EPA/600/R-94/150

SUMMARY REPORT

Evaluation of a New Fluorometric Technique that Uses Highly Selective Interference Filters for Measuring Chlorophyll *a* in the Presence of Chlorophyll *b* and Pheopigments

by

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FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring Systems Laboratory - Cincinnati (EMSL-Cincinnati) conducts research to:

- Develop and evaluate analytical methods to identify and measure the concentration of chemical pollutants in marine and estuarine waters, drinking waters, surface waters, groundwaters, wastewaters, sediments, sludges, and solid wastes.

- Investigate methods for the identification and measurement of viruses, bacteria and other microbiological organisms in aqueous samples and to determine the responses of aquatic organisms to water quality.

- Develop and operate a quality assurance program to support the achievement of data quality objectives in measurements of pollutants in marine and estuarine waters, drinking water, surface water, groundwater, wastewater, sediment and solid wastes.

- Develop methods and models to detect and quantify responses in aquatic and terrestrial organisms exposed to environmental stressors and to correlate the exposure with effects on chemical and biological indicators.

Spectral interferences, caused by the presence of pigments such as chlorophyll *b* and pheophytin *a*, degrade the accuracy of conventional fluorometry for determining chlorophyll *a* extracted from algae. This EMSL-Cincinnati report, "Evaluation of a New Fluorometric Technique that Uses Highly Selective Interference Filters for Measuring Chlorophyll *a* in the Presence of Chlorophyll *b* and Pheopigments," was prepared to inform environmental monitoring organizations of a recently developed technique that improves the fluorometric measurement of chlorophyll *a*.

ABSTRACT

A new fluorometric technique was compared to conventional fluorometry with and without pheophytin *a* (pheo *a*) correction and to spectrophotometry using Lorenzen's modified monochromatic equations and Jeffrey and Humphrey's trichromatic equation to calculate chlorophyll *a* (chl *a*). The new technique uses excitation and emission filters that are highly selective for chl *a*, eliminating the interference caused by accessory pigments and pheopigment degradation products.

Four method parameters were evaluated using the new technique, conventional fluorometry and spectrophotometry. They were (1) sensitivity, (2) linear dynamic range, (3) precision, and (4) accuracy. Controlled studies of the interference caused by chl *b* were conducted, and real world samples of varying taxonomic composition were analyzed. In laboratory solutions, the new technique was comparable to conventional fluorometry with respect to sensitivity and accuracy. The linear dynamic range for the new technique exceeded that of conventional fluorometry by a factor of three. Interference caused by chl *b* was +6% at the highest chl *a*:chl *b* likely to occur in nature. Chl *a* values obtained using the new technique compared well with conventional fluorometry when pheo *a* was the only interfering pigment present in the sample.

ACKNOWLEDGMENTS

The valuable assistance and technical advice of Susan Mokelke of Turner Designs (Sunnyvale, CA) was much appreciated throughout this work. Many thanks also go out to Don Schultz of the USEPA for organizing the Chlorophyll Workshop in Athens, GA, in February, 1993 and for seeing this report through to its completion.

FIGURES

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INTRODUCTION

The high sensitivity of fluorometry makes it a choice technique for measuring chlorophyll *a* (chl *a*). However, spectral interferences caused by pheophytin *a* (pheo *a*) and chlorophyll *b* (chl *b*) can cause substantial over- or underestimation of chl *a*. Conventional fluorometry with acidification correction for pheo *a* results in underestimation of chl *a* and overestimation of pheo *a* if chl *b* is present (caused by the conversion of chl *b* to pheo *b* which is determined as pheo *a*), whereas if no pheo *a* correction is applied, overestimation of chl *a* results.[1-3] A simple fluorometric method for measuring chl *a* in the presence of pheo *a* and chl *b* recently has been developed[4] and commercially introduced (Turner Designs, Sunnyvale, CA). The new technique utilizes excitation and emission filters that are highly selective for chl *a*, thereby eliminating the need for acidification (Figures 1-3). The result is a direct relationship between chl *a* and fluorescence with a maximum interference from chl *b* or pheo *a* of +10%.

At a chl *a* measurement and interpretation workshop held by USEPA Region 4 in Athens, GA, Turner Designs (a leading manufacturer of fluorometers) presented laboratory results obtained by Dr. Nicholas Welschmeyer of Moss Landing Marine Laboratories, CA using the newly selected interference filters.[4] EMSL-Cincinnati personnel present at the workshop arranged to evaluate the new technique using a Turner Designs Model 10-AU digital fluorometer. Solutions of pure pigments, mixtures of pigments and natural sample extracts were analyzed using the 10-AU fluorometer, a Turner Designs Model 10 analog fluorometer and a Beckman DU-6 spectrophotometer. The results of those analyses are presented here.

EXPERIMENTAL

Instrumentation Specifications

Spectrophotometer - Beckman DU-6, multiwavelength. Wavelength accuracy - ±0.5 nm, Wavelength resolution - 2 nm, Lamp - tungsten. Unless otherwise indicated, a 1-cm glass cell was used for all analyses.

Analog Fluorometer - Turner Designs Model 10. F4T5 blue lamp, red-sensitive photomultiplier and filters for excitation (CS-5-60) and emission (CS-2-64). 13 mm diameter borosilicate glass culture tubes were used for all analyses.

Digital Fluorometer - Turner Designs Model 10-AU. Daylight white lamp, red-sensitive photomultiplier, and filters for excitation (436FS10) and emission (680FS10). 13 mm diameter borosilicate glass culture tubes were used for all analyses.

Preparation of Standards and Samples

Chl *a* and chl *b* obtained from Sigma Chemical were prepared in 90% acetone:10% water. Pheo *a* was prepared by the mild acidification of chl *a* solutions with .1 N HCl and subsequent 1:1 molar neutralization with 0.1 NaOH. All standard solutions were stored in the dark at -70°C.

The technique that was used for sample collection, handling and extraction is described in detail in USEPA Method 445.0.[5] Natural water samples were vacuum-filtered through 47-mm Whatman GF/F glass fiber filters. Filters were folded once with particulate matter inward, blotted with a laboratory tissue to remove excess moisture, and stored in petri dishes in the dark at -70°C until extraction.

Samples were extracted with 90% acetone using a motor-driven Teflon pestle and glass grinding tube. Final dilution volume in all cases was 10 mL. Extracted filters were allowed to steep from 4 to 18 hours. The supernatant of centrifuged samples was analyzed by the three techniques.

Calibration

Chl *a* and chl *b* were determined using the trichromatic equations of Jeffrey and Humphrey.[6] The spectrophotometer was used to verify the concentration of chl *a*. Lorenzen's[7] modified monochromatic equation was used to determine corrected chl *a* for fluorometer calibration.

The fluorometers were always calibrated on the day of use and with the same dilutions of stock chl *a* solution. The following set of equations were used for calibration of the analog fluorometer:

$$F_s = C_a/R_s$$

where:

F_s = response factor for sensitivity setting, S.

R_s = fluorometer reading for sensitivity setting, S.

C_a = concentration of chl *a* (from spectrophotometric analysis).

and, $r = R_b/R_a$

where:

R_b = fluorescence of pure chl *a* standard solution before acidification.

R_a = fluorescence of pure chl *a* standard solution after acidification.

The following equations were used for sample data reduction:[8]

Uncorrected chl *a*, $\mu g/L = R_{sb} \times F_s$

Corrected chl *a*, $\mu g/L = F_s\ (r/r-1)\ (R_{sb} - R_{sa})$

Pheo *a*, $\mu g/L = F_s(r/r-1)\ (rR_{sa} - R_{sb})$

where:

F_s = response factor for the sensitivity setting used.

R_{sb} = fluorescence of sample extract before acidification.

R_{sa} = fluorescence of sample extract after acidification.

The digital fluorometer was calibrated directly with a single standard solution. Readout was in concentration units of µg/L. Pheo *a* is optically excluded, for the most part, from the chl *a* measurement. For users of analog instrumentation equipped with the new interference filters, calibration is affected as with conventional fluorometry *without acidification and subsequent corrections* (i.e., "uncorrected" chl *a* equation).

RESULTS

Four parameters were evaluated for the spectrophotometric and fluorometric techniques as a baseline comparison. They were (1) sensitivity, (2) linear dynamic range, (3) precision, and (4) accuracy. Accuracy of the fluorometers determined by comparison to spectrophotometric chl *a* results. Interference caused by the presence of chl *b* or pheo *a* was also investigated. Finally, comparisons in the determination of chl *a* were made using real samples. Filter samples from Lake Pontchartrain, Louisiana (primarily green and bluegreen algae), San Francisco Bay (unknown mixed assemblage) and New York Bight (mixed assemblage, predominantly diatoms) were extracted and analyzed by all three methods.

Sensitivity (Instrumental Detection Limit) - A stock solution of chl *a* (50 mg/L) was serially diluted and analyzed until a response of .005 AU was obtained using the spectrophotometer. Dilutions were continued and analyzed fluorometrically until the normalized FU response for the analog fluorometer was .00013 (.4 FU non-normalized on the most sensitive setting) and the direct readout response of the digital fluorometer was .026. The results can be summarized as follows:

	Instrumental Detection Limit
DU-6 Spectrophotometer	.08 mg/L*
Model 10 Analog Fluorometer	.05 µg/L
Model 10-AU Digital Fluorometer	.03 µg/L

* Determined using a 1-cm cell.

