

AN ABSTRACT OF THE THESIS OF

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Title: NON-BIOLOGICAL UPTAKE OF ZINC-65 FROM A MARINE
ALGAL NUTRIENT MEDIUM

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The nature and magnitude of experimental errors due to ^{65}Zn adsorption by inorganic surfaces was examined in a laboratory system designed to measure ^{65}Zn uptake by marine phytoplankton. In the pH range, $6.3 \pm .1$ to $7.5 \pm .1$, a precipitate formed in the algal nutrient medium selected for the system. To this seawater-base medium, zinc was added only as carrier-free ^{65}Zn . The precipitate increased in both volume and tendency for ^{65}Zn uptake as the pH increased. At a pH of $7.5 \pm .1$ the particles, predominantly orthophosphates, accumulated 70 percent of the ^{65}Zn in the medium in 24 hours. It was therefore concluded that ^{65}Zn adsorption by undetected precipitates could result in serious errors in measurements of ^{65}Zn uptake by marine phytoplankton.

Equilibrium of ^{65}Zn adsorption by Pyrex glass surfaces was attained between .50 and 2.0 hours elapsed time. Found to be negligible

at pH values less than $6.0 \pm .1$, such uptake was a linear function of the hydrogen ion concentration in the pH range, $6.7 \pm .1$ to $8.2 \pm .1$. Pretreatment of the glass surfaces with dimethyldichlorosilane (General Electric "Dry Film") reduced ^{65}Zn adsorption by over 80 percent.

The relationship between percent ^{65}Zn sample adsorption and wetted glass surface area/pipette sample volume was shown to be linear for volumetric pipettes of the size range, 1-15 ml. At a pH of $7.5 \pm .1$, ^{65}Zn adsorption was negligible for most laboratory glassware, but increased with increased pH. At a pH value of about 8.0, glassware having surface area/sample volume ratios as small as those of 15 and 20 ml volumetric pipettes adsorbed 7 to 11 percent of the contained sample activity. Two prerinses with the sample liquid were required to reduce such errors by a factor of one half.

Non-Biological Uptake of Zinc-65 from
A Marine Algal Nutrient Medium

by

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THE CALMING THOUGHT OF ALL

That coursing on, whate'er men's speculations,
Amid the changing schools, theologies, pholosophies,
Amid the bawling presentations new and old,
The round earth's silent vital laws, facts, modes continue.

-WALT WHITMAN

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NON-BIOLOGICAL UPTAKE OF ZINC-65 FROM A MARINE ALGAL NUTRIENT MEDIUM

INTRODUCTION

In recent years, radioisotopes are being used with increasing frequency as tracers in ion transport studies. As such, the use of radioactive materials has raised the sensitivity of ion detection by as much as 10^8 times over that of previous methods (Wang and Willis, 1965). Associated with improved techniques for ion detection is the ability to follow smaller amounts of specific materials through biological systems. Laboratory studies dealing with ion uptake by marine phytoplankton have benefitted considerably from this circumstance. At the same time, adsorption of radioelements on inorganic surfaces can be a potential source of error in such studies. A qualitative and quantitative analysis of this effect is necessary to interpret data from subsequent experiments. This thesis deals with the evaluation of the error due to non-biological uptake of ^{65}Zn from a marine algal nutrient medium.

In both natural and artificial aqueous environments, micronutrients such as zinc may be taken up by marine algae in excess of their metabolic needs. Although theoretical calculations give a figure of 2.7 ppm as an approximate zinc requirement per unit weight of soft tissue (Pequegnat, Fowler and Small, 1969), both laboratory and

environmental measurements have demonstrated large zinc concentration factors in marine algae. The concentration factor is defined as total zinc per unit weight of algae/total zinc per unit weight of culture medium, where the units are the same for the algae and the medium. For a species of filamentous blue-green algae, Harvey (1967) determined experimental zinc concentration factors ranging from 22,000 at 25°C to 33,500 at 40°C. Cultures of the marine diatom, Nitzschia closterium, showed a ^{65}Zn concentration factor of 50,000, calculated on a volume basis (Chipman, Rice and Price, 1958). On a live-weight basis, Polikarpov (1966) lists environmental zinc concentration factors of 127 and 290 for two species of green algae, and a range of 420 to 1400 for seven species of brown algae. Similarly, Gutknecht (1965) gives an experimental range of concentration factors of 30 to 3300 for ^{65}Zn accumulated by nine species of seaweed.

Some authors have accorded such high zinc concentration factors to algal cell surface adsorption. This explanation has elicited both contention (Bachmann and Odum, 1960) and support (Gutknecht, 1965) on the basis of ^{65}Zn tracer studies. Bachmann and Odum, however, failed to use carrier-free ^{65}Zn , and therefore conjectured that the sensitivity of their measurements was probably insufficient to detect adsorption. Chipman, Rice and Price (1958) used carrier-free ^{65}Zn and found that most of the uptake by Nitzschia closterium was due to surface adsorption.

Zinc adsorption is not restricted to tissue surfaces alone. Depending on such controlling factors as pH and zinc concentration, adsorption within an experimental laboratory system may occur on a wide variety of surfaces. These include precipitates and laboratory glassware. Indeed, this phenomenon imposes severe limitations on studies seeking zinc uptake data in general. Nutrient media often contain abnormally high levels of nutrients in comparison to the natural environment (Fogg, 1966). To maintain solution of these added salts, chelating compounds such as sodium ethylenediamine tetraacetate (Na_2EDTA) are introduced into the media. However, various workers (Chipman, Rice and Price, 1958; Polikarpov, 1966; Bernhard and Zattera, 1967) have indicated that EDTA strongly chelates zinc, and renders it unavailable for algal uptake. Thus, the addition of extra zinc salts and a chelating agent to a seawater-based nutrient medium contribute to an unnatural level of available zinc.

Any serious attempt to duplicate environmental levels and rates of zinc uptake under laboratory conditions must use environmental zinc concentrations and eliminate chelating agents from the artificial nutrient medium. These limitations can often result in further complications, depending on the composition of the medium. Omission of the chelator may engender chemical precipitation. The problems accompanying precipitation in such systems are basically twofold: 1) the availability of nutrients to the algae is seriously altered, and 2) the

precipitates themselves are often readily capable of adsorbing zinc. Any further measurements of zinc levels associated with the suspended particulate fraction can then no longer be considered representative of algal uptake alone.

Even more serious are the effects of precipitates that may form in a culture already clouded with algal cells. Conceivably, the formation of such particles might go unnoticed and result in serious errors in reported zinc uptake data. Adsorption must be considered a potential source of major error in laboratory studies of zinc uptake by marine algae.

Zinc adsorption may occur on most surfaces in contact with the liquid medium. In this sense, the laboratory glassware cannot be ignored. The general properties of ion adsorption on glass surfaces have been recorded by a number of authors. Polikarpov (1966) has stated that such adsorption is directly proportional to the concentration of the element for a wide range of concentrations up to 10^{-3} moles/l. The adsorption is reduced by further increase of concentration, owing to saturation of the capacity of the adsorbent. This agrees with a statement by Freundlich (1926).

Adsorption of zinc by glass surfaces is generally considered negligible by workers using ^{65}Zn plus carrier zinc in relatively large containers such as beakers. However, as a function of both zinc concentration and available glass surface area, adsorption may be

significant when carrier-free ^{65}Zn (involving extremely low zinc concentrations) is transferred to containers having high surface area to volume ratios, such as pipettes. At moderate activity levels, seawater spiked with carrier-free ^{65}Zn would experience no significant increase in total zinc concentration. Any study using such a medium would measure zinc uptake at oceanic zinc concentrations. The possibility of significant glassware zinc adsorption must be considered if such studies are to be accurate.

The purpose of this thesis was to evaluate, both qualitatively and quantitatively, the non-biological adsorption of zinc in a laboratory system designed to measure zinc uptake by a marine alga. To this end, carrier-free ^{65}Zn was used as a zinc radiotracer. Four general areas of investigation received special emphasis. These were: 1) the physiochemical nature of the nutrient medium precipitate, 2) adsorption of ^{65}Zn by the nutrient medium precipitate, 3) adsorption of ^{65}Zn by laboratory glassware, and, as a factor potentially influencing adsorption, 4) pH levels in algal culture conditions.

METHODS AND MATERIALS

General Experimental Preparations

All radiozinc used in these experiments was carrier-free ^{65}Zn prepared by New England Nuclear by the reaction, $^{65}\text{Cu} (p, n) ^{65}\text{Zn}$. It was received as .20 mCi ^{65}Zn in .19 ml of 1.0N HCl, and was subsequently diluted with deionized distilled water to a total volume of approximately 5 ml. This stock solution was used to spike the experimental cultures, which consisted principally of seawater filtered through a .45 μ pore membrane filter. For the studies of ^{65}Zn uptake by algal growth culture precipitates, inorganic nutrients and vitamins were added as specified below.

The algal nutrient medium used in these studies was similar to that first characterized by Davis (1961). The Davis medium was modified as shown in Table 1. Because the present studies were designed to deal with problems encountered in algal uptake experiments involving ^{65}Zn , EDTA was eliminated from the medium in an attempt to duplicate the conditions of algal growth studies simulating natural conditions. Similarly, the quantity of FeCl_3 was maintained at a reduced level previously established in this laboratory as an attempt to reduce precipitation in ^{65}Zn uptake experiments using algae. Zinc was deleted from the trace-metal complement to prevent its interference with uptake measurements using carrier-free ^{65}Zn . The

Table 1. Algal Nutrient Medium

Component	Concentration	
	Davis Medium	* Modified Davis Medium
KH_2PO_4	200 mg/l	200 mg/l
Thiamine HCl	.2 mg/l	.2 mg/l
Biotin	1.0 $\mu\text{g/l}$	1.0 $\mu\text{g/l}$
Vitamin B ₁₂	2.0 $\mu\text{g/l}$	2.0 $\mu\text{g/l}$
Na_2EDTA	7.3 mg/l	---
FeCl_3	6.88 mg/l	1.28 mg/l
NaNO_3	150 mg/l	150 mg/l
$\text{CuCl}_2 \cdot 5\text{H}_2\text{O}$	19.5 $\mu\text{g/l}$	19.5 $\mu\text{g/l}$
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	44.0 $\mu\text{g/l}$	---
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	22.0 $\mu\text{g/l}$	22.0 $\mu\text{g/l}$
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	360 $\mu\text{g/l}$	360 $\mu\text{g/l}$
$(\text{NH}_4)_2\text{MoO}_4$	12.5 $\mu\text{g/l}$	12.5 $\mu\text{g/l}$
NH_4Cl	25 mg/l	25 mg/l

*Medium base: seawater filtered through a .45 μ pore membrane filter.

level of total zinc in the base seawater was determined by atomic absorption spectrometry to be $12.4, \mu\text{g/l} \pm 5$ percent.

The experimental culture vessels consisted of 2l Pyrex beakers modified to facilitate maintenance of constant pH values. As shown by Figure 1, this was accomplished by fitting the beakers with rubber-sealed caps of acrylic plastic. These lids had stoppered ports large enough to permit pH measurements and sampling with a pipette, but sufficiently small to prevent significant mixing of the internal and external atmospheres when opened for a short time. This setup enabled maintenance of a near-constant partial pressure of CO_2 within the cultures. The pH could be adjusted downward by bubbling the medium with CO_2 and adjusted upward by bubbling with air.

All of the culture beakers were treated with dimethyldichlorosilane, a commercially produced liquid (General Electric "Dry Film", #SC-300V) which purportedly reduces adsorption of ions by glass surfaces. This contention was verified experimentally with respect to zinc, and the related data are presented in the results section of this thesis.

Figure 2 shows the configuration of the plastic cage used to hold Pyrex glass rods for the glass adsorption experiments. Its open construction permitted free flow of the radioactive seawater over the glass surfaces during periods of uptake.



Figure 1. Experimental culture apparatus used in nutrient medium precipitate studies.

