RAPID AND PRECISE DETERMINATION OF ATP USING A MODIFIED PHOTOMETER

U.S. GEOLOGICAL SURVEY

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CONTENTS

ILLUSTRATIONS

TABLES

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ABSTRACT

An inexpensive delay timer was designed to modify a commercially available ATP photometer which allows a disposable tip pipette to be used for injecting either enzyme or sample into the reaction cuvette. The disposable tip pipette is as precise and accurate as a fixed-needle syringe but eliminates the problem of sample contamination and decreases analytical time.

INTRODUCTION

The light producing reaction of ATP with the luciferin-luciferase enzyme substrate system is the basis for the ATP assay of microbial biomass. The light emission is commonly measured using a sensitive photometer, of which there are several commercial models available.

The Aminco[1/] Chem-Glow Photometer, model J4-7441, and Integrator Timer, model J4-7462, is a commonly available instrument which has been used for several years in this laboratory. As supplied by the manufacturer, this instrument uses a fixed-needle micro-syringe to penetrate a light-tight septum before injecting the sample into a 6X50 mm cuvette which contains the enzyme-substrate system positioned before the photomultiplier

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[1/] The use of the brand names in this report is for identification purposes only and does not imply endorsement by the U.S. Geological Survey.

tube. Using a single syringe for multiple samples in an analysis as sensitive as the ATP assay creates the real possibility of sample carry-over unless a time-consuming, stringent rinsing procedure is followed. This report deals with the use of a disposable tip microliter pipette with the Chem-Glow Photometer which 1) eliminates the sample carry-over and bubble formation inherent with fixed-needle syringes, 2) markedly speeds the analysis by eliminating the rinsing step, and 3) is as precise and accurate as injections performed with a fixed-needle syringe.

INSTRUMENT MODIFICATION

Due to the design of the Chem-Glow Photometer, the use of the disposable tip pipette requires that the sample be injected into the enzyme-substrate outside of the photomultiplier tube chamber. This necessitates introducing a precision time delay between mixing the reactants and starting the measurement of the emitted light. This time delay is accomplished by an inexpensive manually triggered timer, the construction of which is also discussed. The timer could be used with any photometer where a precise time delay is desired prior to peak integration. Various pin selections on the 4020 counter chip would permit a wide range of delay times.

The modification to the Chem-Glow Photometer involves installation of a delay timer in-line between a push-button switch and the integrator (fig. 1). The delay timer (fig. 2) utilizes a color television oscillator crystal and additional integrated circuitry with a rectified power