Linear Dynamic Range (LDR) - The LDR is the concentration at which the instrument's response to chl *a* is no longer linear. Sixteen serial dilutions of chl *a* (.09 to 50 mg/L) were analyzed spectrophotometrically and 38 serial dilutions (.15 to 730 µg/L) were analyzed fluorometrically. Linear regressions of absorbance/fluorescence versus concentration were plotted and the concentration at which the response deviated more than 10% of the expected response was judged to be the upper limit of the LDR (Figures 4 - 6). The

upper limit of the LDR for the digital fluorometer was the first dilution from a 1000 µg/L solution that was on scale. (NOTE: *The LDR should not be confused with a calibration range.* Calibration relying upon a non-weighted linear regression should be over 1 to 2 orders of magnitude, at most, to minimize lack-of-fit inaccuracies. This type of inaccuracy becomes especially apparent at low concentrations.) The results can be summarized as follows:

Linear Dynamic Range

DU-6 Spectrophotometer	25 mg/L
Model 10 Analog Fluorometer	250 µg/L
Model 10-AU Digital Fluorometer	700 µg/L

The higher LDR for the Model 10-AU fluorometer can probably be attributed to the interference filters, which block out all but a narrow band of excitation and emission light. Quenching effects caused by the presence of accessory pigments and other compounds not of interest are thus minimized.

The upper limit of the LDR for the digital fluorometer also proved to be an acceptable calibration point (direct readout of concentration agreeing well with the calculated concentration from serial dilution). (NOTE: Quenching effects caused by the presence of other compounds can cause underestimation of the true chl *a* concentration. Since the presence of these compounds cannot be predicted, real world samples should not be analyzed at concentrations approaching the LDR.)

Precision - Precision was calculated as the percent relative standard deviation (%RSD) of repeated measurements of standard solutions and natural samples. Precision for real world samples are presented later in this summary. Precision obtained using pure solutions of chl *a* obtained from the USEPA is summarized below:

	Corrected Chl *a*	Uncorrected Chl *a*
	%RSD	%RSD
DU-6 Spectrophotometer*	5.1%	2.2%
(N=9)	mean chl *a* = 69 µg/L	mean chl *a* = 74 µg/L
Model 10 Analog Fluorometer	4.7%	4.5%
(N=3)	mean chl *a* = 67 µg/L	mean chl *a* = 69 µg/L
Model 10-AU Digital Fluorometer	4.6%	
(N=3)	mean chl *a* = 68 µg/L	

* Determined using a 10-cm cell.

The fluorometers were calibrated on the day of use using the same standard solutions (chl *a* prepared from a pure pigment obtained from Sigma Chemical). Three fluorometric QC samples obtained from the USEPA were then analyzed. The spectrophotometric results were obtained from the analyses of nine fluorometric QC samples using a 10-cm cell.

Accuracy of Fluorometric Techniques (versus spectrophotometry) - Measurement of pure chl *a* by fluorometry is only as accurate as the spectrophotometer used to standardize the calibration solutions. As a baseline comparison, however, the data gathered from the sensitivity study is used to compare the accuracy of the two fluorometers. The spectrophotometric result for corrected chl *a* was regarded as the nominally "true" concentration, 69 µg/L (±5%). Comparing the mean fluorometric values we obtained: -2.9% error using the analog fluorometer with pheo *a* correction, and -1.4% error with the digital fluorometer (no pheo *a* correction is necessary).

Effect of Chl *b* on the Measurement of Chl *a*

Spectrophotometry - The trichromatic equation of Jeffrey and Humphrey is fairly accurate in determining chl *a* in the presence of chl *b*.[9] However, spectrophotometry often is not used because of poor sensitivity.

Fluorometry - Six concentrations of chl *a* ranging from 1.8 to 180 µg/L were evaluated. Chl *b* was present at varying ratios up to a chl *a*:chl *b* ratio of 1:1, the maximum likely to occur in nature. Figures 7 and 8 illustrate the error observed over a 1:0 to a 1:1 ratio, at 180 µg/L and 1.8 µg/L (the highest and lowest concentrations used in the study). "Corrected" and "uncorrected" chl *a* results refer to pheo *a*-corrected and non-corrected chl *a*, respectively, as determined by the analog fluorometer. No error would be indicated by a line with zero slope. In Figure 7 note that the conventional fluorometric method, with or without pheo *a* correction, results in significant error (-19% and +30%, respectively). The underestimation of chl *a* is caused by the overestimation of pheo *a* which is actually pheo *b*. The overestimation of pheo *a* caused when chl *b* is converted to pheo *b* is illustrated in Figure 9. The slightly nonlinear trend observed in Figure 8 cannot be explained, although it could be a dilution error since it is observed for all three techniques. Chl *a* as determined by the new fluorometric method was overestimated by ca. +6%. In summary, the effect of chl *b* on the determination of chl *a*, by the new fluorometric method, across six concentrations ranging from 1.8 to 180 µg/L, was approximately +6%.

Effect of Pheophytin *a* on the Measurement of Chl *a*

Spectrophotometry - When the trichromatic equation was used to calculate chl *a* in the presence of pheo *a*, a positive bias was observed (Figure 10). The monochromatic equation, on the other hand, was fairly accurate even in the presence of chl *b*.

Fluorometry - The results obtained when chl a was calculated in the presence of pheo *a* at various ratios up to a 1:1 ratio were erratic. These erratic results are thought to be due to the manner in which the pheo *a* was prepared. Solutions of pure chl *a* were acidified to convert the chl *a* to pheo *a*. The solutions were then neutralized 1:1 molar with NaOH. The results indicated that residual acid was present which converted the chl *a* to pheo *a* when the two solutions were mixed. Regrettably, the borrowed digital fluorometer was returned prior to evaluating the data gathered in that study. Dr. Welschmeyer, however, reports an error no greater than +10% at a chl *a*:

pheo *a* ratio of 1:1, the maximum likely to occur in nature. The results obtained in this study using real world samples containing chl *a* and pheo *a*, do not contradict those findings.

Comparison of Techniques Using Real World Samples

Filtered samples from various locales and of various taxonomic composition were analyzed by the three techniques. In most cases the samples that contained a sufficient concentration of chl *a* were analyzed first by spectrophotometry, then diluted and analyzed fluorometrically. In all cases the samples that were analyzed by the two fluorometers were the exact same dilution.

The results are presented in Figures 11 - 15. The following annotation is used:

Digital Fl. - the new fluorometric method under evaluation.

Fl.Uncorra. - conventional fluorometry without pheo *a* correction.

Fl.Corra - conventional fluorometry with pheo *a* correction.

Methods - will indicate the mean or %RSD of the results from all the methods that are presented on that particular figure.

Sp.Tri. - spectrophotometry, trichromatic equations.

Sp.Mono. - spectrophotometry, monochromatic equations.

Macerated - indicates that filters were macerated using a tissue grinder prior to steeping.

Unmacerated - indicates that samples were not macerated prior to steeping.

Unless otherwise indicated, all samples were macerated prior to steeping in 90% acetone.

Figure 11 - San Francisco Bay, 1000 mL samples. The taxonomic composition of these samples was unknown. Previous laboratory experiments have indicated that the spectrophotometric, monochromatic equation (Sp.Mono.) performs well even in the presence of chl *b*. Previous studies also have indicated that the spectrophotometric, trichromatic equation (Sp.Tri.) overestimates chl *a* in the presence of pheo *a*. There was pheo *a* present in the sample and the extremely low results for chl *a* using the Fl.Corra method indicated the presence of chl *b*. This information leads to the conclusion that the most confidence may be placed in the Sp.Mono results. The Digital Fl. results agree closely with the Sp.Mono. results. The relative percent difference (RPD) between the Fl.Corra and Digital Fl results was 34%.

Figure 12 - Lake Pontchartrain, 300 mL samples. These samples were primarily green and blue-green algae. Blue-green algae do not contain chl *b* or chl *c* and the green algae do not contain chl *c*. Four filtered samples were and three filters were not macerated prior to steeping in 90% acetone. Concentrations of chl *a* were normalized to the spectrophotometric results (actual concentrations for fluorometric analyses were 10X less). Spectrophotometric results indicated the presence of chl *a* and pheo *a* but very little chl *b*. In this case close agreement was observed between the Digital Fl. and FL.Corra methods (RPD = 7%, for macerated samples). Maceration of the filters increased the quantity of pigment that was extracted. The fact that calculated pheo *a*, relative to calculated chl *a*, was considerably different for the FL.Corra and the Sp.Mono techniques gives reason to believe that either chl *b* is present in the sample in a higher concentration than indicated by the spectrophotometric method or another pigment that interferes with conventional fluorometry is present in the sample. Previous work performed in this laboratory has demonstrated that chl *b* is underestimated when the chl *a* concentration is greater than 4X the chl *b* concentration.

Figure 13 - New York Bight, 300 mL samples. This was a mixed assemblage with diatoms being the predominant species. Diatoms contain the pigments chl *a* and *c*, however, chl *c* is not a major spectral interferant for the spectrophotometric method or the fluorometric methods. Three filter samples were extracted. For this sample all the techniques performed comparably. The RPD between the Digital Fl. technique and the Fl.Corra technique was 5.6%.

Figure 14 - New York Bight, 900 mL sample. This was a one month old frozen extract that was analyzed once spectrophotometrically, diluted three times and analyzed fluorometrically. Results were normalized to the fluorometric concentrations. The Sp.Tri. results reflect the fact that the trichromatic equation overestimates chl a in the presence of pheo *a*. The RPD between the Digital Fl. and Fl.Corra methods was 7.7%.

Figure 15 - New York Bight, 600 mL. This was a one month old frozen extract that was analyzed once spectrophotometrically, diluted three times and analyzed fluorometrically. The chl *a*:pheo *a* ratio was approximately 3:1. Concentrations were normalized to the fluorometric results. The %RPD between the Digital Fl. results and the Fl.Corra results was 10%.