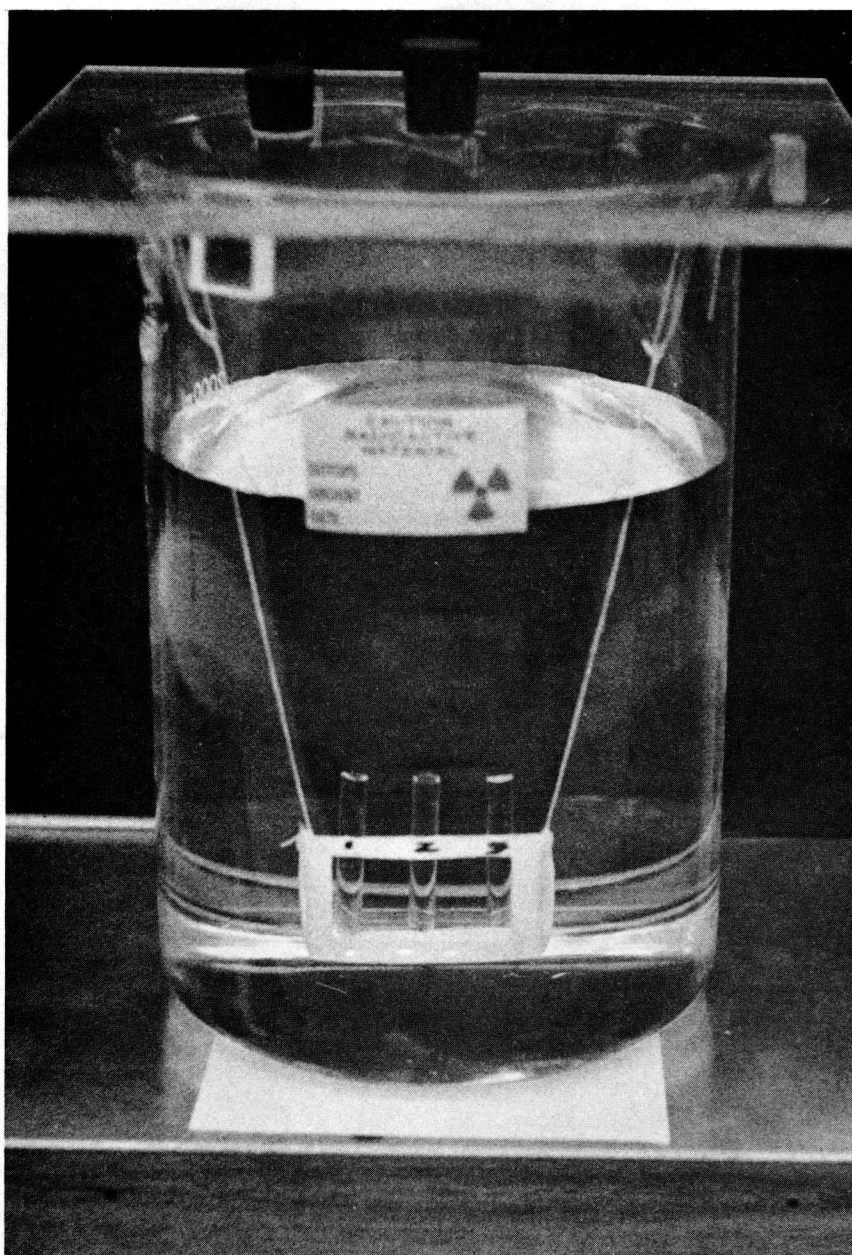


Figure 2. Nutrient medium culture vessel showing configuration of the plastic cage used to hold Pyrex glass rods for the ^{65}Zn adsorption experiments.

Physiochemical Studies: Nature of the Nutrient Medium Precipitate

The qualitative and quantitative effects of pH on precipitate formation were examined in two experiments. In the first, the pH in the 2 l culture vessels was adjusted prior to precipitation. In the second, the pH was adjusted after precipitation, and the system was then allowed to equilibrate for several days. Each experiment involved four pH values: $6.3 \pm .1$, $6.7 \pm .1$, $7.1 \pm .1$ and $7.5 \pm .1$. All of the cultures were continuously stirred on a magnetic stirrer at a rate sufficient to keep all solids in suspension. When equilibration of the systems was assured, the relative volume of suspended solids was measured by centrifuging a 50 ml sample from each vessel and transferring the solid portion to a capillary tube which had been sealed at one end and calibrated to measure contained volumes (mercury was used to calibrate each tube at successive $1 \mu\text{l}$ intervals). The samples were again centrifuged, at 1500 rpm for 10 minutes, and their relative packed volumes recorded. The color and consistency of the precipitates was examined under a microscope using white light, and reported along with the other observations.

The precipitate formed at a pH of $7.5 \pm .1$ was analyzed for chemical composition using a variety of methods. X-ray diffraction was used to determine the crystalline structure. Suggestions were then made as to possible chemical components, considering crystalline

structure, physical consistency, color, relative solubility, the concentration of chemicals added to the seawater, and the probable composition of the seawater itself. On this basis, pre-weighed samples of precipitate that had been filtered, washed with deionized distilled water and dried at 110°C were subjected to various analyses, including atomic absorption spectrometry and photometric methods. The results were reported as percent by weight of the original precipitate.

Zinc-65 Uptake Studies: Adsorption by the
Nutrient Medium Precipitate

The uptake of ^{65}Zn by the nutrient medium precipitate was studied using the same cultures examined with respect to the physical nature of the precipitate. Four 1.5 l cultures were involved, having initial pH values of $6.3 \pm .1$, $6.7 \pm .1$, $7.1 \pm .1$ and $7.5 \pm .1$. The pH was monitored throughout the experiment. To each beaker, enough ^{65}Zn stock solution (1.0 ml) was added to give a final activity level of approximately $4\mu\text{Ci } ^{65}\text{Zn/l}$. The level of total zinc was $12\mu\text{g/l}$.

Samples were taken from each of the four cultures at 1, 2, 4, 8, 16, 32, 64 and 128 hours elapsed time. The sampling procedure involved taking duplicate samples of two types at each sampling period. One type consisted of .5 ml of the total culture suspension, stirred constantly to maintain homogeneity. The second sample type was .5 ml of the centrifugate of a larger volume of the total suspension, which

had been centrifuged at 1500 rpm for five minutes. All samples were diluted to 12 ml volume in polypropylene counting tubes and radio-analyzed in a 3" x 5" NaI(Tl) well crystal coupled to a Nuclear Data Series 130 pulse height analyzer. The radioactivity associated with the precipitate itself was calculated as the difference between these two sample types. All of the values were reported as pCi ^{65}Zn /ml suspension.

To minimize loss of ^{65}Zn through adsorption to the equipment, polypropylene tubes were used for centrifugation. All pipetting was done with a 500 μl Eppendorf push-button microliter pipette with disposable polypropylene tips (Brinkmann Instruments, Inc. of Westbury, N. Y.). The adsorption of ^{65}Zn to the polypropylene surfaces in each case was found by direct measurement to be less than one percent of the contained sample. The accuracy of the pipette was given by the manufacturer as $\pm .3$ percent at three standard deviations.

Zinc-65 Uptake Studies: Adsorption by Laboratory Glassware

The glassware adsorption studies were conducted on two basic levels--general and specific. In a general sense, borosilicate (Pyrex) glass rods were used to determine adsorption as a function of time and adsorption-surface area relationships which could potentially be applied to specific laboratory apparatus. To supplement these results, a varied size range of Pyrex volumetric pipettes was

examined to determine the potential error resulting from adsorptive uptake from contained sample volumes.

The glass rods used in all of the ^{65}Zn uptake experiments were scored and broken in 40 ± 1 mm lengths from a standard Pyrex glass shaft 5 mm in diameter. In each of the related experiments, three of these rod segments were used as triplicate samples for each sampling condition or period. The corresponding experimental values were reported as the mean ^{65}Zn activity determined by averaging individual radioanalyses of the three rods.

During each period of uptake the glass rods were held suspended in the radioactive solution by a plastic cage, which contacted a minimum of glass surface area and permitted free circulation around the rods (Figure 2). The liquid medium, which was constantly stirred, was seawater containing approximately $4 \mu\text{Ci } ^{65}\text{Zn/l}$, with a total zinc concentration of about $12 \mu\text{g/l}$. At the end of each uptake period, the cage was removed from the solution and lowered into a non-radioactive seawater rinse medium. The rods were then rinsed thoroughly with a wash bottle also containing seawater, which in all instances was drawn from the same polyethylene carboy as that used for the uptake medium. The glass rods were then placed in individual counting tubes for radioanalysis, and three more pieces were lowered into the radioactive water. The entire rinse procedure took about three minutes for each triplicate set of rods.

A series of studies was done using the Pyrex rods. The first was designed to measure the rate of uptake of ^{65}Zn by borosilicate glass. For this experiment, seven sets of triplicate samples were used. The rods were initially soaked in acetone and then dried. Following this pretreatment, the sample sets were exposed to ^{65}Zn -spiked seawater ($4\ \mu\text{Ci } ^{65}\text{Zn/l}$, $\text{pH} = 8.0 \pm .1$) for periods of .13, .25, .50, 1.0, 2.0, 4.0 and 8.0 hours, rinsed and radioanalyzed. The activity of the seawater was measured several times during the experiment to determine if it was significantly reduced by loss of ^{65}Zn to the glass surfaces and the plastic cage. Uptake of zinc by these surfaces was found to be below detection limits for radioanalyses of the uptake solution.

An attempt was also made to evaluate the effectiveness of the rinse procedure. Following the first radioanalysis, the rods were again rinsed using a seawater-filled wash bottle, and then radioanalyzed a second time.

To determine if a dilute wash of HF prior to ^{65}Zn uptake would more effectively clean and standardize the glass surfaces, i.e., clarify experimental trends by decreasing the percent sample standard deviation of the ^{65}Zn activity/unit glass surface area, the same seven rod sets were used in a second uptake experiment. The rods were this time soaked in a .3N HF solution for one minute, rinsed with deionized

distilled water, and dried. The rest of the experimental procedure was identical to that described previously.

The relationship between pH and ^{65}Zn adsorption was studied using a second set of glass rods. These rods were pretreated with a one minute soak in .3N HF, followed by a deionized distilled water rinse. They were then dried and exposed to ^{65}Zn -spiked seawater ($4\mu\text{Ci } ^{65}\text{Zn/l}$) for one hour. Six sets of rods were used--at pH values of 5.0, 6.0, 6.7, 7.2, 7.5 and 8.2. In each case, the pH of the rinse water matched that of the uptake solution.

The effect of various types of chemical pretreatments on ^{65}Zn adsorption by borosilicate glass was also examined. Following an acetone wash and treatment with .3N HF, five sets of rods were exposed overnight to the following conditions: a .1M solution of CuCl_2 , a saturated solution of ZnCl_2 , $\text{K}_2\text{Cr}_2\text{O}_7$ glassware cleaning solution, the vapors of dimethyldichlorosilane (General Electric's "Dry Film") and air (control sample). The rods were subsequently rinsed with deionized distilled water, dried and exposed to ^{65}Zn -spiked seawater ($4\mu\text{Ci } ^{65}\text{Zn/l}$, $\text{pH} = 8.1 \pm .1$) for one hour. The post-uptake rinse and radioanalysis procedures were as described previously.

The adsorptive uptake of ^{65}Zn by Pyrex volumetric pipettes was measured indirectly by determination of the decrease in the value of $n\text{Ci } ^{65}\text{Zn/g}$ solution, from the stock solution to the pipetted sample. The value of $n\text{Ci } ^{65}\text{Zn/g}$ for the stock solution was established as an

average value for seven decay-corrected samples taken over a period of several days. The standard samples were individually dipped out of the stock container using a polypropylene tube, and poured into a preweighed second tube. This aliquot was then weighed, brought to a total volume of 12 ml, and radioanalyzed. Each uptake sample was drawn by a pipette from the stock container and delivered into a preweighed polypropylene tube. Part of this aliquot was then poured into a second tube, and both samples were weighed and radioanalyzed. The results were reported as the mean value of $n\text{Ci } ^{65}\text{Zn/g solution}$. In each case, the liquid was not allowed to rise above the volume mark on the pipette. Therefore, only that surface area associated with the sample volume was wetted by the liquid and became involved in the adsorptive uptake process. The stock solution was seawater spiked with ^{65}Zn ($4\mu\text{Ci } ^{65}\text{Zn/l}$, $\text{pH} = 8.0 \pm .1$, $12\mu\text{g total Zn/l}$). It was stirred throughout the experiment.

For purposes of rough comparison of adsorptive potential, the geometric inner surface area of each pipette was approximated by a combination of cylinders and cones. This parameter is not to be confused with the specific surface area, which is the total surface area involved in adsorptive processes, including microscopic and submicroscopic surface irregularities. The specific surface area may be much greater than the geometric surface area, and requires special techniques for determination (Stryker and Matijević, 1968).

One set of pipettes was prepared by washing several times with 6N HCl, deionized distilled water and acetone. The sample volumes delivered by these instruments were given by the manufacturer as 2 ml, 3 ml, 6 ml, 10 ml, 15 ml and 20 ml. A second set was rinsed with .3N HF, deionized distilled water and acetone. The stated volumes in this case were 1 ml, 2 ml, 3ml, 5 ml, 10 ml and 15 ml.