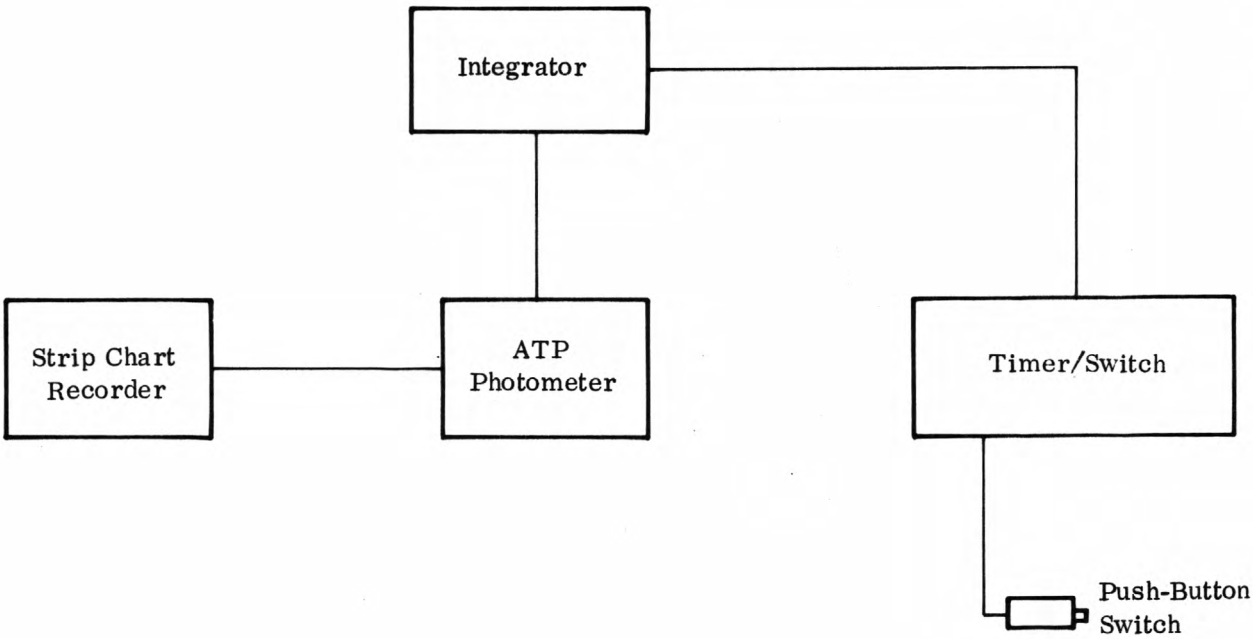

Figure 1.--Relation of the delay timer to other electronic components of the ATP analytical system.