CONCLUSIONS

A new fluorometric technique that uses highly selective interference filters to optically exclude the fluorescence of pheo *a*, chl *b* and pheo *b* was compared to the spectrophotometric and conventional fluorometric techniques for determining chl *a*. A baseline comparison was made using the following parameters: (1) sensitivity, (2) linear dynamic range, (3) precision, and (4) accuracy. The new technique was comparable in sensitivity to conventional fluorometry. The upper limit of the linear dynamic range was 700 µg/L, compared to 250 µg/L for conventional fluorometry. Precision compared with spectrophotometry and conventional fluorometry. Accuracy, using standard solutions of chl *a* that were verified spectrophotometrically, compared well with conventional fluorometry.

Interference from chl *b* was evaluated over six concentrations and varying chl *a*:chl *b* ratios, up to 1:1. The superiority of the new technique was most pronounced in these comparisons. Whereas conventional fluorometry severely underestimated chl *a* in the presence of chl *b*, by overestimating pheo *a*, the new fluorometric technique exhibited an average +6% bias. Interference studies of pheo *a* were unsuccessful due to incorrect preparation of pheo *a*, however, real world samples containing pheo *a* and negligible quantities of chl *b* allowed an estimate of error of the new method in the presence of pheo *a*. The bias of the technique was less than +10% for real world samples.

Real world samples from three locales and of varying taxonomic composition were analyzed by all three techniques. The degree of difference in the results was correlated with taxonomic differences in the samples. If chl *b* was present, the new fluorometric technique compared well with the spectrophotometric technique using the monochromatic equation. In those cases, however, chl *a* was severely underestimated by conventional fluorometry. For a sample that was known to contain chl *a* and pheo *a*, the new fluorometric method compared well to pheopigment-corrected conventional fluorometry.

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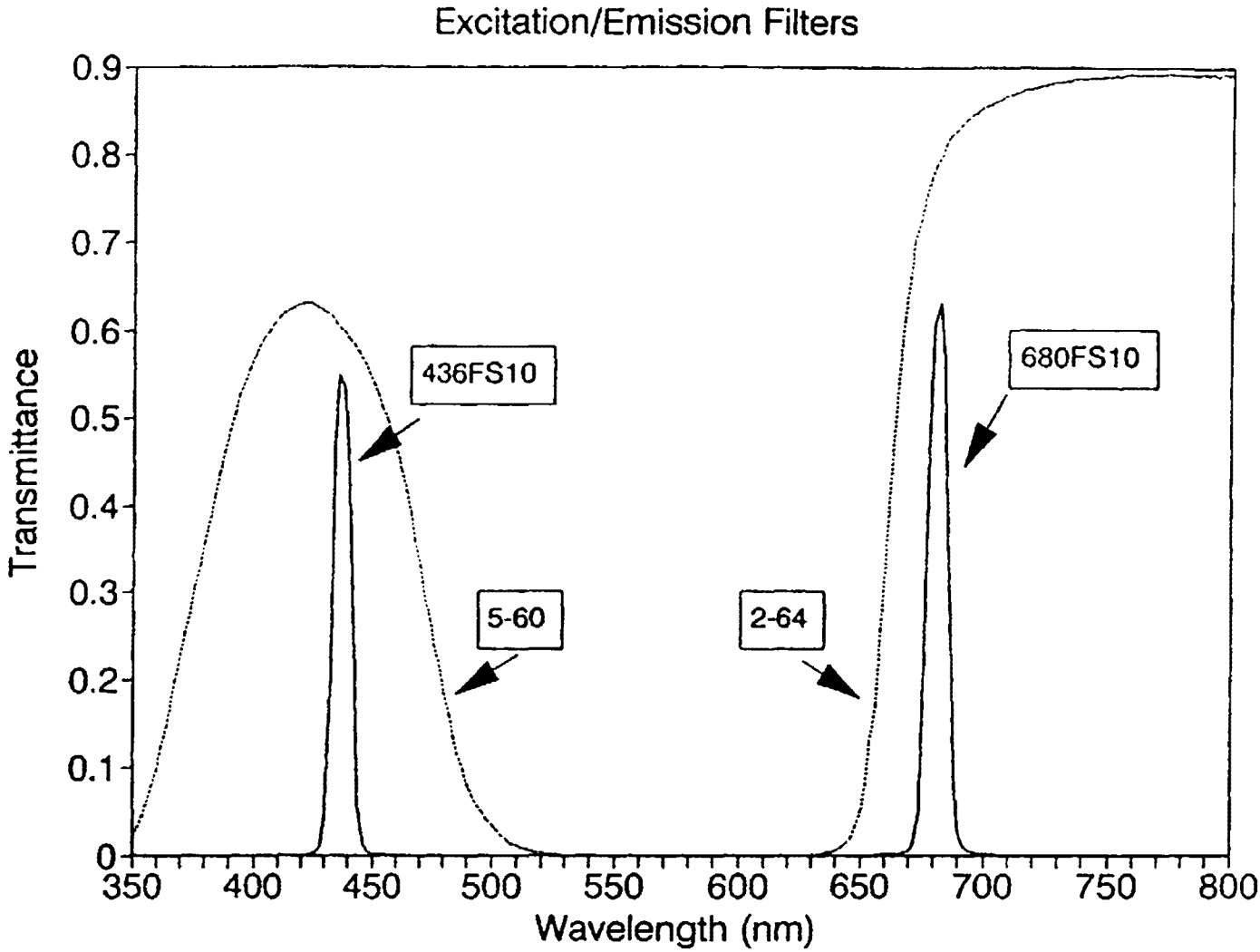


Figure 1. Transmittance Characteristics of Excitation/Emission Filters used in Conventional Fluorometric Acidification Technique (Corning 5-60/Corning 2-64) and Newly Described Fluorometric Method (436FS10/680FS10 Interference Filters, Andover Corp.).

Reprinted courtesy of Nicholas Welschmeyer, Moss Landing Marine Laboratories, CA.

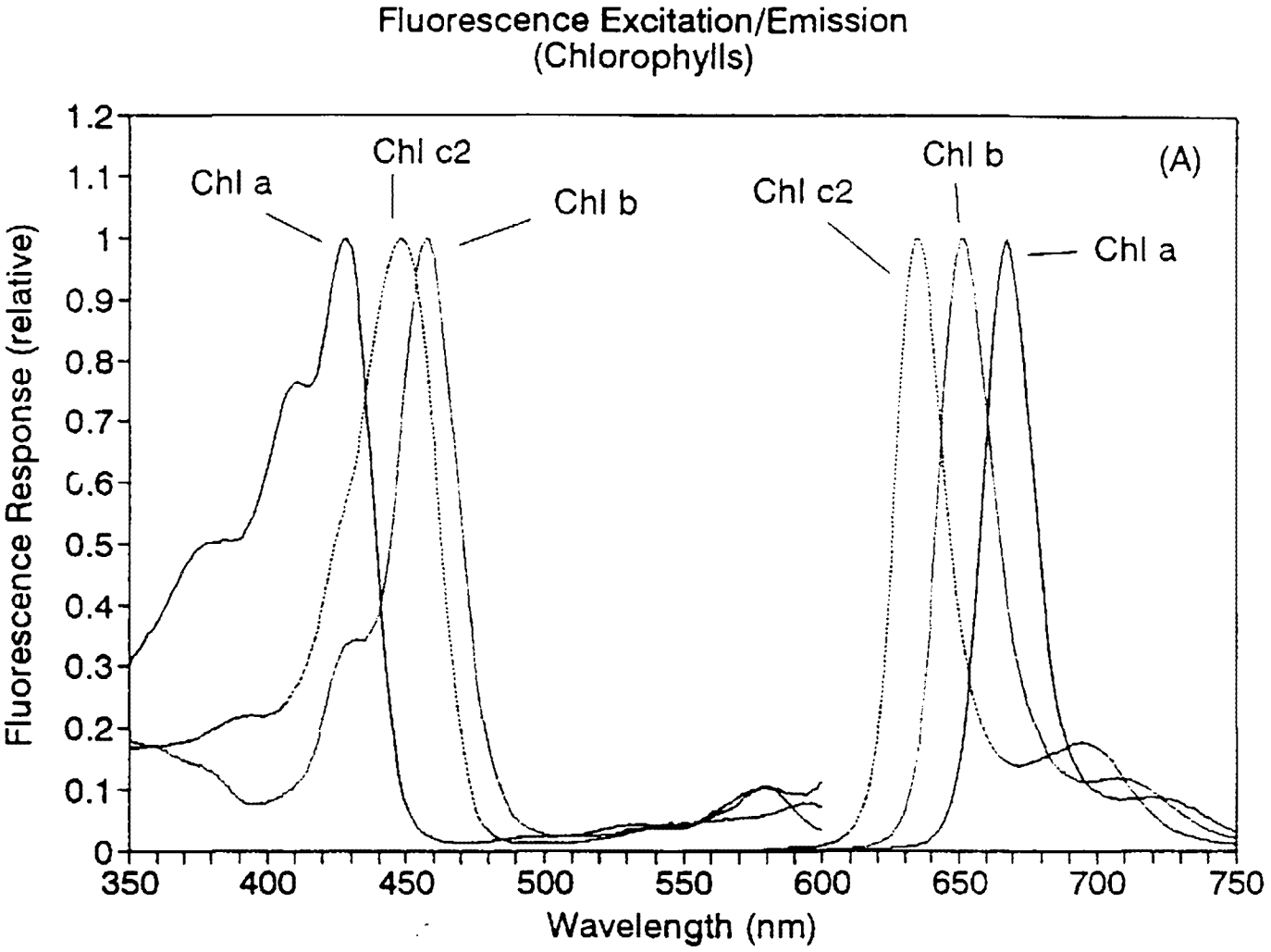


Figure 2. Excitation/Emission Spectra of the Chlorophylls

Reprinted courtesy of Nicholas Welschmeyer, Moss Landing Marine Laboratories, CA.