One additional pipette adsorption experiment was carried out. This study was done to test the effect on zinc adsorption of a series of rinses with the sample liquid prior to sampling. The procedure was the same as outlined above, with the exception that three successive samples were taken with each pipette. The sample volumes were 3 ml and 6 ml.

pH Study: pH Levels in Algal Culture Conditions

To place the results of the precipitate and glassware adsorption studies on a practical basis, the pH values generated under algal culture conditions were measured using a unicellular, motile species of green algae, Isochrysis galbana. To this end, the pH and cell density of a single 1.5 l culture were monitored for a period of 18 days. The cell suspension was not stirred, but was mixed by bubbling with laboratory air. During the experiment, the culture temperature was $14 \pm 2^{\circ}$ C. The cells were grown in modified Davis medium (Table 1). Cell densities were determined as relative light absorbance values using

a Coleman Hitachi Perkin-Elmer 111 uv-vis spectrophotometer. The measured light wavelength was 430 m μ . The system was equilibrated with respect to precipitation prior to inoculation with the algal cells, which were prepared by centrifuging and washing twice with the culture medium.

RESULTS

Physiochemical Studies: Nature of the Nutrient Medium Precipitate

The results of the qualitative and quantitative examinations of the algal nutrient medium precipitate are summarized in Table 2. The values recorded in Section A were observed regardless of whether the medium pH was adjusted before precipitation, or after. X-ray diffraction indicated that the precipitate was amorphous, i.e., it lacked a definite crystalline structure. Elimination of FeCl_3 from the nutrient medium formula resulted in the formation of a white precipitate. At the same pH ($7.5 \pm .1$), the complete medium gave a yellow-brown precipitate.

As the pH increased from $6.3 \pm .1$ to $7.5 \pm .1$, the relative packed volume of precipitate increased from a value of $1.5 \mu\text{l}$ precipitate/50 ml suspension, to $4.0 \mu\text{l}$ precipitate/50 ml suspension. The physical consistency of the precipitate was seen to vary from gelatinous to particulate with an increase of pH over this range. The precipitate color range was from red-brown at $\text{pH} = 6.3 \pm .1$ to yellow-brown at $\text{pH} = 7.5 \pm .1$. At a pH of $7.5 \pm .1$, PO_4^{3-} -P was found to be the prevalent chemical species in the precipitate (Table 2, Section B). Calcium and magnesium were also found to be major constituents.

Table 2. Qualitative and quantitative analysis of the algal nutrient medium precipitate.

A) Physical characteristics of the precipitate as a function of pH				
pH	Relative packed volume (μ l ppt/50 ml suspension)	Color	Consistency	Crystalline structure
$6.3 \pm .1$	1.5	red-brown	gelatinous	---
$6.7 \pm .1$	2.0	red-brown	gelatinous	---
$7.1 \pm .1$	4.0	orange-brown	gelatinous to particulate	---
$7.5 \pm .1$	4.0	yellow-brown	particulate	amorphous
B) Chemical composition of precipitate formed at pH = $7.5 \pm .1$				
Component	Percent (by weight) of total precipitate		Method of Analysis	
$\text{PO}_4^{3-} - \text{P}$	54.3 ± 3.3		*spectrophotometry	
Ca	14.4 ± 1.4		**AAS	
Mg	$6.8 \pm .3$		AAS	
Fe	$1.7 \pm .1$		AAS	
Zn	$.2 \pm .01$		AAS	
Mn	not detectable		AAS	
Unknown	22.6 ± 5.1			

* Strickland and Parsons (1965)

** Atomic Absorption Spectrometry

Zinc-65 Uptake Studies: Adsorption by the
Nutrient Medium Precipitate

The data presented in Table 2, Section A represent analyses performed on four cultures of algal nutrient medium which were also studied with respect to the effect of pH on ^{65}Zn uptake by the precipitate. A synopsis of the results of the latter measurements is given in Figure 3. In each case, the ^{65}Zn activity of the total suspension and the centrifugate are experimentally measured values, while the ^{65}Zn activity of the particulate fraction was determined as the calculated difference between the two measured values at each sample time.

Corresponding to an increase in pH over the range, 6.3 to 7.5 pH units, there was an increase in the amount of ^{65}Zn associated with the particulate fraction (i. e., the nutrient medium precipitate) at uptake equilibrium. At pH values of $6.3 \pm .1$ and $6.7 \pm .1$, the uptake of ^{65}Zn by the precipitate amounted to less than 10 percent of the initial value of ^{65}Zn activity in the total suspension (right scale, Figure 3 (a-d)). This uptake equilibrium level increased to about 25 percent at $\text{pH} = 7.1 \pm .1$, and nearly 70 percent at $\text{pH} = 7.5 \pm .1$. In relative figures, the ^{65}Zn activity associated with a unit volume of precipitate at equilibrium was 1.3 at $\text{pH} = 6.3 \pm .1$, 1.0 at $\text{pH} = 6.7 \pm .1$, 1.3 at $\text{pH} = 7.1 \pm .1$ and 3.5 at $\text{pH} = 7.5 \pm .1$.

The corresponding ^{65}Zn activity levels for the total suspension

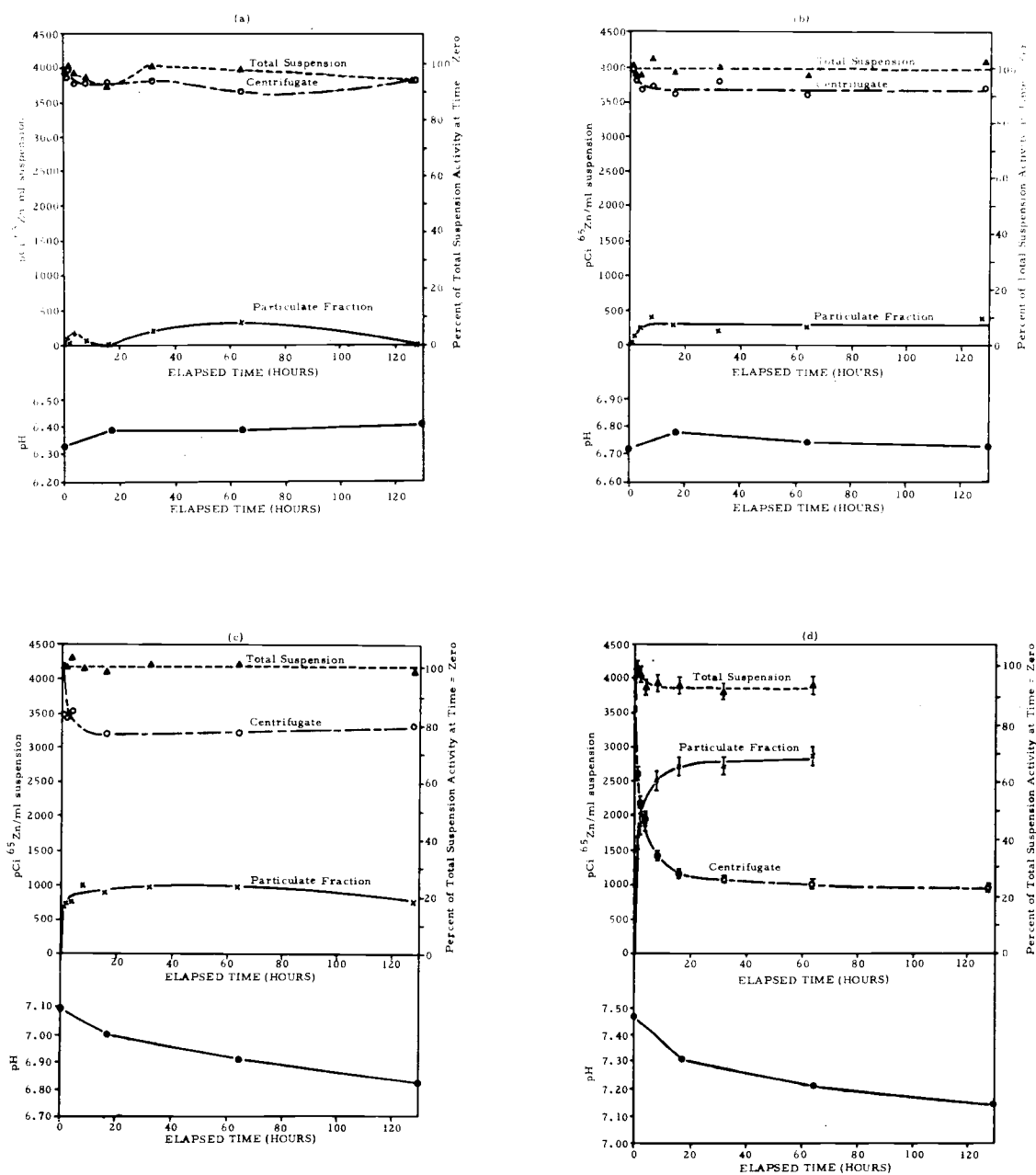


Figure 3. The zinc-65 activity of the total suspension, the centrifugate and the particulate fraction at four pH levels as a function of time. The pH of each culture is also shown as a function of time. The error brackets around the values in part (d) represent a range of \pm three standard deviations of the mean, weighted according to the inverse of the individual sample variances (Harley, 1967)

were nearly constant for the duration of the experiment at pH values of $6.7 \pm .1$ and $7.1 \pm .1$. At $\text{pH} = 6.3 \pm .1$ and $\text{pH} = 7.5 \pm .1$, there was a pronounced initial drop in these activity values.

At each of the four pH levels, uptake equilibrium for the system was essentially complete by 24 hours elapsed time. The experimental value for the ^{65}Zn activity of the total suspension at 128 hours time in Figure 3 (d) was lost. The corresponding value of the ^{65}Zn level associated with the particulate fraction was therefore impossible to calculate.

Curves depicting the relationship between pH and elapsed time for each of the four cultures are also given in Figure 3. The pH remained essentially constant for the systems having initial values of $6.3 \pm .1$ and $6.7 \pm .1$, but showed a decrease for those having values of $7.1 \pm .1$ and $7.5 \pm .1$ at zero elapsed time.

Section A of Table 2 lists the physical characteristics of the precipitate at $\text{pH} = 7.5 \pm .1$ (Figure 3 (d)) as determined at both zero and 128 hours elapsed time. There was no perceptible change in the results of these measurements during the ^{65}Zn uptake experiment.

Zinc-65 Uptake Studies: Adsorption by Laboratory Glassware

The first of a series of experiments using Pyrex glass rods was devised to measure the rate of adsorptive uptake of ^{65}Zn by borosilicate glass surfaces. The rods were prepared by cleaning with

acetone. The results of the corresponding ^{65}Zn adsorption experiment are given in Figure 4. Two curves are shown for cpm $^{65}\text{Zn}/\text{rod}$ as a function of time. The filled circles indicate initial sample activity values. The open circles are those values obtained from a second analysis of the same glass rods, after they had been dried and then rinsed again with seawater. In general, both curves show that ^{65}Zn uptake equilibrium was attained between .50 and 2.0 hours elapsed time. In addition, it is evident that the dry-and-rinse procedure was quite effective in the removal of a significant portion of the original sample activity.