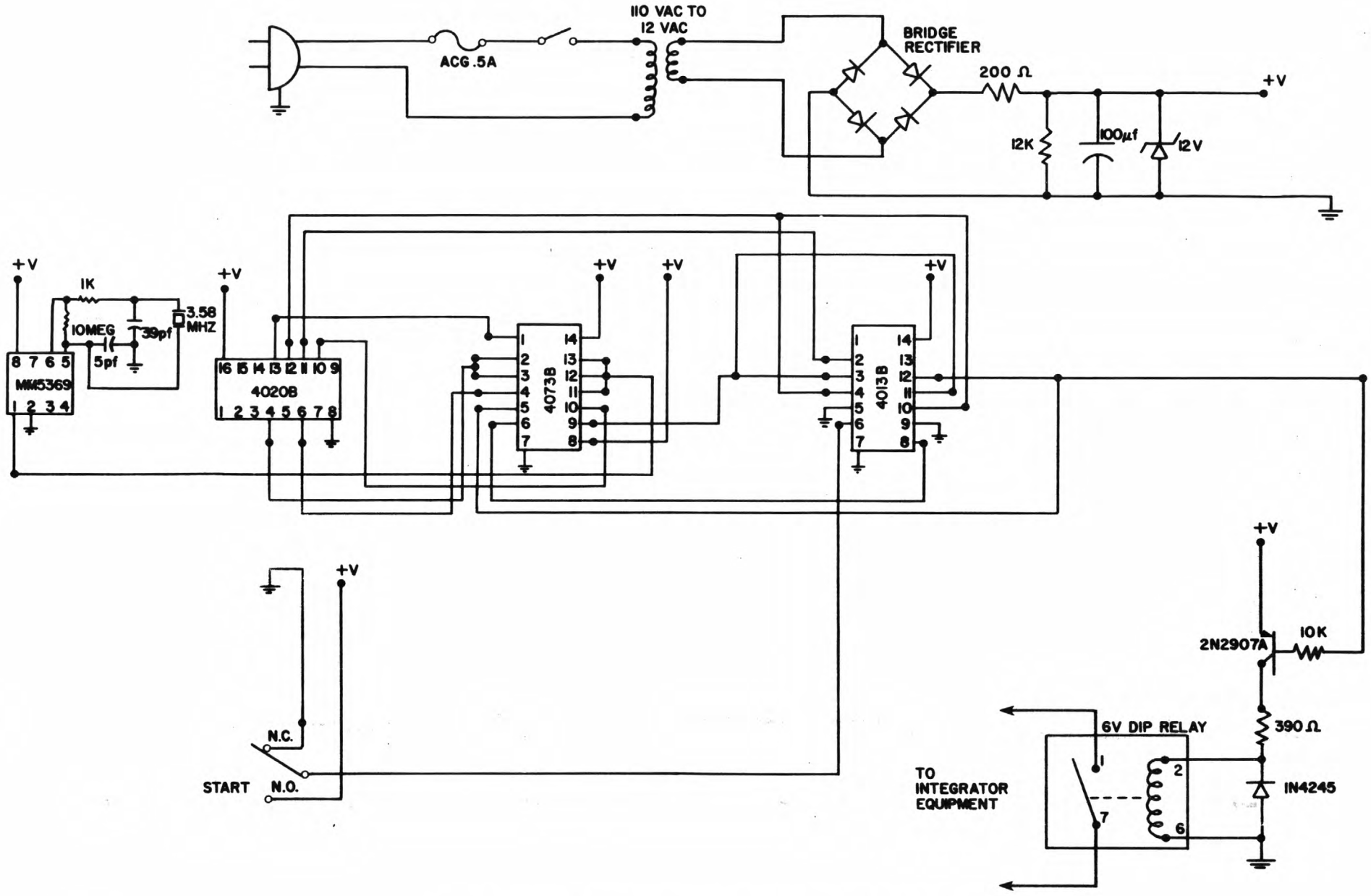

Figure 2.--Schematic of the delay timer.

supply which was designed for our particular application by Wayne Pourciau and Bill Rapp of the Geological Survey's Hydrologic Instrumentation Facility. It can be constructed with readily available components at an approximate cost of $25 (table 1).

At the start of a series of analyses, the delay timer is turned on and the circuitry is set for an analysis using a push-button switch. The sample is injected into the cuvette containing the luciferin-luciferase enzyme-substrate mixture and the push-button switch is again depressed. The timer pauses for 3.19 seconds then completes the circuit to the integrator which then initiates the integration. This interval is sufficient to withdraw the pipette and rotate the cuvette into the photometer chamber. The timer resets and re-opens the in-line internal switch. (This is necessitated by the design of the integrator. If the in-line switch is not re-opened, the integrator will not stop counting after the preselected counting interval.) When the integrator completes the preselected counting interval, which is variable depending on the instrument used, the cumulative number of counts of the light emission reaction is displayed.

SAMPLE ANALYSIS

A review of the literature shows that opinions differ concerning the method used to mix the sample with the enzyme preparation. Levin and others (1975) advocated injecting the enzyme into the sample because it resulted in better mixing of the reactants. Similarly, Eiland and Nielsen (1979) and Karl and LaRock (1975) have followed this procedure. On the other hand, there is some evidence that injection of the sample

Table 1. Parts list for on/off timer.

Item	Manufacturer or supplier	Part no.	Quantity	Approximate price, each
National oscillator 17 stage divider	Quest Electronics (Santa Clara, Ca.)	MM 5369	1	2.10
Crystal 3.58 MHz (color TV-oscillator	Quest Electronics	--	1	1.20
Binary counter, 14 bit, CMOS I.C., 16 pin	Motorola	14020BE	1	1.75
Dual D flip-flop	Motorola	14013BCP	1	.90
Triple input and gate	RCA	CD4073BE	1	.35
DIP 69 relay, SPST-NO	Magnecraft	W117DIP-69	1	3.60
Bridge rectifier, 1.0 AMP, 50 V.PIV.	Motorola	MDA100-A	1	2.00
Zener diode, 12 V, 1 watt	Motorola	1N4742	1	1.50
Transistor	Texas Instruments	2N2907	1	1.08
Diode	General Electric	1N4245	1	.38
Toggle switch, SPST	Alco	TT13A-6T	1	1.75
Momentary switch, push button, SPDT	Alco	MSP105F	1	3.25
Transformer, 12 V filament	Local supplier	--	1	2.00
Capacitor, 5 pfd	Local supplier	--	1	.18
Capacitor, 39 pfd	Local supplier	--	1	.32
Capacitor, 100 µfd, 25 WV	Local supplier	--	1	1.25
Resistor, 10 MEG Ω, carbon	Local supplier	--	1	.25
Resistor, 10K Ω, carbon	Local supplier	--	1	.25
Resistor, 1K Ω, carbon	Local supplier	--	1	.25
Resistor, 12K Ω, carbon	Local supplier	--	1	.25
Resistor, 200 Ω, carbon	Local supplier	--	1	.25
Resistor, 390 Ω, carbon	Local supplier	--	1	.25
			TOTAL PRICE	$25.11