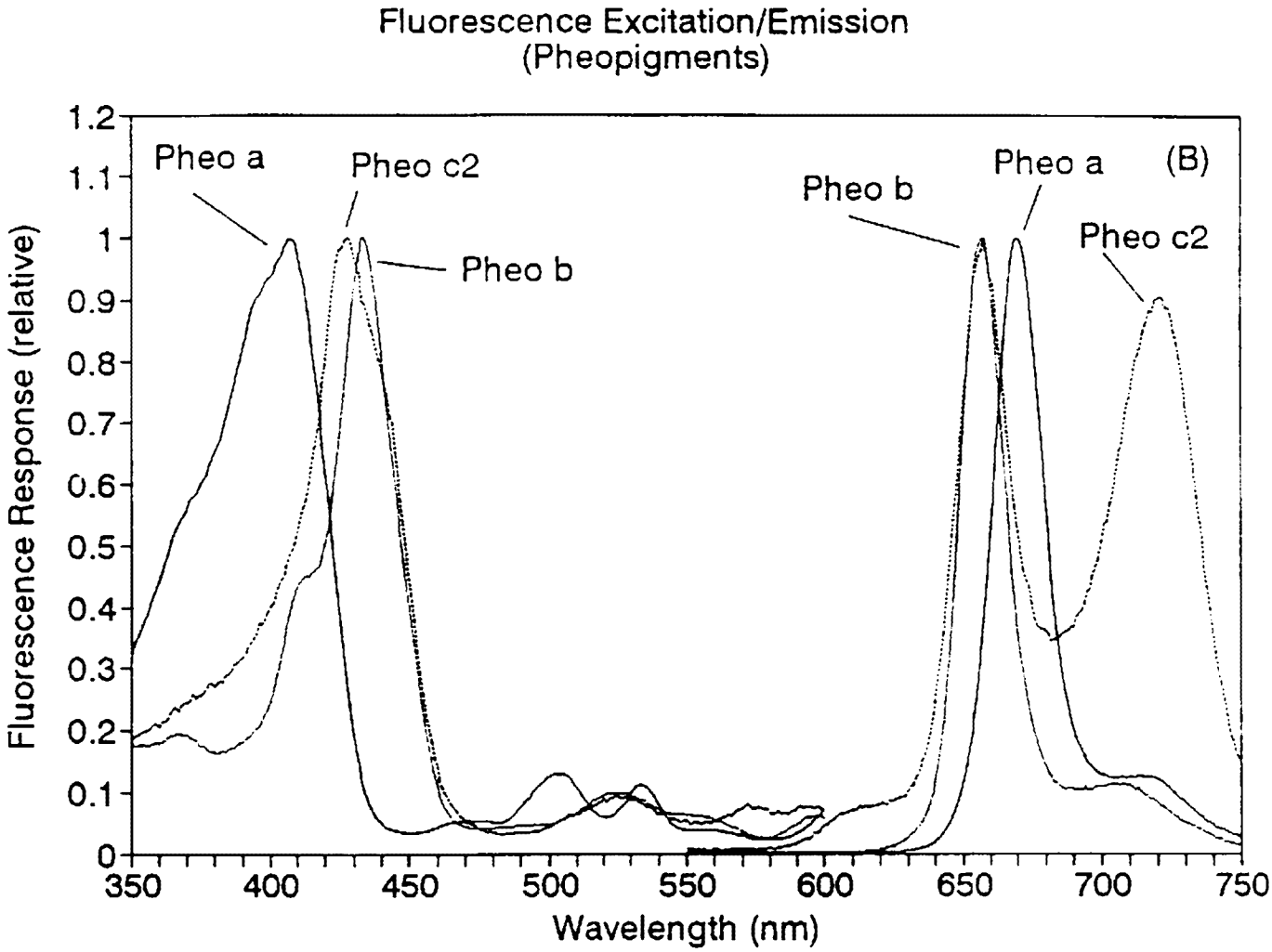


Figure 3. Excitation/Emission Spectra of the Pheopigments

Reprinted courtesy of Nicholas Welschmeyer, Moss Landing Marine Laboratories, CA.

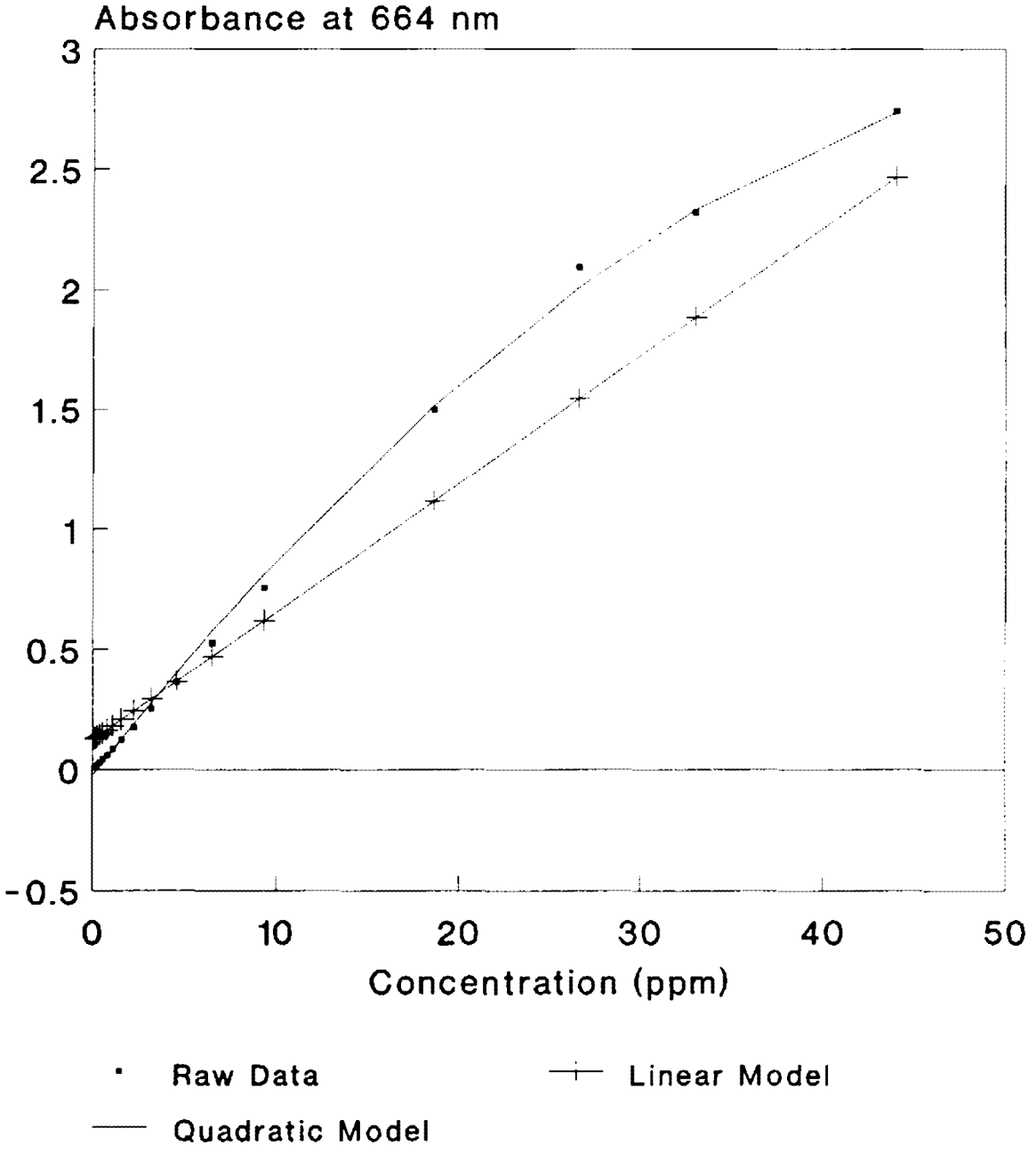

Chlorophyll a - Spectrophotometry
Linear Dynamic Range (.08 - 25 ppm)
Absorbance at 664 nm
3
2.5
2
1.5
1
0.5
0
-0.5
0
10
20
30
40
50
Concentration (ppm)
Raw Data
Linear Model
Quadratic Model