The same glass rods were then soaked in .3N HF for one minute, rinsed with deionized distilled water and dried. Radioanalysis indicated that this procedure removed the remainder of the detectable ^{65}Zn activity from the glass surfaces. The ^{65}Zn adsorption experiment was subsequently repeated. These results are shown in Figure 5. A comparison of Figure 5 with Figure 4 suggests that pretreatment with dilute HF solution greatly increased the zinc adsorptive capacity of the borosilicate glass. This phenomenon may have been temporary, as evinced by the failure of the system to reach a state of equilibrium by eight hours elapsed time. The ^{65}Zn activity level reached a maximum at about one hour elapsed time, and then began a decline which continued for the remainder of the experiment. As observed in the previous experiment, the dry-and-rinse procedure

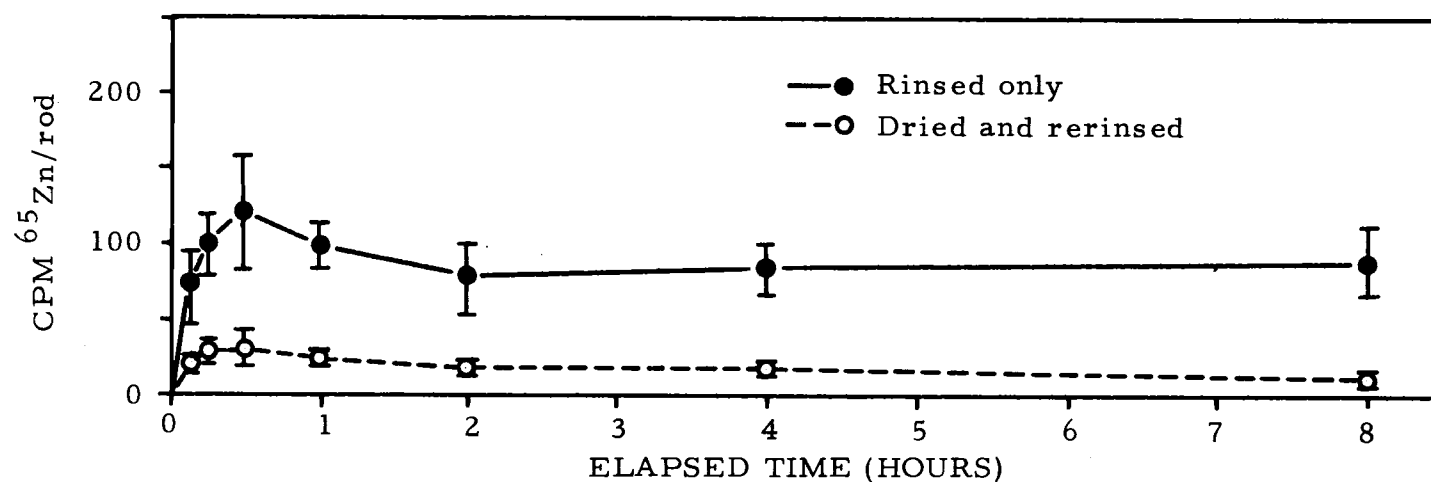


Figure 4. Adsorptive uptake of ^{65}Zn on acetone-pretreated Pyrex glass rods, expressed as ^{65}Zn activity per rod as a function of time exposed to the radioactive medium. The brackets around the sample means represent a range of \pm one standard deviation.

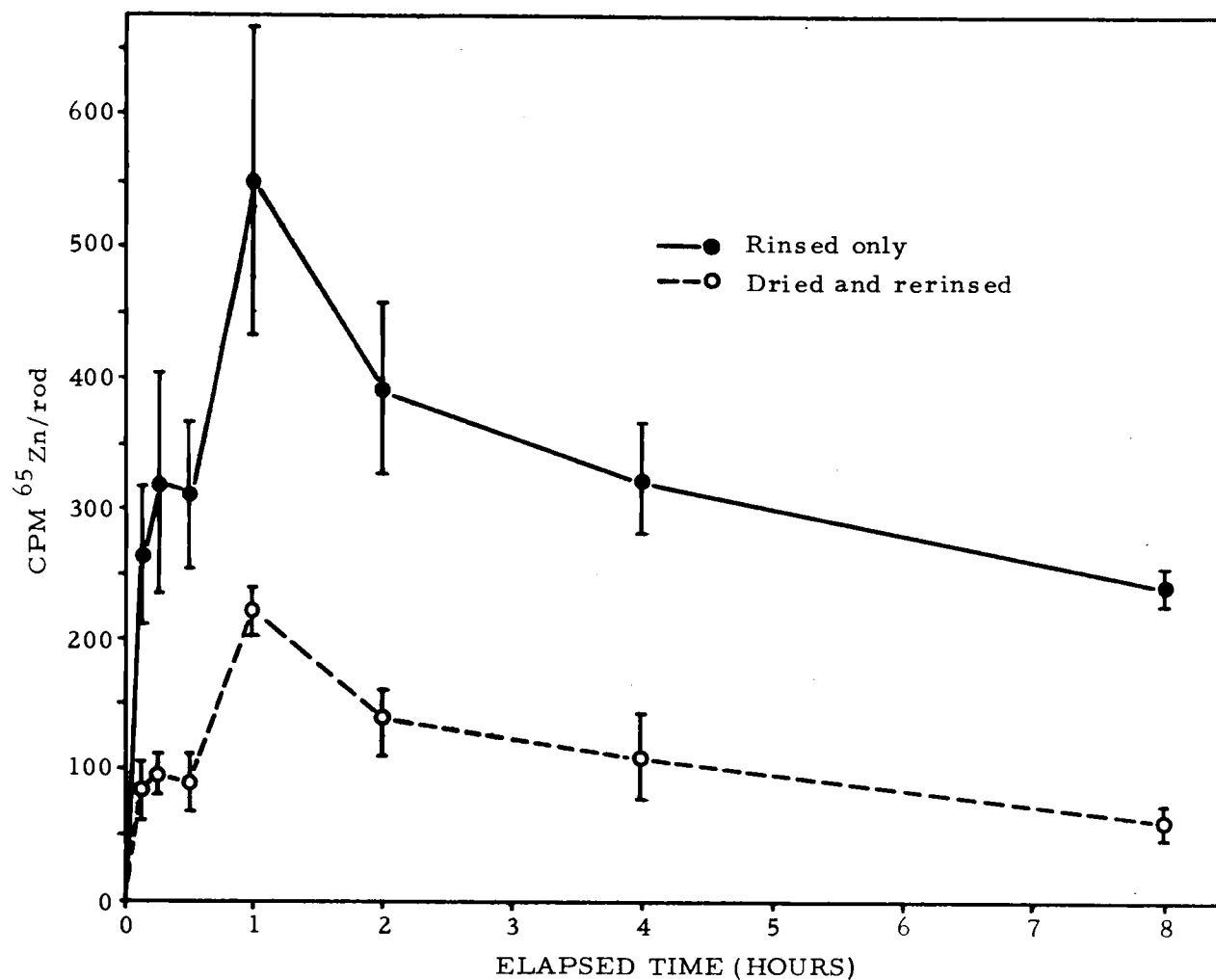


Figure 5. Adsorptive uptake of ^{65}Zn on HF-pretreated Pyrex glass rods, expressed as ^{65}Zn activity per rod as a function of time exposed to the radioactive medium. The brackets around the sample means represent a range of \pm one standard deviation.

removed a substantial amount of the adsorbed ^{65}Zn from the glass rods. The general trend of zinc accumulation, with a maximum level of activity at one hour followed by a decline, was unaffected by this secondary treatment.

The somewhat unexpected effects of the HF pretreatment on the ^{65}Zn adsorption rates greatly increased the difficulty of testing the relative extent of glass surface standardization. The large differences between comparable (according to exposure time) sample means rendered unrealistic the use of Snedecor's F test for the variance ratio (Snedecor, 1934). Evidence for surface standardization is therefore presented in a more practical form in conjunction with the pipette adsorption studies. The present results have been retained for their value in the discussion of the chemical nature of zinc adsorption by glass.

The second major study related to ^{65}Zn adsorption by glass surfaces gave information concerning the effect of solution pH on adsorptive uptake. ^{65}Zn uptake was found to be negligible at pH values of $5.0 \pm .1$ and $6.0 \pm .1$. An increase in pH above 6.0 resulted in increased adsorption of ^{65}Zn . The range of pH examined was $5.0 \pm .1$ to $8.2 \pm .1$. Figure 6 indicates that the relationship between pH and $\log (\text{pCi } ^{65}\text{Zn}/\text{mm}^2 \text{ glass surface})$ is linear for the pH range $6.7 \pm .1$ to $8.2 \pm .1$.

The third ^{65}Zn adsorption study was of a more practical nature.

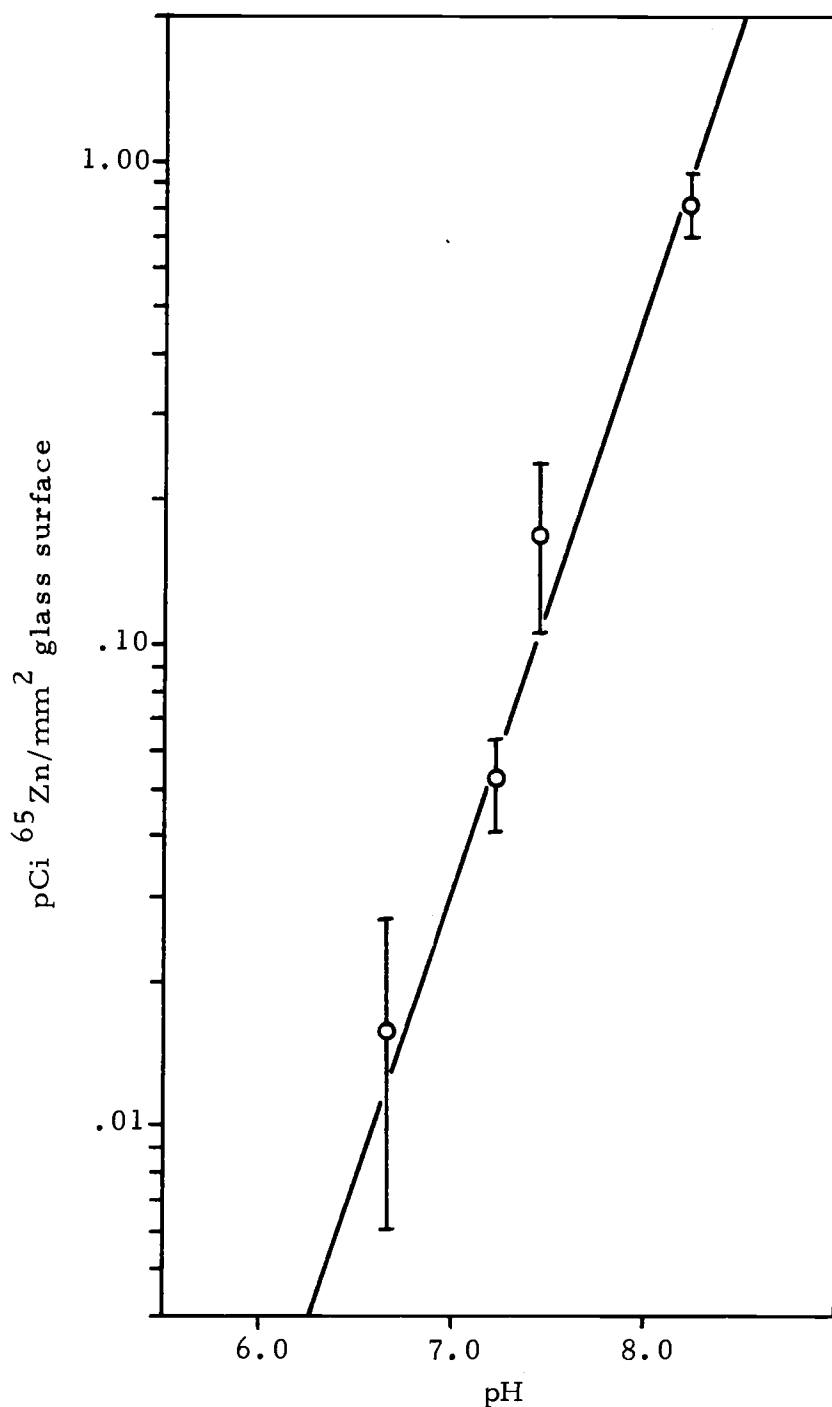
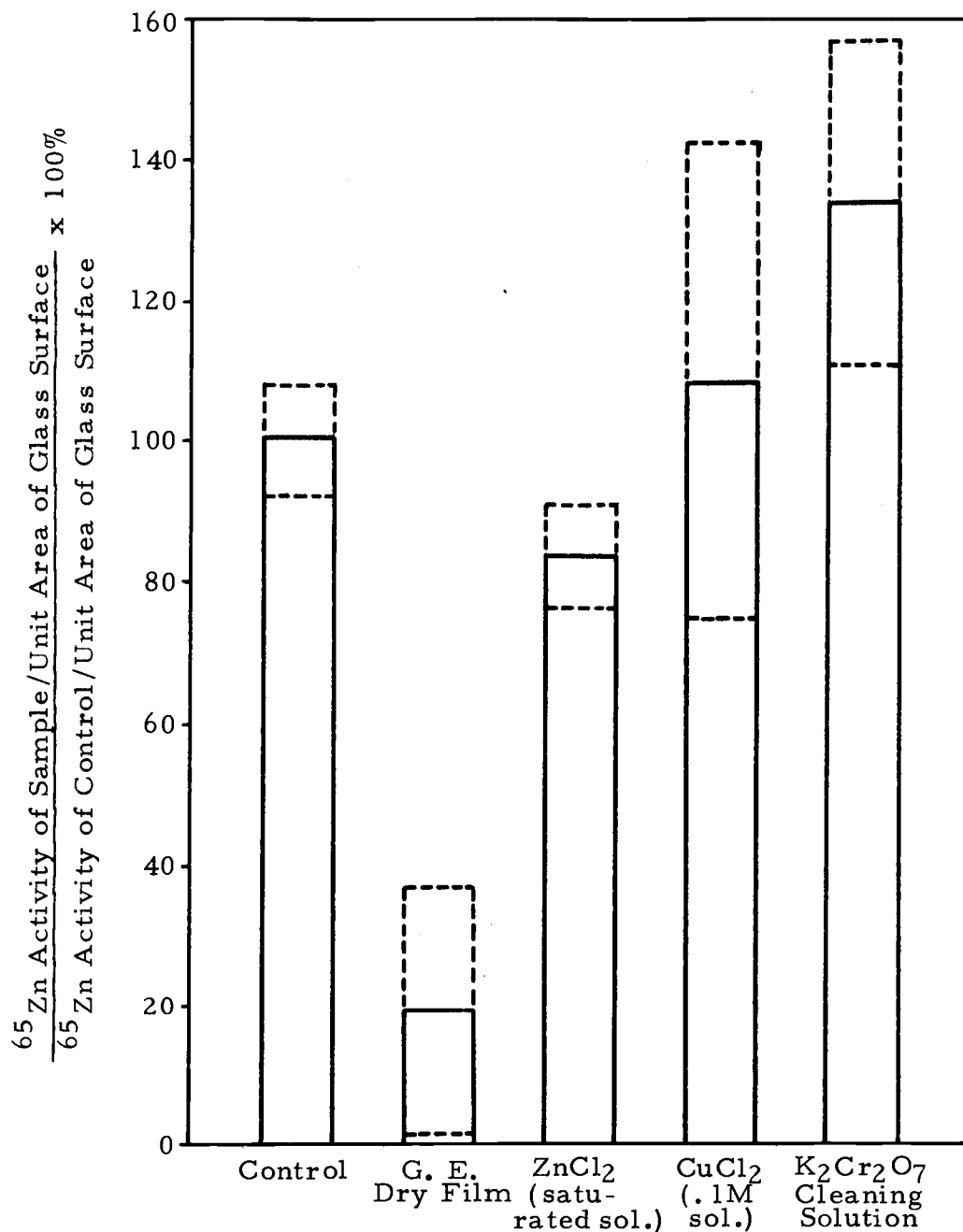


