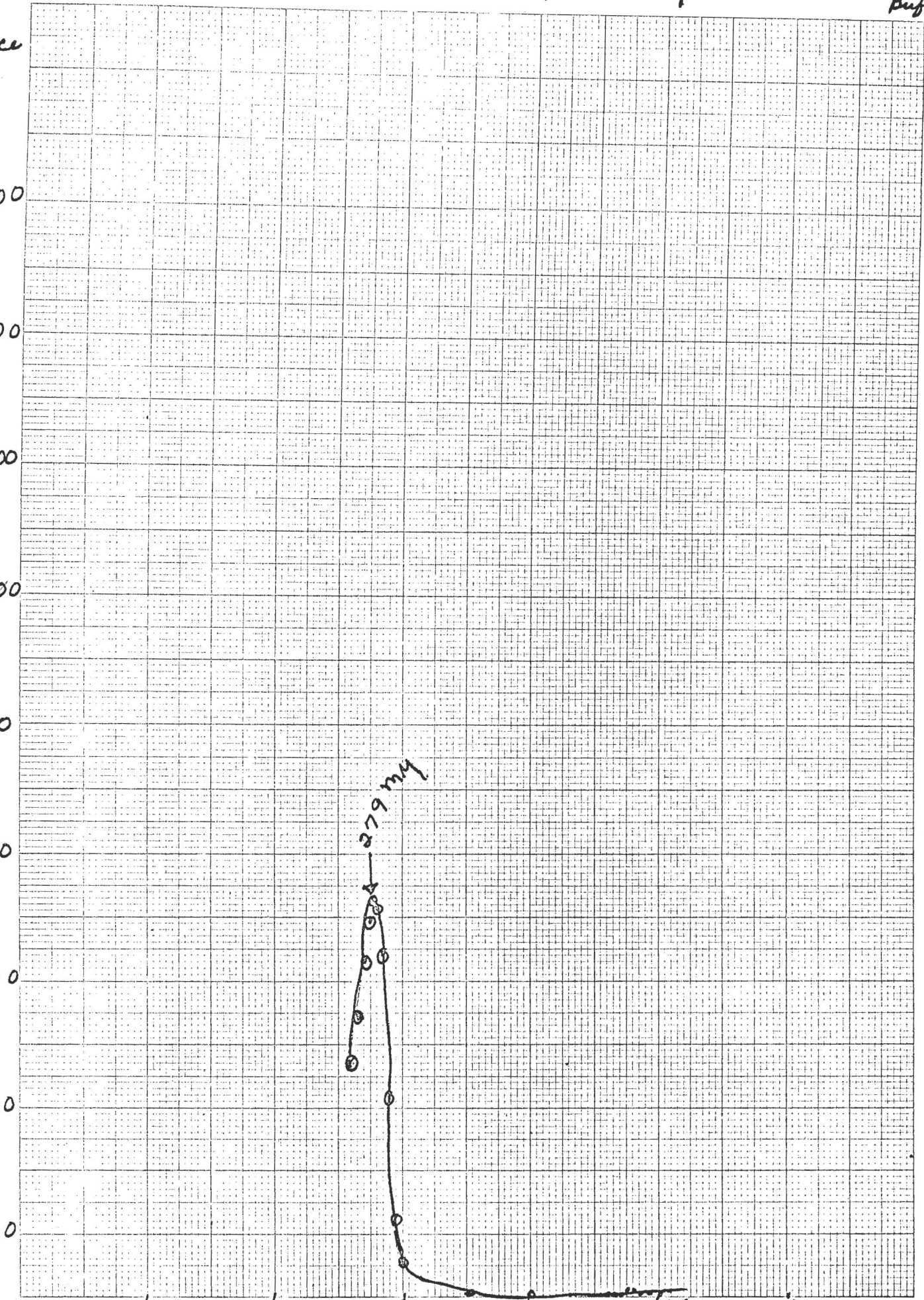
.900

.700

.500

.300

.100



1100

1200

1300

400

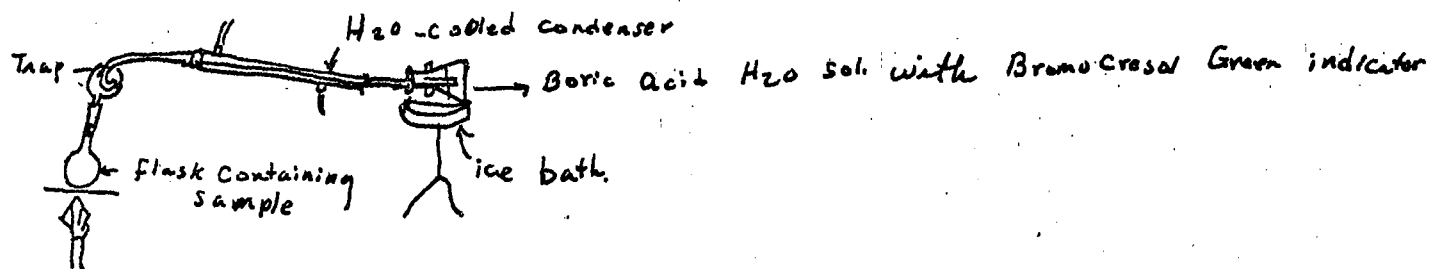
500

600

mμ

failed to yield samples which could be read on the DU. Library research was done in hopes of finding material concerning the dye in question. These attempts were, however, futile.

As a final task, there was an attempt to determine the amount of labelled protein in the two effluents. This student's advisor suggested trying a semimicro Kjeldahl method on a known amount of BPA, and if this proved successful, then applying this method to the labelled protein. The semi-micro Kjeldahl analysis as described in Organic Quantitative Chemical Analysis, ©1958, pp 642-645, was attempted with modification in the distilling technique. Instead of the suggested method of distillation, the following (hopefully more accurate) set-up was used



However, after five attempts, the method was still unsuccessful. This student will continue to work on improving this method, for it is believed that the amount of labelled protein can be determined accurately, once the Kjeldahl (semi-micro) analysis has been improved.

The initial goal was accomplished, the BPA had been labelled as indicated by the fluorescent samples collected. But that was only the initial step. There are unanswered questions which will be dealt with further.

✓ End ✓
C.E.N.