Figure 4

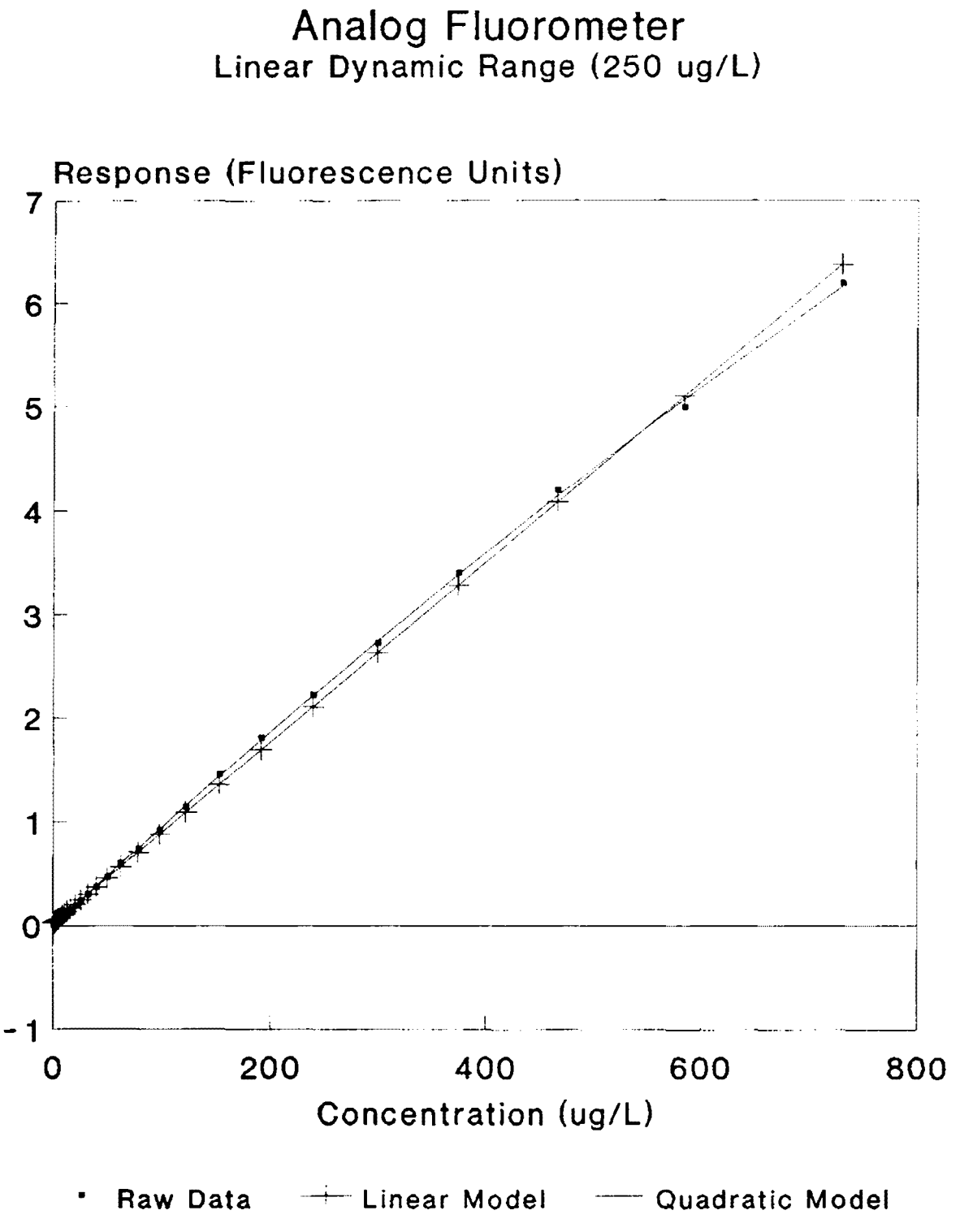

Analog Fluorometer
Linear Dynamic Range (250 ug/L)
Response (Fluorescence Units)
7
6
5
4
3
2
1
0
-1
0
200
400
600
800
Concentration (ug/L)
Raw Data
Linear Model
Quadratic Model

Figure 5

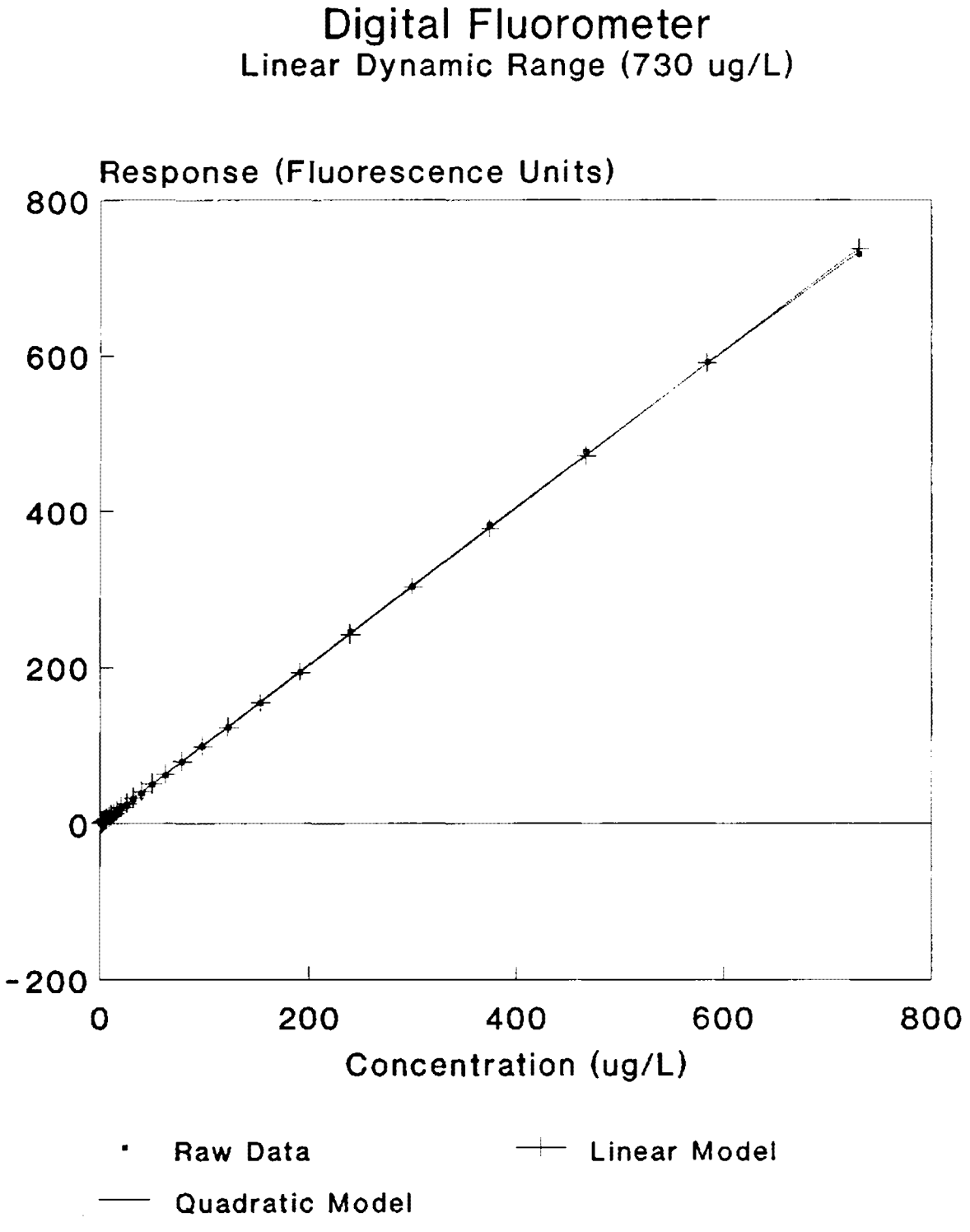
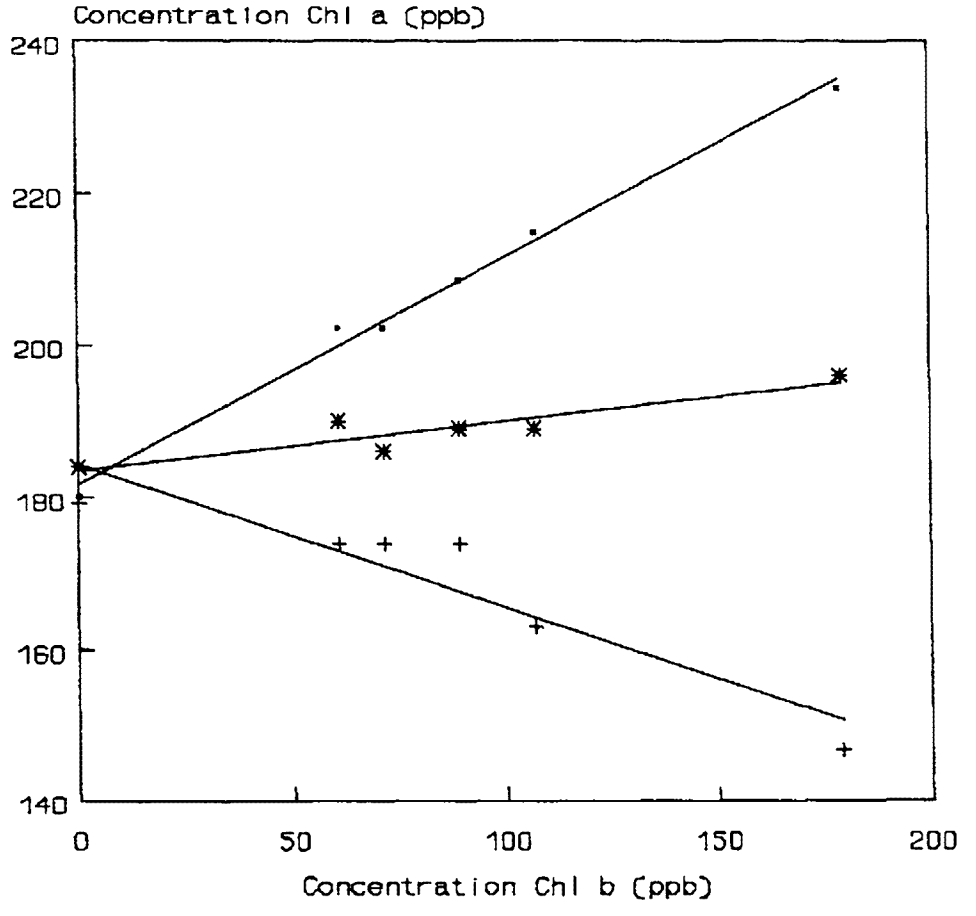

Digital Fluorometer
Linear Dynamic Range (730 ug/L)
Response (Fluorescence Units)
800
600
400
200
0
-200
0
200
400
600
800
Concentration (ug/L)
Raw Data
Linear Model
Quadratic Model