Figure 6. Zinc-65 uptake by Pyrex glass as a function of pH. Sample exposure time was one hour in all cases. Zinc-65 activities measured at pH values of 5.0 and 6.0 were below detectable limits for the analyzer. Error limits are plotted as \pm one standard deviation about the mean.

The questions to be answered were: what are the effects of various glass surface pretreatments on ^{65}Zn adsorption in a seawater medium, and, would one or more of these pretreatments be significantly effective in reducing such adsorption? Figure 7 gives the results of an experiment conducted as an attempt to provide answers to these queries.

Both the ZnCl_2 and the G. E. "Dry Film" were found to significantly (at the 95 percent level) reduce ^{65}Zn adsorption by the glass surfaces. In this sense, the G. E. "Dry Film" was the most effective pretreatment tested, lowering the level of adsorbed activity to 19.2 percent of that measured for the control samples. The CuCl_2 solution did not significantly affect the level of ^{65}Zn adsorption. Pretreatment with the glassware cleaning solution ($\text{K}_2\text{Cr}_2\text{O}_7 + \text{H}_2\text{SO}_4$) resulted in a 34 percent increase in adsorption, found to be significant at the 95 percent confidence level.

Two sets of Pyrex volumetric pipettes were examined with regard to adsorption of ^{65}Zn during pipetting procedures using radioactive seawater. One group was pretreated with 6N HCl, deionized distilled water, and acetone. Figure 8 summarizes the results of this study. Two relationships were examined--adsorbed ^{65}Zn activity vs. geometric glass surface area exposed to the ^{65}Zn solution, and percent sample adsorption vs. exposed glass surface area/pipette volume. In each case the linear relationship between the two



Chemical Treatment of Glass Surface Prior to ^{65}Zn Uptake

Figure 7. The effect of chemical pretreatment of a Pyrex glass surface on ^{65}Zn uptake from solution. The ^{65}Zn activity per unit glass surface area of the various samples is expressed as a relative percent, with the activity of the control sample set at 100%. The dashed lines represent a range of \pm two standard deviations around the sample means.

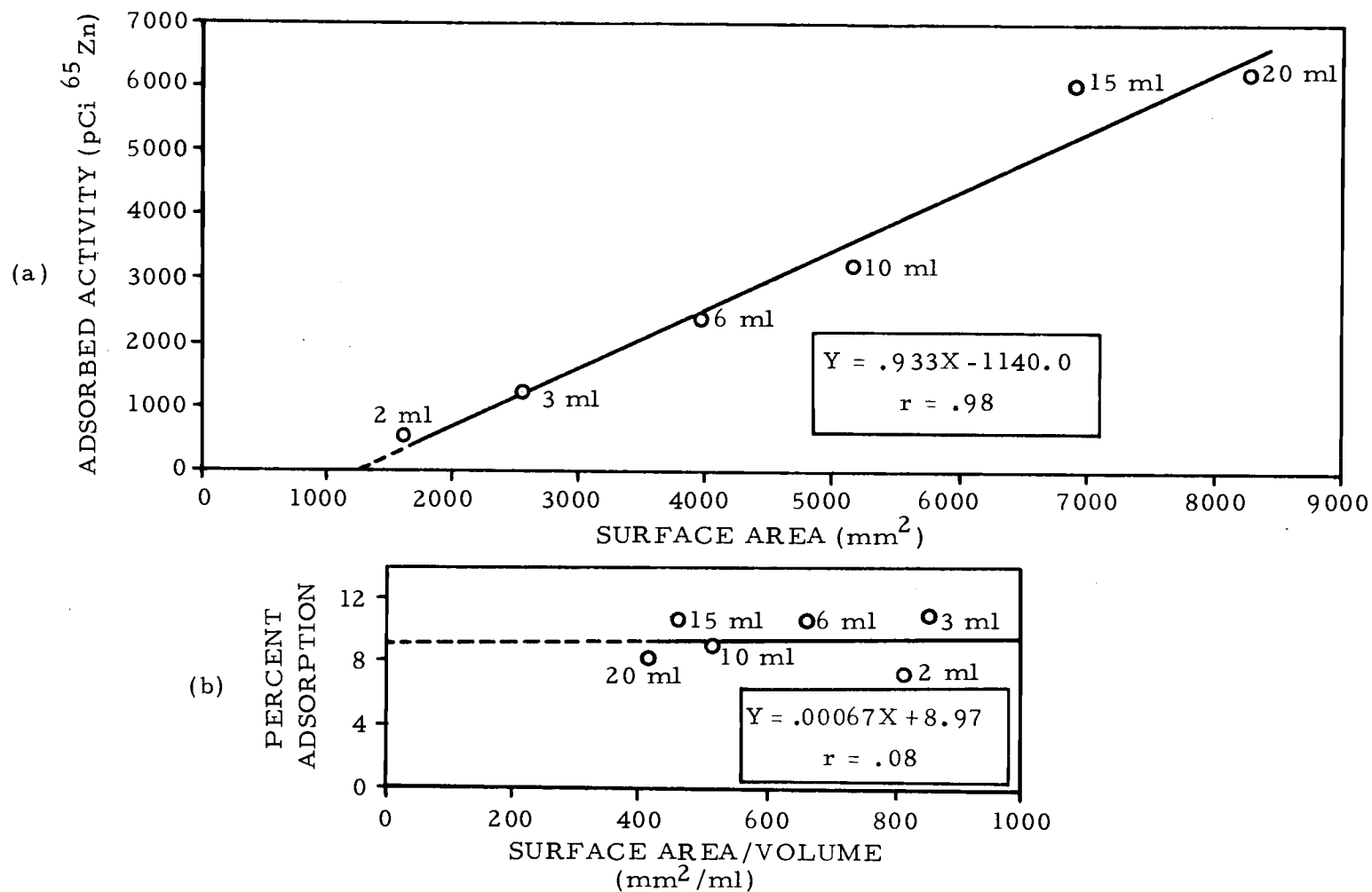


Figure 8. Zinc-65 uptake by acetone-pretreated Pyrex volumetric pipettes.
 (a) Zinc-65 uptake as a function of exposed glass surface area.
 (b) Percent adsorption of ^{65}Zn in the contained sample as a function of pipette surface area/sample volume.

parameters was obtained by the method of least squares, with no attempt to force the line through zero. The plot of adsorbed ^{65}Zn activity vs. geometric glass surface area showed a high level of correlation, with $r = .98$. The Y-intercept determined for this relationship was $-1140 \text{ pCi } ^{65}\text{Zn}$. Percent sample adsorption vs. glass surface area/pipette volume had a low correlation, with $r = .08$, indicating that $1 - r^2 = .99$ was the fraction of uncorrelated values (Snedecor, 1956). In this instance, the Y-intercept was 9.0 percent sample adsorption.

The effect of pretreating the second set of pipettes with dilute hydrofluoric acid becomes immediately apparent in a comparison of the results just described with those given in Figure 9 for the HF-pretreated samples. The Y-intercept for the adsorbed ^{65}Zn activity-glass surface area relationship of the HF-pretreated pipettes was nearer to the expected value of zero, with $y = 160 \text{ pCi } ^{65}\text{Zn}$. The correlation coefficient of the X and Y values was .99. Similarly, the Y-intercept in the plot of percent sample adsorption vs. glass surface area/pipette volume was nearer to the theoretical value of zero than for the HCl-pretreated samples, with $y = .31$ percent sample adsorption. The X-Y correlation for this relationship was also comparatively improved--the calculated value of the correlation coefficient was $r = .93$.

The efficiency of reducing ^{65}Zn adsorption by prerinsing pipettes with the sample solution was measured in an experiment involving a

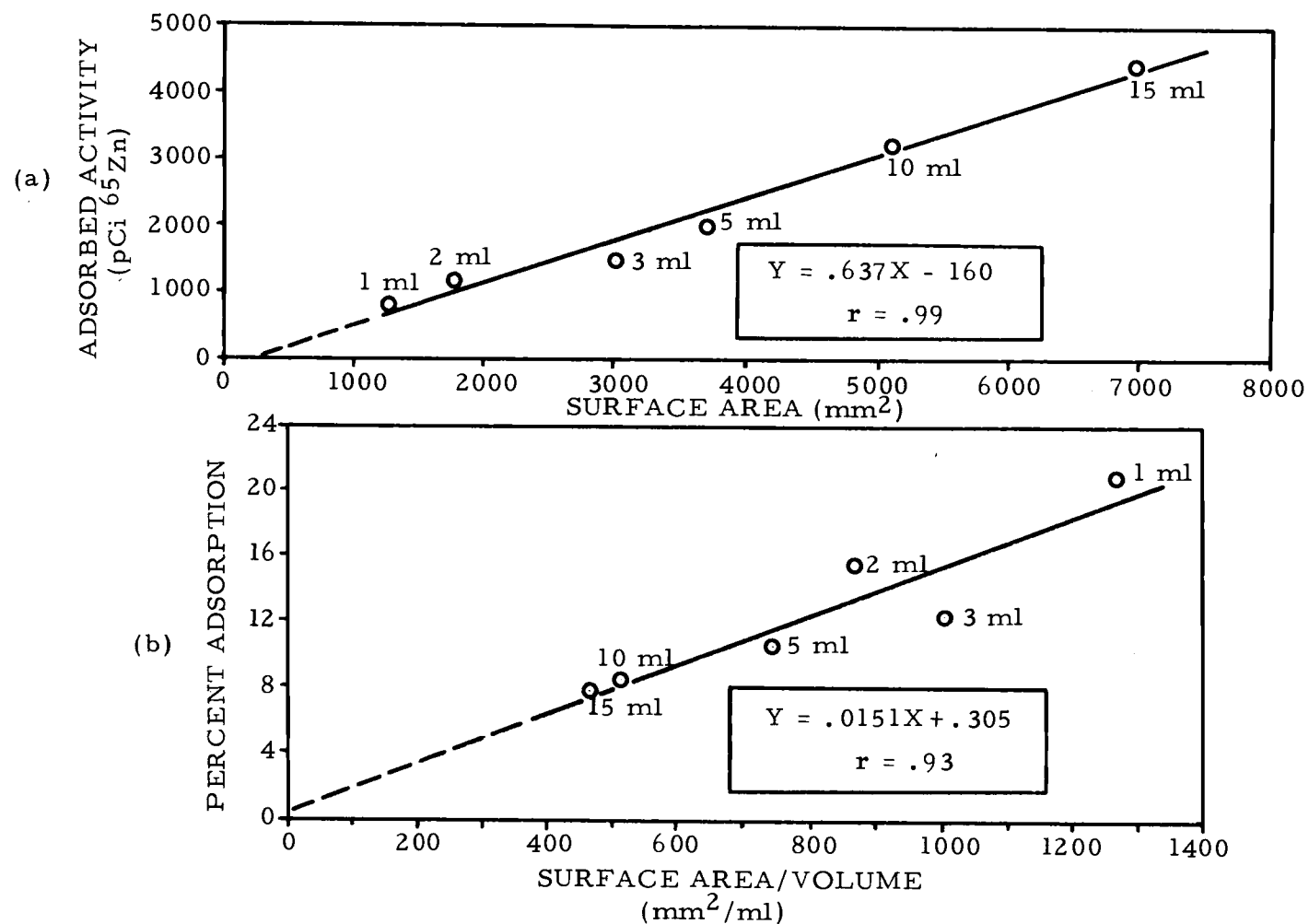


Figure 9. Zinc-65 uptake by HF-pretreated Pyrex volumetric pipettes.
 (a) Zinc-65 uptake as a function of exposed glass surface area.
 (b) Percent adsorption of ^{65}Zn in the contained sample as a function of pipette surface area/sample volume.