into the enzyme is a better methodology for the following reason. In measurement of very low levels of ATP, it is important to subtract the endogenous light level of the enzyme from the light produced by the ATP luciferin-luciferase light emission reaction. With crude enzyme preparations, this endogenous light level decreases with time (Booth, 1975) and must be measured before each sample is assayed. By preloading the reaction cuvettes with the enzyme, it is very easy to measure this endogenous light level and then inject the sample into the enzyme and measure the light emission due to the ATP in the sample. Many workers thus choose to inject the sample into the enzyme (Cheer and others, 1974; Shoaf and Lium, 1976; Qureshi and Patel, 1976). Dupont Instruments, which markets a highly purified enzyme preparation, recommends injecting the sample into the reconstituted enzyme preparation.

A Centaur Sciences 10 µL pipette is used for the injection of the sample into the enzyme. This syringe was chosen because long, narrow tips are available which will extend to the bottom of the standard 6X50 mm cuvette used with the Chem-Glow Photometer ensuring rapid and thorough mixing of the sample and enzyme solution. The reproducibility of the pipette is given as 0.3 percent by the manufacturer with an accuracy of ±1 percent.

Before the above modifications were made, a Hamilton CR 700-20 push-button fixed-needle syringe was used for injecting the sample through the septum into the enzyme in the cuvette. A comparison of 10 replicate injections of a 1 ng/mL ATP standard between the Centaur pipette and the Hamilton push-button syringe gave coefficients of variation of 4.03 percent and 3.57 percent, respectively. A t-test on

the means of these two sets of replicate injections indicated there was no difference at the 99 percent confidence limit.

An experiment was designed to illustrate the problem of cross contamination between samples. Three replicates of 0.2, 1.0, 5.0, and 10 ng/mL ATP standards were analyzed with the Centaur pipette. The same series of replicates was repeated with the Hamilton syringe except that before each analysis an aliquot of a 100 ng/mL ATP standard was drawn into the Hamilton syringe and discharged. The syringe was then rinsed five times with distilled water and five times with a sample to be analyzed. The results are shown in table 2. Any cross contamination should result in a higher mean for the syringe-injected samples. Therefore, a one tailed t-test at the 95 percent level of significance was used. For all four samples the means of the Hamilton syringe-injected samples were significantly higher than the Centaur-injected samples. Also, the coefficients of variation were considerably smaller for the Centaur pipette for the 0.2 and 1.0 ng/mL concentrations. For the 5.0 and 10 ng/mL concentrations, the coefficients of variation were similar for the two syringes.

Using the disposable tip pipette eliminates bubble formation which is a common problem with a fixed-needle syringe. Elimination of rinsing markedly speeds assay time and tips can be autoclaved if the user desires.

Table 2. Effect of cross contamination by a 100 ng/mL ATP standard on lower concentration samples using a rinsed syringe. Coefficient of variation calculated as (standard deviation/mean) X 100.

Sample concentration (ng ATP/mL)	Mean (relative peak area units X 10^5)	Standard deviation	Coefficient of variation (percent)
	Disposable tip pipette		
0.2	.297	.00663	2
1.0	1.45	.120	8
5.0	6.61	.416	6
10	13.5	.427	3
	Cross contaminated and rinsed syringe		
0.2	1.86	.258	14
1.0	2.49	.419	17
5.0	8.16	.351	4
10	14.9	.448	3

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