Figure 6

Effect of Chl b on Chl a

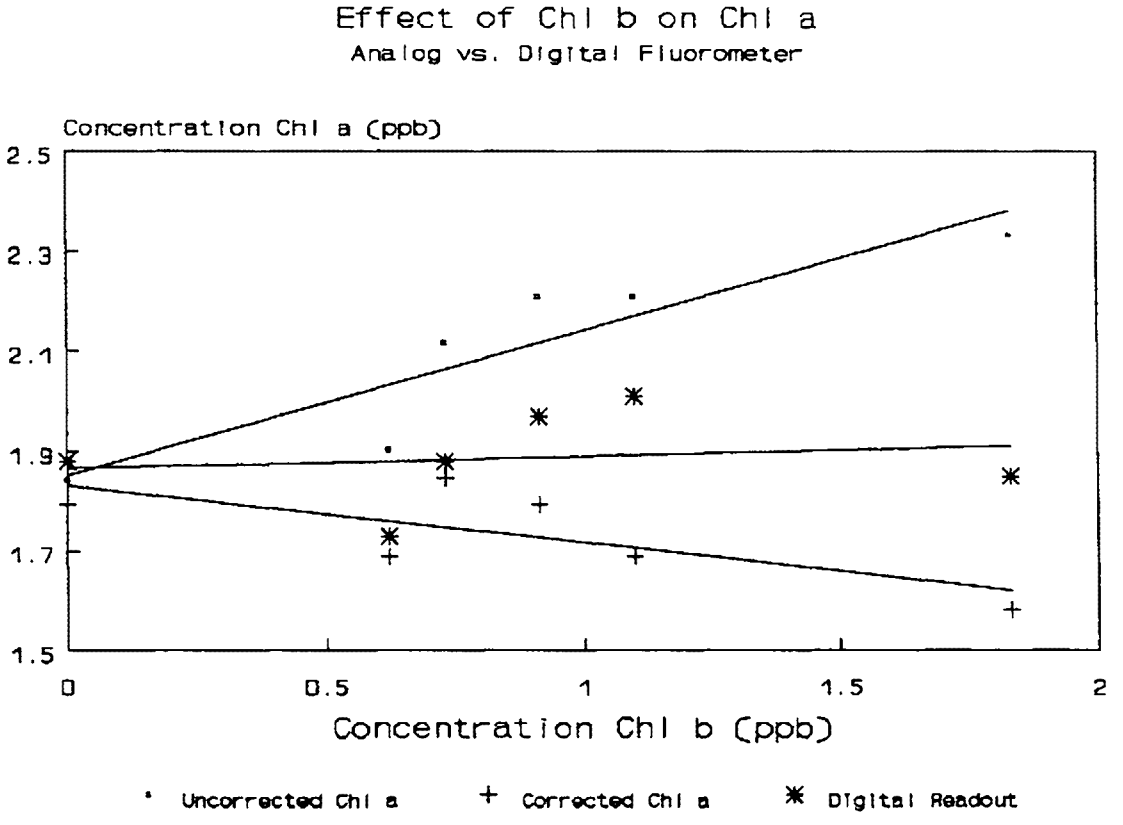
Analog vs. Digital Fluorometer



	Average %Error (Slope X 100)
Uncorrected chl *a*	+29.8%
Corrected chl *a*	-18.9%
Digital chl *a*	+ 6.3%

Chlorophyll *a* True Value = 180 ppb

Figure 7



	Slope X 100 (Average %Error)
Uncorrected chl *a*	+28.9%
Corrected chl *a*	-11.5%
Digital chl *a*	+ 2.3%

Chlorophyll *a* True Value = 1.8 ppb

Ratios chl a:chl *b* were 1:0, 3:1, 2.5:1, 2:1, 1.7:1 and 1:1

Average %Error for digital method for six concentrations (1.8 ppb to 180 ppb) was +6%

Figure 8

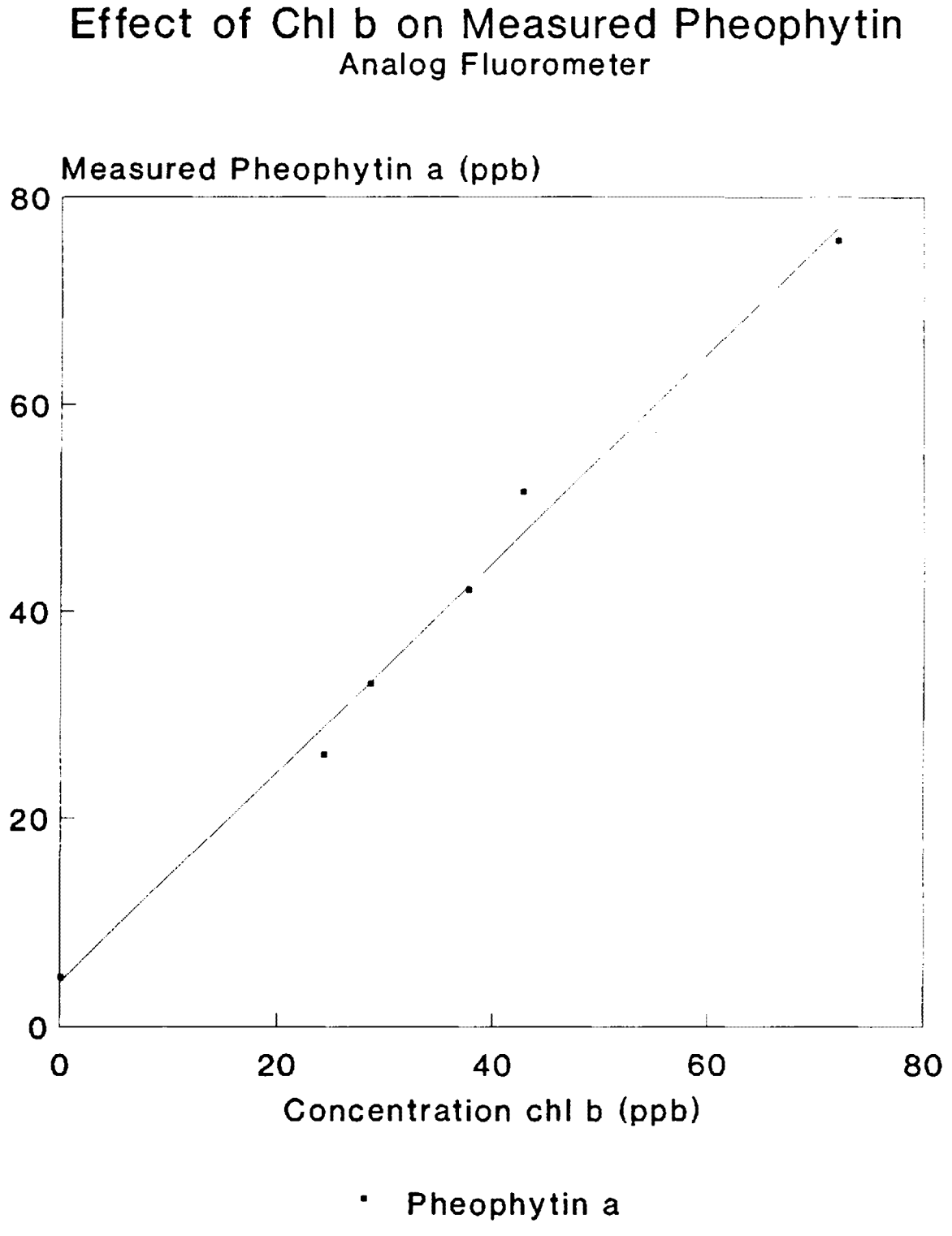

Effect of Chl b on Measured Pheophytin
Analog Fluorometer
Measured Pheophytin a (ppb)
80
60
40
20
0
0
20
40
60
80
Concentration chl b (ppb)
Pheophytin a