3 ml and a 6 ml pipette. Table 3 contains a summary of the experimental results. The pattern of reduction of sample ^{65}Zn adsorption was similar for the two pipettes tested. The initial adsorption amounted to greater than 10 percent of the sample in each case. A single prerinse with the sample liquid reduced adsorption by an average of 1.1 percent of the contained sample activity. Two such rinses cut adsorption by an average of 5.4 percent of the sample activity.

pH Study: pH Levels in Algal Culture Conditions

Consideration of previous experimental results indicated that pH was a major factor controlling ^{65}Zn adsorption levels. On this basis, a study of pH levels in algal culture conditions was deemed desirable as a means of increasing the applicability of this information to laboratory studies of ^{65}Zn uptake by marine algae. Figure 10 summarizes the resultant data. Immediately following its nutrient inoculation, the seawater-based culture medium had a pH of $6.3 \pm .1$. Bubbling with laboratory air over a period of one week resulted in an increase in pH to a value of $7.5 \pm .1$. These measurements were repeated numerous times in connection with the precipitate adsorption experiments, and were never found to vary significantly from these values. The alga was then added to the culture, and according to light absorption data presented in Figure 10 (b), entered an exponential growth phase sometime between three and six days later. Nine

Table 3. The effect of prerinsing with the sample solution on ^{65}Zn adsorption by Pyrex volumetric pipettes.

Pipette Volume	Number of Prerinses with Sample Liquid	Percent Sample Adsorption	Percent Adsorption Reduction
3 ml	0	13.1	
	1	12.1	.9
	2	7.0	6.1
6 ml	0	11.5	
	1	10.2	1.3
	2	6.8	4.7

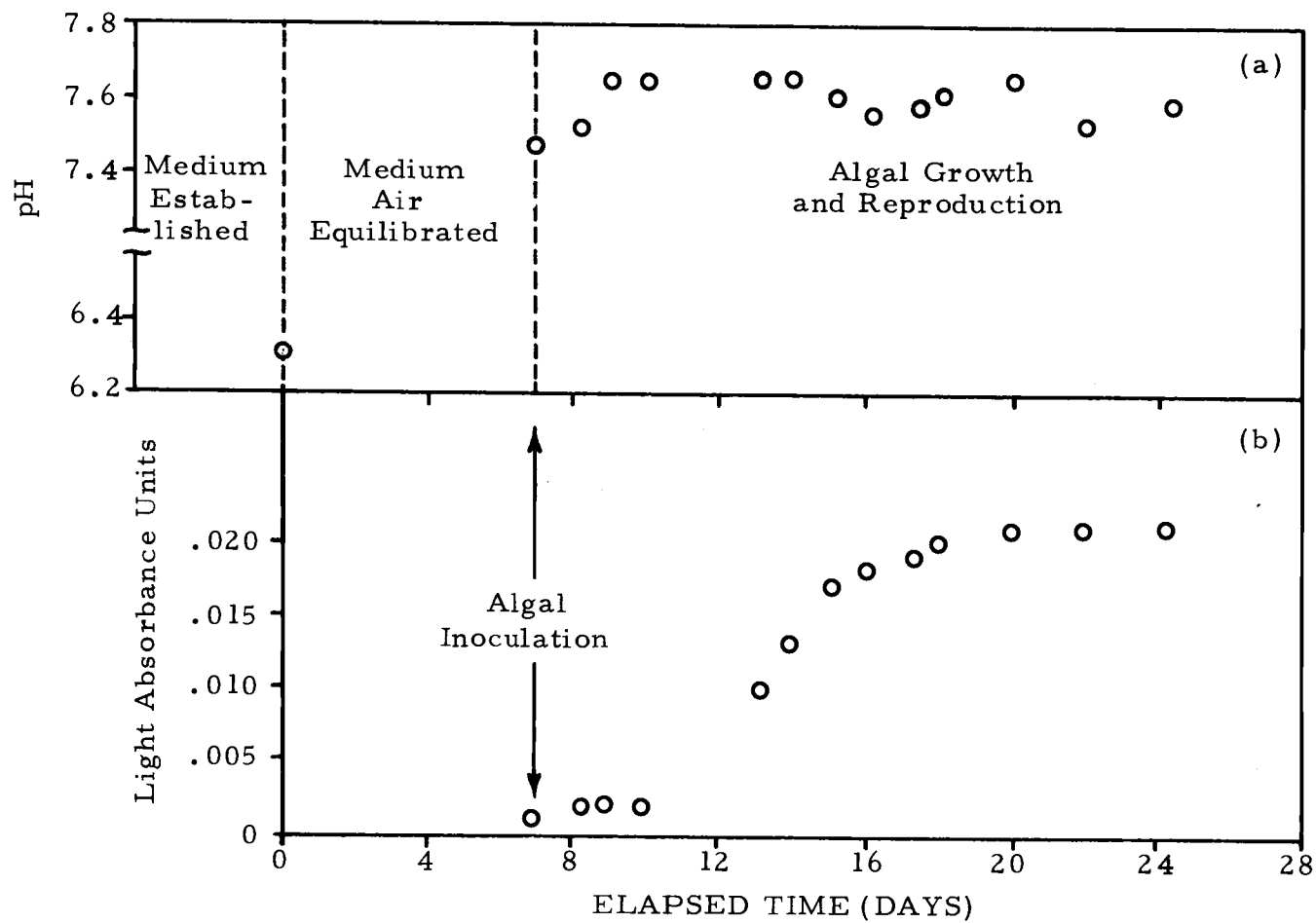


Figure 10. The effect of algal growth on culture pH levels.
 (a) Culture pH as a function of time.
 (b) Culture cell density, measured in light absorbance units, as a function of time.

days from the time of cell inoculation, the culture began a growth rate decline and entered a senescent phase. Corresponding to this growth pattern, the pH oscillated by less than .1 pH unit around an approximated mean value of 7.6. This represents a statistically insignificant change from the pH value of $7.5 \pm .1$ measured at one week elapsed time and prior to algal inoculation.

DISCUSSION

The Physiochemical Nature of the Nutrient Medium Precipitate,
and Its Role in Zinc Uptake

The omission of EDTA from the nutrient medium sufficiently lowered the solubility of certain ionic species to permit their precipitation. The ferric ion was apparently responsible for giving the precipitate its red-brown to yellow-brown color, since the particles were found to be white when FeCl_3 was not added. The precipitate consisted primarily of orthophosphates. On the basis of its composition, low solubility, amorphous form and white color, calcium orthophosphate ($\text{Ca}_3(\text{PO}_4)_2$) is one possible constituent. The consistency and color of the precipitate suggest that an iron compound, perhaps hydrated ferric oxide, predominates at pH values of 6.3 to 6.7. Further increases in pH resulted in a lighter, more particulate phosphate precipitate. It should be observed, according to the values of relative packed volume (Table 2, Section A), and the corresponding equilibrium levels of ⁶⁵Zn adsorption (Figure 3), that the precipitate formed at pH values greater than 7.1 had a greater tendency for zinc adsorption than a similar volume of the more gelatinous substance formed at pH's less than 7.1.

Neglecting the phosphate already present in the seawater base, the level of phosphate added as KH_2PO_4 to the nutrient medium was

approximately 1.47 mmoles/l. This is 10^3 times higher than natural phosphate concentrations in highly productive coastal waters off Oregon (Park, 1967). Therefore, it would seem reasonable to reduce the amount of added KH_2PO_4 as a first step toward eliminating precipitation in the medium. The concentration of added nutrients in algal nutrient media is generally planned according to desired culture densities (Fogg, 1966). For some purposes, then, it may be possible to avoid precipitation by using lower nutrient concentrations such as those specified for Chu medium no. 10 (Chu, 1942). High levels of phosphate are not unique to the modified Davis medium used in the present studies, but seem to be relatively common in many nutrient media used for algal culture. Accordingly, the utility of these observations is not strictly limited to the present case.

Figures 3 and 10 complete the picture of the role of the nutrient medium precipitate in ^{65}Zn adsorption. Figure 3 (a) indicates that precipitation in a newly established medium at $\text{pH} = 6.3 \pm .1$ results in very little depletion of soluble zinc levels. However, as the P_{CO_2} of the solution approaches that of the air, the pH rises to a value near 7.5 and the relative packed volume of the precipitate increases by a factor of 2.7 (Figure 10 and Table 2, Section A). Simultaneously, the physiochemical nature of the precipitate changes and its capacity to adsorb ^{65}Zn increases. Subsequent algal growth further increases

the pH value by only about .1 unit.

Figure 3 shows an increase in adsorption of ^{65}Zn by the precipitate, with increases in pH over the range, $6.3 \pm .1$ to $7.5 \pm .1$. These are initial pH values in each case. The plots of pH vs. time in each figure give pH changes in the closed containers due to addition of the ^{65}Zn in acidic solution. The decrease in pH seen in Figure 3 (c and d) gives evidence that the associated measurements of the particulate fraction ^{65}Zn activity are conservative in comparison to projected results for ^{65}Zn uptake at a constant pH equal to the initial value. Even so, the culture having an initial pH of $7.5 \pm .1$ shows an equilibrium adsorption level of about 70 percent of the added ^{65}Zn (Figure 3 (d)). The pH of this medium is near to that of the growing algal culture (Figure 10). The resultant conclusion is that such a precipitate can compete with a marine alga in the accumulation of zinc from an aqueous medium.

The presence of such precipitates may go undetected under some circumstances. They may cause significant error in measurements of zinc accumulation by unicellular algae, which are often assumed to comprise 100 percent of the suspended particulate fraction. In such cases, relative particulate surface area and surface charge are two of the most significant factors affecting the level of error (Weiser, 1950; Blaedel and Meloche, 1957).

Using similar levels of zinc, i. e., 10 $\mu\text{g}/\text{l}$, Boroughs, Chipman and Rice (1957) found that the marine diatom, Nitzschia closterium, removed nearly 90 percent of the zinc from a culture in four hours. At a pH of $7.5 \pm .1$, the present studies show that a nutrient medium precipitate is capable of taking up over 50 percent of the zinc in the same length of time. This comparison is somewhat limited, due to lack of pH and particle density data for the alga, but it is not unreasonable to suggest that the precipitate and the alga could be present in a single culture, each accumulating zinc. Under such conditions considerable error could result from the assumption that loss of zinc from the solution was due to uptake by the alga alone.

As a corollary to the previous discussion it is worthwhile to note the values of ^{65}Zn associated with the total suspension in Figure 3. For the most part, they remain relatively constant with time. This is to be expected, for these suspensions were essentially homogeneous, and as will be discussed later, glassware adsorption of zinc by vessels of this configuration is negligible under these conditions of pH and zinc concentration. However, the level of ^{65}Zn in the total suspension at $\text{pH} = 7.5 \pm .1$ shows a decrease of about five percent in the first 10 hours. This decrease is statistically significant, since the highest value of three standard deviations calculated for these measurements was 4.4 percent. This contention is further supported by the application of the Student's t test for the difference between the

means of the pertinent samples at one hour and 64 hours elapsed time. The means were found to be significantly different at the 99 percent confidence level.

The values of ^{65}Zn activity of the particulate fraction were calculated for each sampling period as the difference between the measured ^{65}Zn activity of the total suspension and that of the centrifugate. Clearly, this is a more direct and accurate method of calculation than that used by many experimenters, who assume a constant figure for the ^{65}Zn activity of the total suspension. Regardless of the cause of the total suspension activity decrease seen in Figure 3 (d), assumption of a constant value in this case would result in an additional five percent error for the calculated ^{65}Zn equilibrium level of the particulate fraction.

Zinc-65 Adsorption on Pyrex:
The Effect of Glass Surface Pretreatment with HF

The base results of the time-related study of ^{65}Zn adsorption by borosilicate glass rods are straightforward. In general, adsorption equilibrium was attained fairly rapidly--sometime between .50 and 2.0 hours elapsed time (Figure 4). This rate may be considered representative of zinc adsorption under these conditions (12 μg zinc/l, pH = 8.0) by borosilicate glass surfaces in general. However, the rationale and results associated with the use of the dry-and-rinse procedure and the HF pretreatments require clarification. The outcome of each of these manipulations was surprising, and makes unexpected contributions to a practical understanding of the physiochemical nature of zinc adsorption.

The purpose of the pretreatment with dilute HF was to thoroughly clean and standardize the glass surfaces--to decrease the percent sample standard deviation and to make experimental trends more evident. The success of this method is apparent in the results of the pipette adsorption study to be discussed later. HF pretreatment of these surfaces resulted not only in increased adsorption, but also in changes in the general shape of the ^{65}Zn uptake curve. A comparison of the relationships represented by filled circles in Figures 4 and 5 indicates the nature of these changes.

As a prelude to discussion of this phenomenon and its implications, a brief review of the theory of the electrical double layer would be helpful. This subject has been summarized by Laitinen (1960), Wiklander (1964) and Adamson (1967). According to the initial version of this theory of ion adsorption at a solid-liquid interface, Perrin postulated in 1904 that a solid surface may have a net electrical charge, due to the adsorption of lattice ions or other potential-determining ions. The charge balance for the system is maintained by an oppositely-charged counter-ion layer, which is a diffuse region of ions in solution, hovering near the charged surface of the solid. This concept is often referred to as the Helmholtz double layer, on the basis of the erroneous (Laitinen, 1960) assumption that Helmholtz (1879) was its originator. A subsequent modification of the theory made by Gouy in 1910, and Chapman, later (1913) but independently, supplanted the concept of rigid layers with a theory of diffuse double layers. In this conception, the concentration of the counter ions is greatest in the immediate vicinity of the surface and decreases at first rapidly and then asymptotically to a point beyond which the solution has a uniform composition. The most widely accepted version of the double layer theory today represents a combination of the above two concepts, and was advanced by Stern in 1924. According to Stern, the inner part of the counter-ion layer is similar to Helmholtz's layer, while the outer part of the ion swarm has a structure like that

described by Gouy and Chapman.

If one accepts that the cations associated with the glass surfaces in the present experiments reside predominantly in a counter-ion layer, then one must concurrently assume a net negative charge for the adsorbed ion layer. This negatively charged layer is composed of anions accumulated from the solution. Starik and Rozovskaya (1956) have discussed the charge distribution on Pyrex glass surfaces. They describe such surfaces as having a negatively charged adsorbed-ion layer of silicate ions surrounded by a counter-ion layer of cations.

In a subsequent paper, Starik and Rozovskaya (1961) studied the sorption of ^{110}Ag , ^{32}P and ^{95}Zr on a fluorinated glass surface. They found that treatment of Pyrex glass with a 10 percent solution of HF resulted in decreased adsorption of both Ag^+ and HPO_4^- ions. Fluorination of the glass surface is thought to lead to the replacement of OH^- radicals (from Si-OH groups) with covalent, non-polar Si-F bonds (Weyl, 1947). This would make the surface more hydrophobic and decrease the availability of negatively charged Si-O $^-$ groups. Starik and Rozovskaya have interpreted the results for Ag^+ and HPO_4^- as indicating that the adsorption of both cations and anions is lower on fluorinated than on non-etched surfaces.

Similar treatment, however, increased the adsorption of zirconium. On this basis, they assumed that zirconium was adsorbed in a molecular form. They do not offer a mechanism for this phenomenon.

One important aspect of the experimental procedure which generated these results must be noted. The duration of exposure of the glass samples to the radioactive solution was 24 hours. Figures 4 and 5 for the present study show that such uptake for zinc quickly reaches a maximum which is higher for fluorinated than for non-fluorinated surfaces. The values for the fluorinated glass then begin to decline. Extrapolation of the curves in Figure 5 to 24 hours exposure time might show results similar to those obtained for Ag^+ and HPO_4^- by Starik and Rozovskaya. The question is: does increased adsorption of zinc by fluorinated glass surfaces indicate uptake of a molecular form of the element? If so, then one might assume, in view of the interpretation of surface fluorination effects offered by Weyl, that zinc is also adsorbed in a molecular form on non-fluorinated glass surfaces. The only apparent effect of fluorination according to this view is a decrease in the net negative charge in the adsorbed-ion layer. It would seem that the length of exposure time is critical for full interpretation of the results of the zinc uptake study. Unfortunately, the experiment was terminated prior to 24 hours. Indeed, the interpretation of molecular uptake by Starik and Rozovskaya on the basis of single samples may require further investigation using periodic sampling techniques.

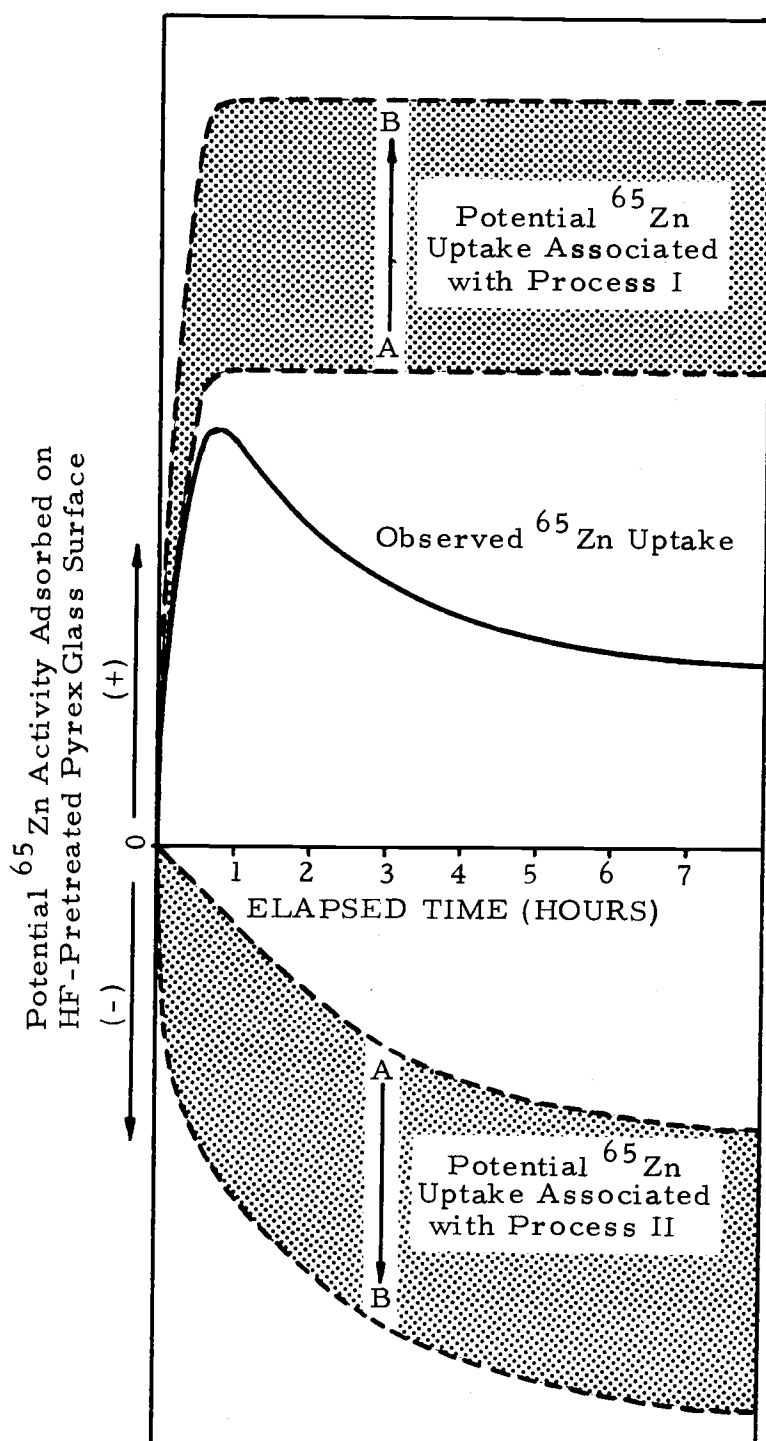
It should also be realized that whereas the present Pyrex adsorption experiments were carried out in a seawater medium, those of

Starik and Rozovskaya were done in a medium having a distilled water base. Due to the presence of many unidentified ion species in the seawater, this difference may be of considerable importance in interpretation of the results of one study in light of the other. Without further data, the nature and magnitude of adsorption effects due solely to the presence of additional ion species cannot be fully evaluated.

The results given in Figure 5 may also be represented as a combination of two processes. Figure 11 is a schematic interpretation of a theoretical synthesis of processes that could produce such an effect. Process I is seen to be similar in form to the uptake of zinc by non-fluorinated surfaces (Figure 4), although of a greater magnitude. Process II represents a tendency for loss of adsorbed zinc. This may denote a decrease of surface adsorption sites, an adsorption exchange involving some cation in solution, a decrease in the negative charge density of the adsorbed-ion layer, or some other systemic alteration affecting the surface attraction for zinc.

According to the effects of fluorination on glass mentioned above (Weyl, 1947; Starik and Rozovskaya, 1961), an etching effect with subsequent surface aging in the uptake solution is another possible explanation for the shape of the initial ^{65}Zn uptake curve in Figure 5. This hypothetical multi-process system is outlined in Figure 11. Alteration of the surface charge distribution would have no measurable influence over the adsorptive uptake of a non-polar, molecular form

Figure 11. Theoretical interpretation of two algebraically additive processes that combine to give an observed relationship of ^{65}Zn uptake by HF-pretreated Pyrex glass as a function of time. The shaded areas denote a family of curves, corresponding for the two processes in the range, A to B, that combine to give the observed uptake relationship. Process I represents a systemic potential for positive ^{65}Zn uptake. Process II represents a potential for negative uptake, implying loss to the solution.



of an element. At the same time, the etching effect of HF would increase the surface area available for adsorption. Process II would then represent the tendency for loss of the element to the solution due to aging of the glass surface, i.e., a decrease in total surface area concurrent with a recrystallization of the glass surface lattice. In sum, examination of ^{65}Zn uptake by fluorinated glass surfaces is a potentially useful method for future studies of the nature of zinc adsorption by glass.

Zinc-65 Adsorption on Pyrex:
The Effects of Glass Surface Drying and Rehydration

The dry-and-rinse procedure was performed to test the effectiveness of the post-uptake seawater rinse of the glass rods prior to radioanalysis. The post-uptake rinse was meant to remove excess radioactive solution adhering to the glass surface, so that only adsorbed radiozinc would remain. The underlying assumption was that zinc would be adsorbed in a discrete quantity not subject to removal by the rinse procedures. If this were true, the length of the rinse time would be inconsequential, and an additional rinse following radioanalysis would not alter the measured level of ^{65}Zn activity. Accordingly, it was found that the duration of the initial rinse was not critical for short time periods. However, a brief additional rinse following an interval of drying removed more than 70 percent of the remaining activity from

the acetone pretreated rods (Figure 4) and at least 60 percent from the HF pretreated rods (Figure 5). For each sample, the amount of ^{65}Zn removed by the dry-and-rinse method was found to bear a distinct time-dependent relationship to the initial level of adsorbed ^{65}Zn . Figure 12 summarizes this effect with an expression of the post-rinse ^{65}Zn sample activity as a percentage of the original activity, vs. elapsed time in the ^{65}Zn uptake solution. The slope of the linear portion of this plot is negative and approximately equal for the acetone pretreated rods and the HF pretreated rods.

The critical part of the additional rinse procedure seems to be the preliminary drying of the glass surfaces. The drying process and the subsequent surface rehydration effectively separate two distinct pools of zinc ions previously held by the glass. According to the adsorption theory of the electrical double layer, ions in the counter-ion layer are held relatively loosely, by electrostatic attraction (Blaedel and Meloche, 1957). The ions of the adsorbed-ion layer are held strongly by sharing of residual valences of ions integral to the surface lattice (Fajans, 1931). Contrary to the theory of molecular adsorption, the fact that a large portion of the zinc can be removed from the glass surface by the dry-and-rinse procedure gives some support to the assertion that the majority of the adsorbed zinc resides as cations in the relatively diffuse counter-ion layer. This configuration matches the general description of Pyrex adsorption outlined by Starik and Rozovskaya (1956).