Figure 9

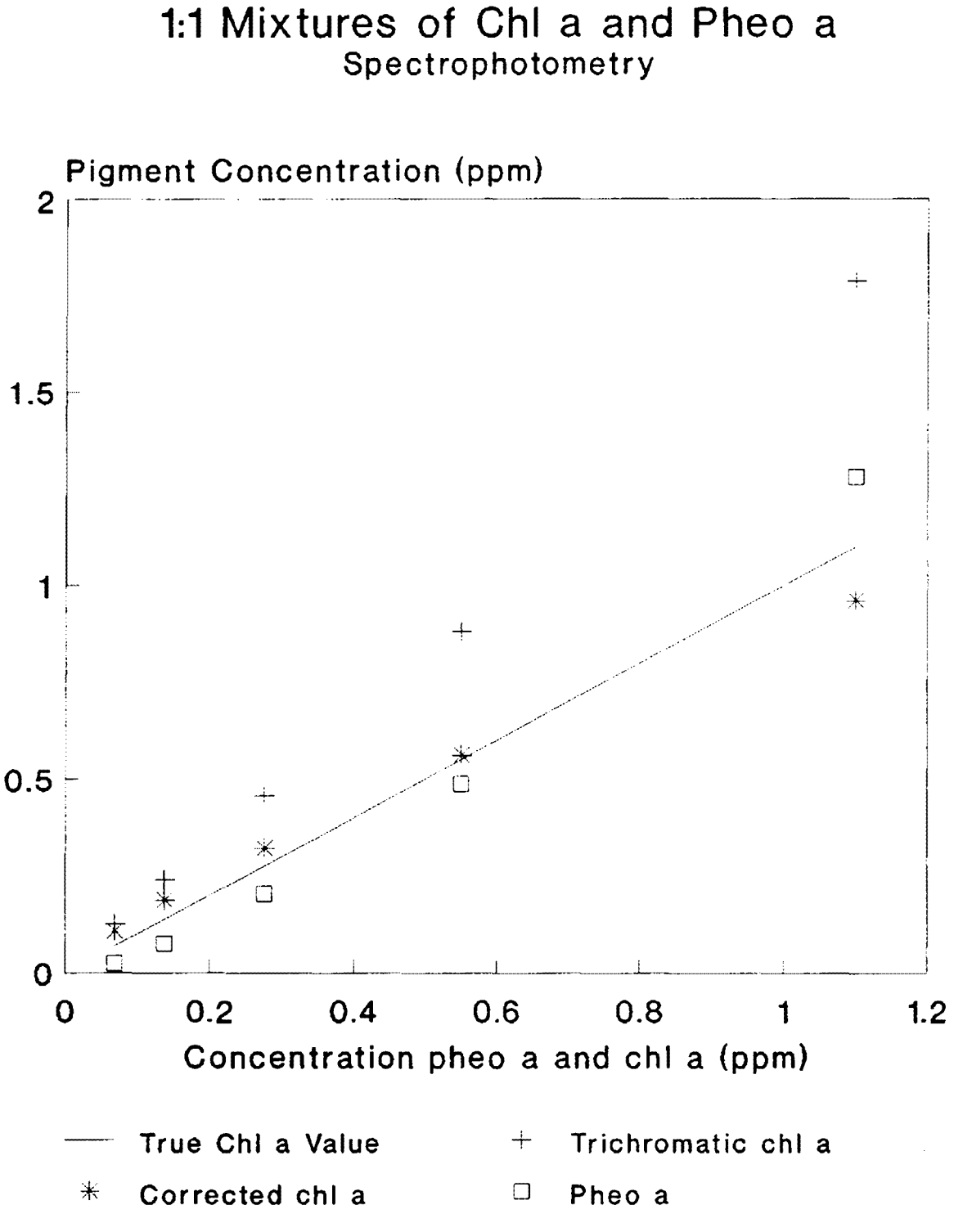
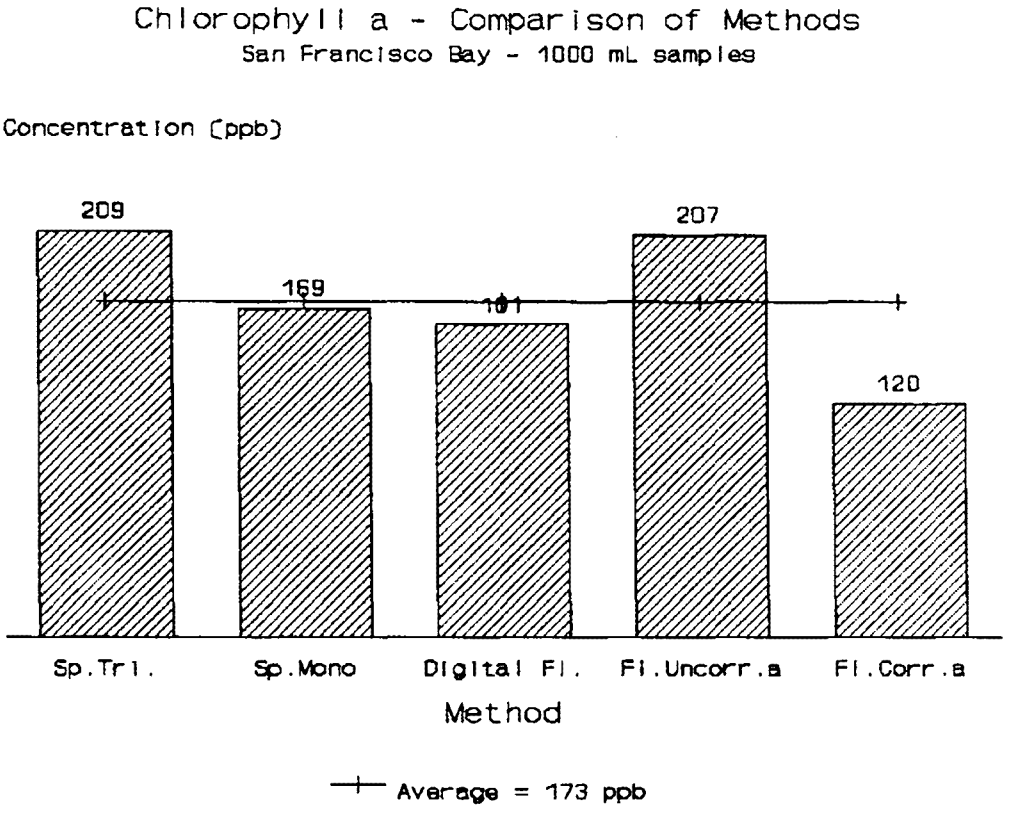

1:1 Mixtures of Chl a and Pheo a
Spectrophotometry
Pigment Concentration (ppm)
2
1.5
1
0.5
0
0
0.2
0.4
0.6
0.8
1
1.2
Concentration pheo a and chl a (ppm)
True Chl a Value
Trichromatic chl a
Corrected chl a
Pheo a

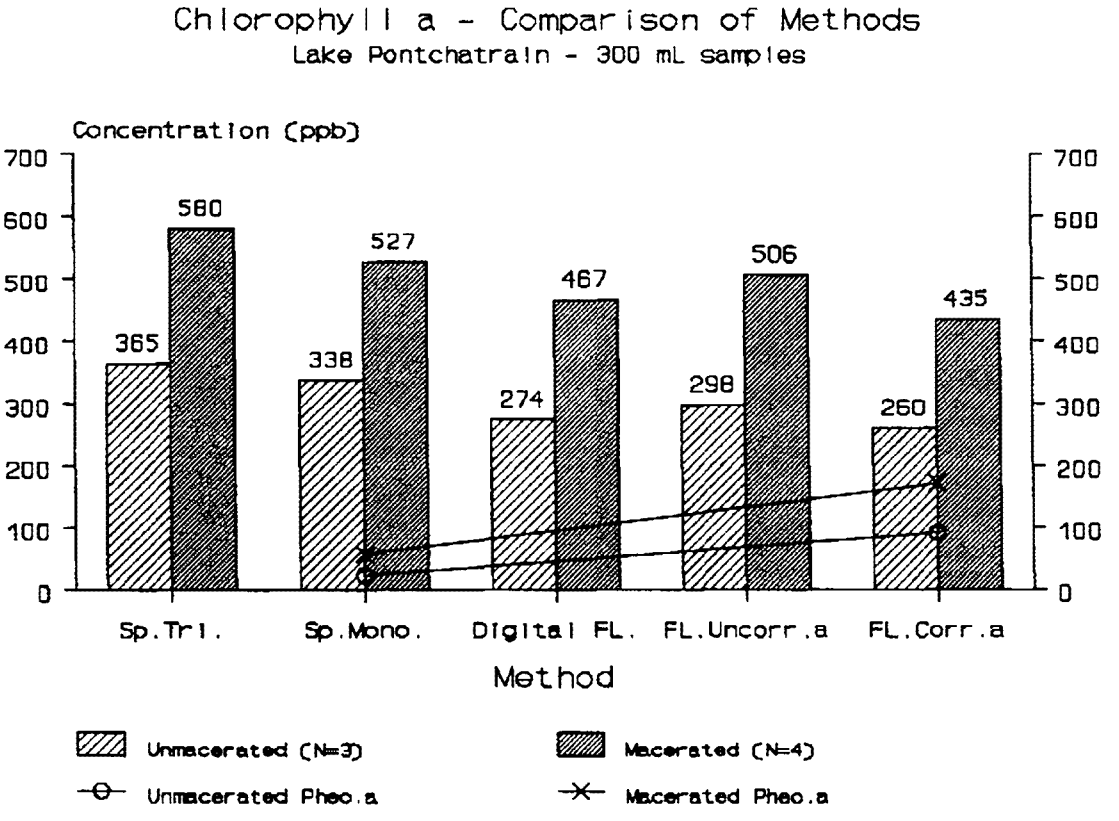
Figure 10



N=3, Samples were macerated

	MEAN (ppb)	RSD
Sp.Tri.	209	6.1%
Sp.Mono	169	9.1%
Digital Fl.	161	4.5%
Fl.Uncorr.a	207	5.7%
Fl.Corr.a	120	4.8%
METHODS	173	21.3%

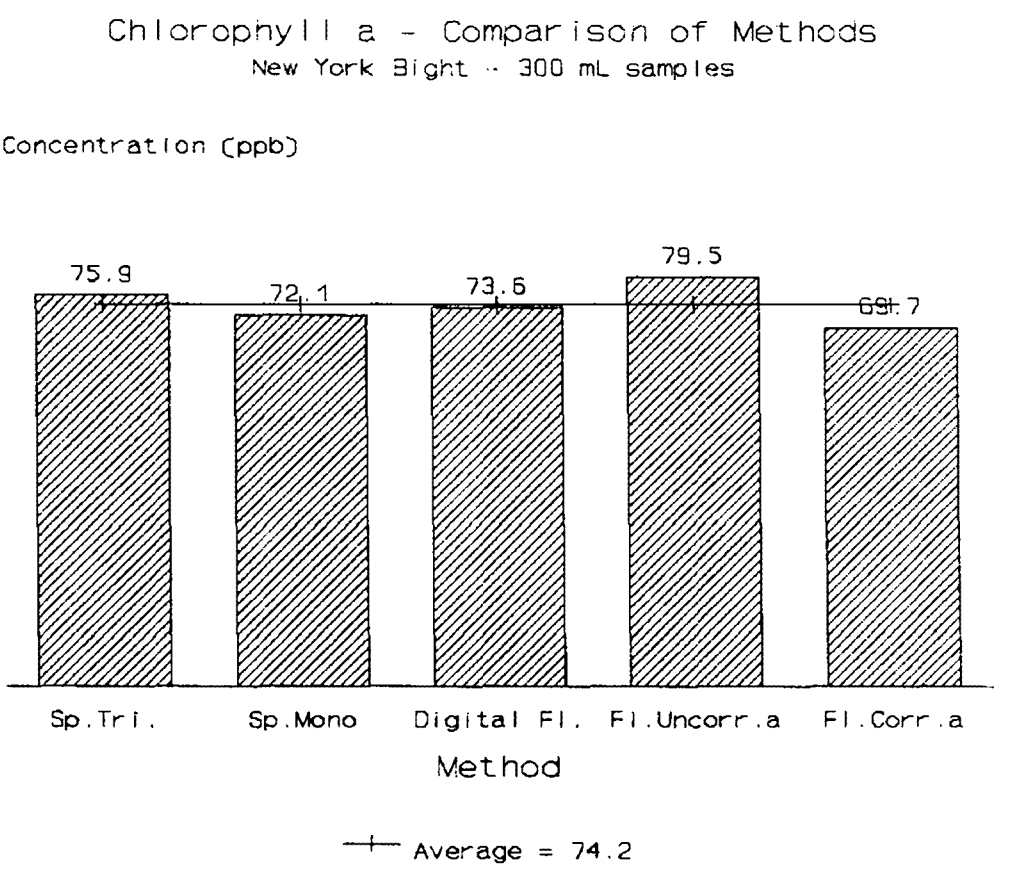
Figure 11



ALL METHODS

	MEAN (ppb)	RSD
Macerated	503	11.1%
Unmacerated	307	14.3%

Figure 12

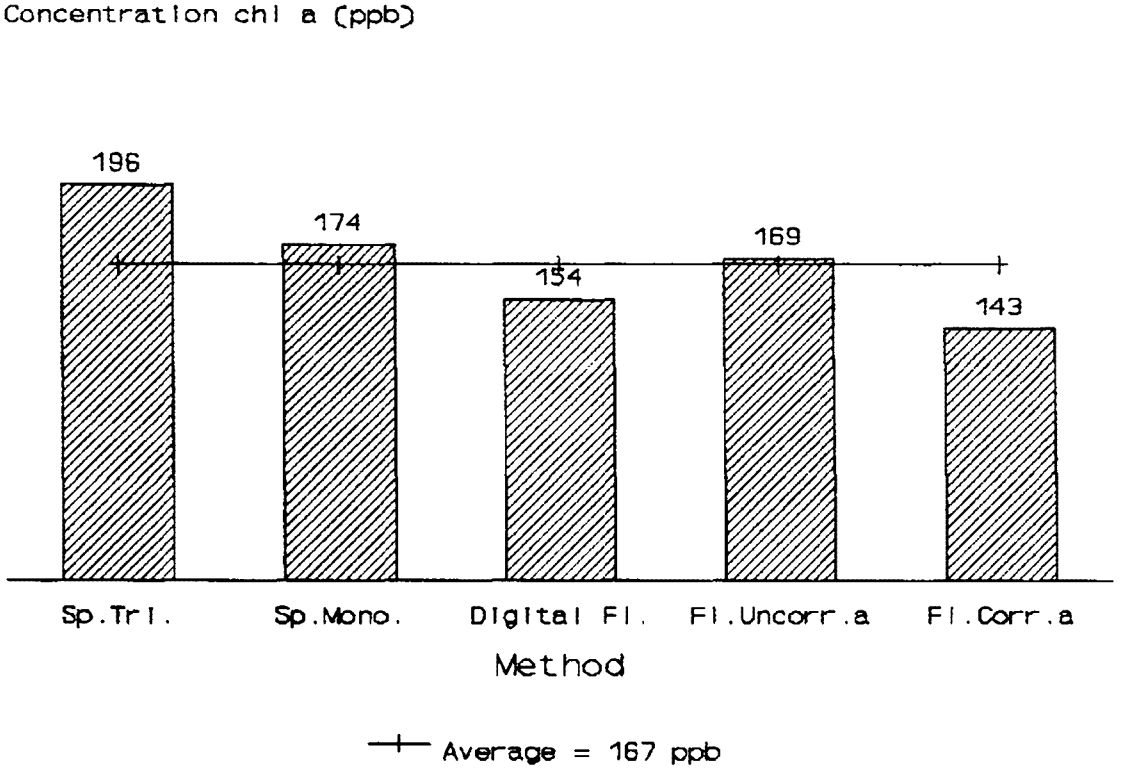


N=3, samples were macerated

	Mean (ppb)	RSD
Sp.Tri.	75.9	6.4%
Sp.Mono.	72.1	9.8%
Digital Fl.	73.6	8.1%
Fl.Uncorr.a	79.5	6.6%
Fl.corr.a	69.7	7.2%
METHODS	74.2	5.1%

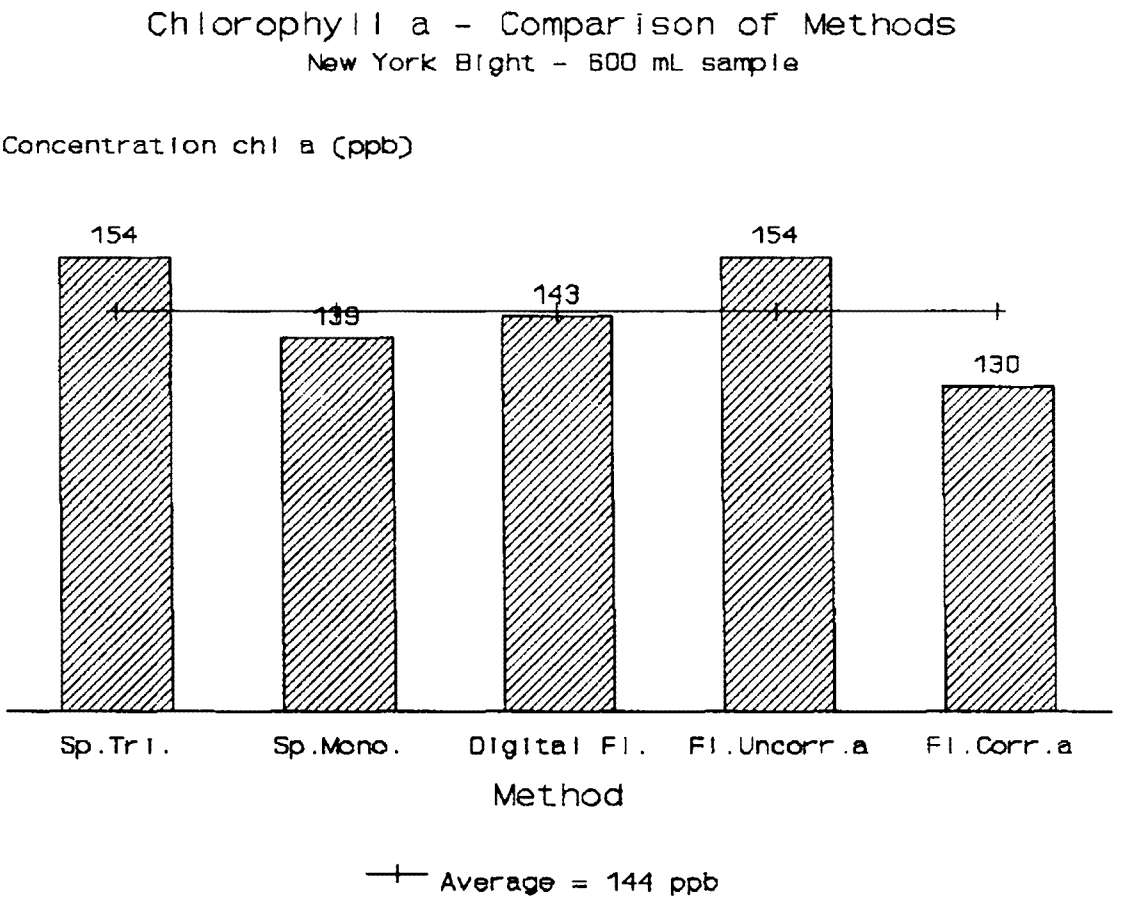
Figure 13

Chlorophyll a - Comparison of Methods
New York Bight - 900 mL sample



	Mean (ppb)	RSD
Digital Fl.	154	2.5%
Fl.Uncorra.	169	2.2%
Fl.Corr.a	143	2.2%
METHODS	167	12.1%

Figure 14



N=3, samples were macerated

	Mean (ppb)	RSD
Digital Fl.	143	1.9%
Fl.Uncorr.a	154	1.2%
Fl.Corr.a	130	0%
METHODS	144	7.1%

Figure 15

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RE
EPA/600/R-94/150		

4. TITLE AND SUBTITLE	5. REPORT DATE
Summary Report: Evaluation of a New Fluorometric Technique that uses Highly Selective Interference Filters for Measuring Chlorophyll a in the Presence of Chlorophyll b and Pheopigments	July 1994
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Elizabeth J. Arar	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Inorganic Chemistry Branch, Chemistry Research Division EMSL-Cincinnati, U.S. Environmental Protection Agency 26 W. Martin Luther King Dr. Cincinnati, Ohio 45268	11. CONTRACT/GRANT NO
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Same as 9	Summary Report
	14. SPONSORING AGENCY CODE
	EPA/600/06

15. SUPPLEMENTARY NOTES

16. ABSTRACT

A new fluorometric technique was compared to conventional fluorometry with and without pheophytin *a* (pheo *a*) correction and to spectrophotometry using Lorenzen's modified monochromatic equations and Jeffrey and Humphrey's trichromatic equation to calculate chlorophyll *a* (chl *a*). The new technique uses excitation and emission filters that are highly selective for chl *a*, eliminating the interference caused by accessory pigments and pheopigment degradation products.

Four method parameters were evaluated using the new technique, conventional fluorometry and spectrophotometry. They were (1) sensitivity, (2) linear dynamic range, (3) precision, and (4) accuracy. Controlled studies of the interference caused by chl *b* were conducted, and real world samples of varying taxonomic composition were analyzed. For laboratory solutions, the new technique was comparable to conventional fluorometry with respect to sensitivity and accuracy. The linear dynamic range for the new technique exceeded that of conventional fluorometry by a factor of three. Interference caused by chl *b* was +6% at the highest chl *a*:chl *b* likely to occur in nature. Chl *a* values obtained using the new technique compared well with conventional fluorometry when pheo *a* was the only interfering pigment present in the sample.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to Public	Unclassified	
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	

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