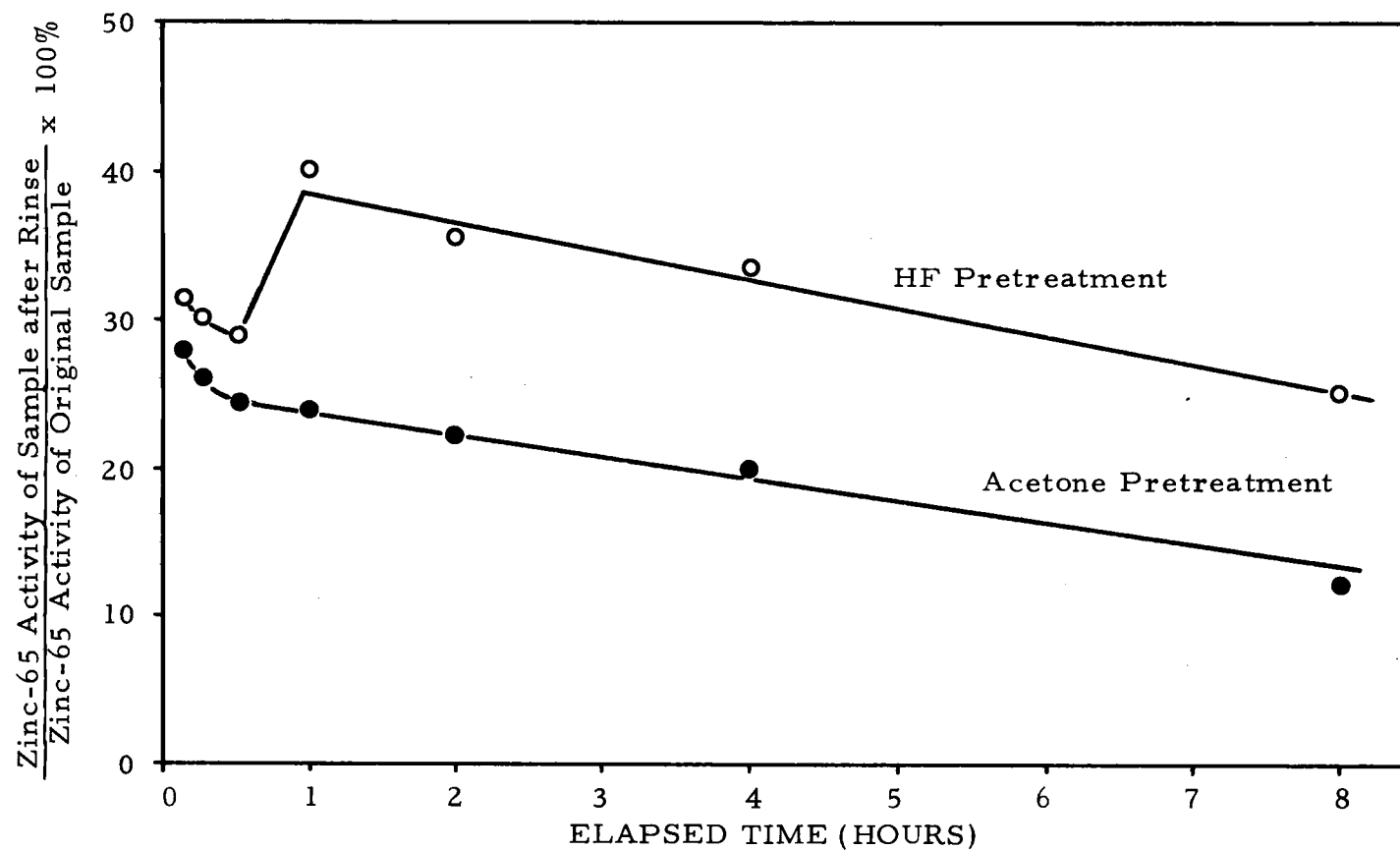


Figure 12. Post-rinse ^{65}Zn sample activity of Pyrex glass rods, expressed as percentage of the original adsorbed ^{65}Zn activity, as a function of exposure time in the uptake solution.

On the basis of the present data, no justifiable explanation can be made for the more tightly bound zinc fraction. It may represent the inner part of the counter-ion layer as theorized by Stern, a region which consists of a condensed layer of counter ions similar to the Helmholtz layer.

Regardless of what the correct explanation may be for the tightly bound zinc fraction, the negative slope of the two curves in Figure 12 gives evidence that there exists a time-related net transfer of zinc from this pool into the more loosely bound one. The rate of this transfer is apparently unaffected by the HF pretreatment, after one hour of exposure to the ^{65}Zn uptake solution. No explanation is offered here for the behavior exhibited by the HF pretreated rods which had less than one hour of exposure to the ^{65}Zn medium.

On the basis of the present discussion it should be clear that the exact nature of zinc adsorption on Pyrex glass is yet uncertain. It is hoped that the observations made here will stimulate further studies on this subject.

Zinc-65 Adsorption on Pyrex:
The Effects of pH and Chemical Pretreatment

The study of the effects of pH on glass adsorption of ^{65}Zn were limited to the range of pH spanning that encountered in the algal nutrient medium. For the range, $\text{pH} = 6.7 \pm .1$ to $\text{pH} = 8.2 \pm .1$, the

linear relationship (Figure 6) between pH and $\log (\text{pCi } ^{65}\text{Zn}/\text{mm}^2 \text{ glass surface})$ shows that zinc adsorption is affected directly or indirectly by the hydrogen ion concentration. This may be due to an increase with increased pH of the number of hydroxyl groups available for the adsorbed-ion layer. An alternative or supplementary explanation might relate decreased zinc adsorption at low pH values to increased displacement of adsorbed zinc ions by hydrogen ions. The effect of pH on glass adsorption of polyvalent metal ions has been discussed briefly by Starik and Rozovskaya (1956), and Stryker and Matijević (1958). Figure 6 indicates that the level of zinc adsorption per unit of glass surface area at the average pH measured for the growing algal culture (pH = 7.6) is about one fourth as great as that determined for the pH of the glassware adsorption experiments (pH = 8.0).

The outcome of the glass pretreatment experiment (Figure 7) demonstrates that it is possible, using G. E. "Dry Film", to reduce the adsorption of zinc by 80 percent. Chromic acid cleaning solution was found to increase adsorption by over 30 percent.

Zinc-65 Adsorption on Pyrex Laboratory Glassware

With respect to adsorptive uptake of zinc by Pyrex volumetric pipettes, the following observations can be made on the basis of data presented in Figures 8 and 9:

- 1) HF pretreatment of previously used pipettes tends to clean and

standardize the glass surfaces according to the potential level of zinc adsorbed per unit area per unit time, all conditions being equal. For each of the two linear relationships examined, adsorbed ^{65}Zn activity vs. glass surface area and percent sample adsorption vs. glass surface area/sample volume, HF pretreatment increased the correlation of X-Y values (in comparison to acetone pretreatment) and placed the Y-intercept nearer to the theoretical zero value. This helped to eliminate individual pipette surface variations and permitted collection of adsorption data on a standard basis (Figure 9).

- 2) For the conditions used ($12\text{ }\mu\text{g}$ total zinc/l, $\text{pH} = 8.0 \pm .1$), zinc adsorption by used pipettes of the size range, 2 ml to 20 ml, amounts to 7-11 percent of the zinc in the contained sample (Figure 8 (b)).
- 3) The relationship, percent sample adsorption vs. glass surface area/sample volume, is confirmed as linear for the range of values tested, with a Y-intercept at or near zero (Figure 9 (b)).

In this light, sample volume is directly proportional to total available zinc. Zinc adsorption by borosilicate glass is therefore shown to be a function of the available zinc and the glass surface area contacted by the liquid.

From a practical viewpoint, these results show that zinc adsorption from a seawater medium is negligible for glass laboratory

apparatus having a low surface area to volume ratio. This includes beakers, flasks, graduated cylinders, etc. This observation agrees with the results obtained by Robertson (1968) for 200 ml Pyrex glass bottles. However, adsorption of zinc by pipettes may result in serious error. At a pH of 7.6, which was the average value measured for the growing algal culture in Figure 10, zinc adsorption by the 10 ml pipette in Figure 8 (b), for which the pH was 8.0, would amount to only about two percent. This can be determined by examination of the pH-adsorption relationship outlined in Figure 6. For smaller pipettes this error would potentially be larger, and would increase for all Pyrex glassware with an increase in pH. At natural oceanic pH values of about 8.0, zinc adsorption errors may be of the order of 7 to 11 percent for even relatively large pipettes. Such errors can be reduced considerably by a minimum of two pre-rinses with the sample liquid and/or treatment of the glass surfaces with various commercial chemicals which produce hydrophobic effects. It should be observed that the latter method may necessitate recalibration of the glassware due to alteration of the shape of the liquid meniscus and the reduction of surface wetting.

SUMMARY

- 1) Omission of Na_2EDTA from the nutrient medium resulted in the formation of a precipitate. The relative centrifuged volume of the precipitate increased with increased pH over the range, $\text{pH} = 6.3 \pm .1$ to $\text{pH} = 7.5 \pm .1$. Simultaneously, the color changed from red-brown to yellow-brown and the consistency went from gelatinous to particulate. The color was found to be due to the addition of FeCl_3 to the medium. At a pH of $7.5 \pm .1$, the precipitate consisted primarily of orthophosphates. Calcium and magnesium were two of the most abundant elements.
- 2) Adsorption by the precipitate of ^{65}Zn from seawater media containing low levels of total zinc ($12 \mu\text{g Zn/l}$) increased with an increase in pH. Such uptake amounted to less than 10 percent of the added radiozinc at a pH of $6.3 \pm .1$. This figure increased to about 70 percent at a pH of $7.5 \pm .1$.
- 3) Adsorption by Pyrex glass of ^{65}Zn from seawater containing $12 \mu\text{g total zinc/l}$ reached equilibrium between .50 and 2.0 hours elapsed time. Pretreatment of the glass surfaces with dilute (.3N) HF resulted in increased uptake of ^{65}Zn , reaching a level of activity at one hour elapsed time that was about five times the maximum activity attained for untreated Pyrex glass having the same surface area. This level declined to twice the activity of

the untreated glass at eight hours, when the experiment was terminated.

- 4) Drying and rehydration of the Pyrex glass surfaces following uptake of ^{65}Zn removed more than 70 percent of the adsorbed activity from the untreated surfaces and at least 60 percent from the HF pretreated surfaces. The amount of ^{65}Zn removed bore a distinct time-dependent relationship to the initial level of adsorbed ^{65}Zn . This relationship indicated a net transfer of adsorbed ^{65}Zn , at a constant rate, from a tightly bound state to one more favorable to ^{65}Zn loss to the solution under mild conditions.
- 5) Adsorption of ^{65}Zn by Pyrex glass was negligible at pH values of 6.0 and less. An increase in pH above 6.0 resulted in greatly increased adsorption of ^{65}Zn . The relationship between pH and $\log (\text{pCi } ^{65}\text{Zn}/\text{mm}^2 \text{ glass surface})$ was linear for the pH range, $6.7 \pm .1$ to $8.2 \pm .1$.
- 6) Pretreatment of Pyrex glass surfaces with dimethyldichlorosilane (General Electric "Dry Film") reduced ^{65}Zn adsorption by 80 percent. Glass pretreatment with a saturated solution of ZnCl_2 resulted in a 16 percent adsorption reduction. CuCl_2 pretreatment was inconsequential in this sense. $\text{K}_2\text{Cr}_2\text{O}_7$ cleaning solution pretreatment resulted in a 34 percent increase in the level of adsorbed ^{65}Zn .

- 7) For the conditions used ($12\mu\text{g}$ total Zn/l, $\text{pH} = 8.0 \pm .1$), ^{65}Zn adsorption by previously-used Pyrex volumetric pipettes of the size range, 2 ml to 20 ml, amounted to 7-11 percent of the radio-zinc in the contained sample. Pretreatment of used pipettes with dilute HF standardized the glass surfaces according to the potential level of ^{65}Zn adsorbed per unit area per unit time. The two relationships, adsorbed ^{65}Zn activity vs. glass surface area, and percent sample adsorption vs. glass surface area/sample volume were shown to be linear, with Y-intercepts at or near zero.
- 8) A single prerinse with the sample liquid of a 3 ml and a 6 ml volumetric pipette reduced adsorption of ^{65}Zn by an average of 1.1 percent of the contained sample activity. Two such rinses cut adsorption by an average of 5.4 percent of the sample activity. The initial level of ^{65}Zn adsorption for each pipette was greater than 10 percent of the contained sample activity.
- 9) Newly made nutrient medium had a pH of $6.3 \pm .1$. Bubbling with laboratory air increased the pH to a value of $7.5 \pm .1$. Following inoculation of the medium with a green alga, Isochrysis galbana, the pH oscillated by less than .1 pH unit around an approximated mean value of 7.6.

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