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RESEARCH AND DEVELOPMENT OF A LUMINOL-CARBON MONOXIDE FLOW SYSTEM

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RESEARCH AND DEVELOPMENT OF A LUMINOL-CARBON MONOXIDE FLOW SYSTEM

ABSTRACT

This report describes attempts to adapt the luminol-carbon monoxide injection system to a flowing type system. Although the flow system did not always produce consistent results, some recommendations such as shortening flow lines and protecting the reaction from light are suggested.

Analysis of actual wastewater samples was carried out and revealed that bacteria can be associated with particles greater than 10 microns in size in samples such as mixed liquor. In such cases, prefiltering the sample with a 10 micron filter would produce erroneous results.

Research into the luminol reactive oxidation state was conducted which supported the previous theories concerning the luminol reaction. Laboratory evidence indicates that the oxidized iron porphyrins, cytochrome-c in particular, produces more luminol chemiluminescence than the reduced form. Correlation exists between the extent of porphyrin oxidation and relative chemiluminescence. In addition, the porphyrin nucleus is apparently destroyed under the current chemiluminescent reaction conditions.

A paper entitled, "Use of the Luminol Assay for the Determination of Bacterial Iron Porphyrins Flow Techniques for Wastewater Effluent" is enclosed. This paper was presented at the Second Bi-Annual SAI ATP Metholology Symposium in San Diego, California during March 1977.

A patent describing the use of the firefly luciferase-ATP and luminol-iron porphyrin reaction system for quantification of bacteria was prepared.

INTRODUCTION

A major advancement in the use of the luminol reaction system for quantitating bacteria was described in Quarterly Reports No. 5 and 6 (NAS 5-22545). This new technique allows the differentiation of live from dead bacteria in real time. The previous luminol methods quantitated total bacteria, live as well as dead; however, it has been discovered that a method employing pretreatment of a bacteria sample with carbon monoxide can be used to differentiate live from dead bacteria. The carbon monoxide pretreatment removes the signal from live bacteria in a sample and the difference between untreated and treated samples can be directly related to the number of viable cells present. The research and development of this technique was exclusively performed using a simple injection type system where a specific volume of treated sample was directly injected into a specific volume of luminol reagent. Since the purpose of this project is to develop an automated real time bacteria detection system, a flowing type system would apparently be much more compatible with this goal.

I. LUMINOL-CARBON MONOXIDE FLOW SYSTEM

Various flow system configurations were examined with varying degrees of success. Figure 1 is a schematic of the initial flow system configuration. The system was designed to selectively meter the sample with carbon monoxide or air to achieve the desired treatment. Some reduction of chemiluminescent signal occurred with a variation of approximately 33%. Although "exhaust" drains were provided to prevent bubbles from reaching the photometer, they were not completely effective and, as a result, the bubbles caused an unacceptably noisy signal. Had this configuration been pursued, some means of completely removing the bubbles would have been a necessity.

To eliminate the inherent problem of bubbles in the sample line in the example above, a second configuration illustrated in Figure 2 was examined. This new configuration allowed carbon monoxide to bubble through the sample without allowing the bubbles to actually enter the sample line. In this study carbon monoxide was bubbled through a third portion of sample for 15 minutes and then assayed. Valve 1 in Figure 2 controlled the amount of sample put into the "bubble chamber" and valve 2 selected the treated or untreated sample. Since the carbon monoxide-iron porphyrin complex disassociates in the presence of light,[1] the system was operated with and without a large black drop cloth covering the entire operation to eliminate light.

Large variations from sample to sample made the data statistically unreliable; however, the basic trends indicate that the flow system protected from light with carbon monoxide bubbling through the sample for 15 minutes or longer produced the best results.

The carbon monoxide-iron porphyrin complex not only disassociates in the presence of light but also in the presence of oxygen. A potential source of oxygen in the luminol flow system is the luminol reagent itself. To determine if elimination of all sources of oxygen would improve the results, the system illustrated in Figure 3 was devised. In this system both the sample and luminol reagent are purged with carbon monoxide for 15 minutes and a large sample volume, 10 ml was used. The data in Table 1 indicates that carbon monoxide purging of the luminol reagent did not increase signal reduction but actually lowered the reduction. This flow system with the large volume of sample in the bubble chamber did show good correlation with the injection system.

The major problem with the luminol flow system at this time concerns repeatability of results. Under identical experimental conditions it was very difficult to reproduce the results. It is difficult to determine if this problem is inherent to a flow system; however, the following recommendations can be made:

1. The reaction system should be completely protected from light to protect the light sensitive carbon monoxide-iron porphyrin complex.

(1) Parr, S. R.; Wilson, M. T.; and Greenwood, C.; The Reaction of Pseudomonas Aeruginosa Cytochrome C. Oxidase with Carbon Monoxide, Biochem. J. Vol. 151, 1975, p. 51

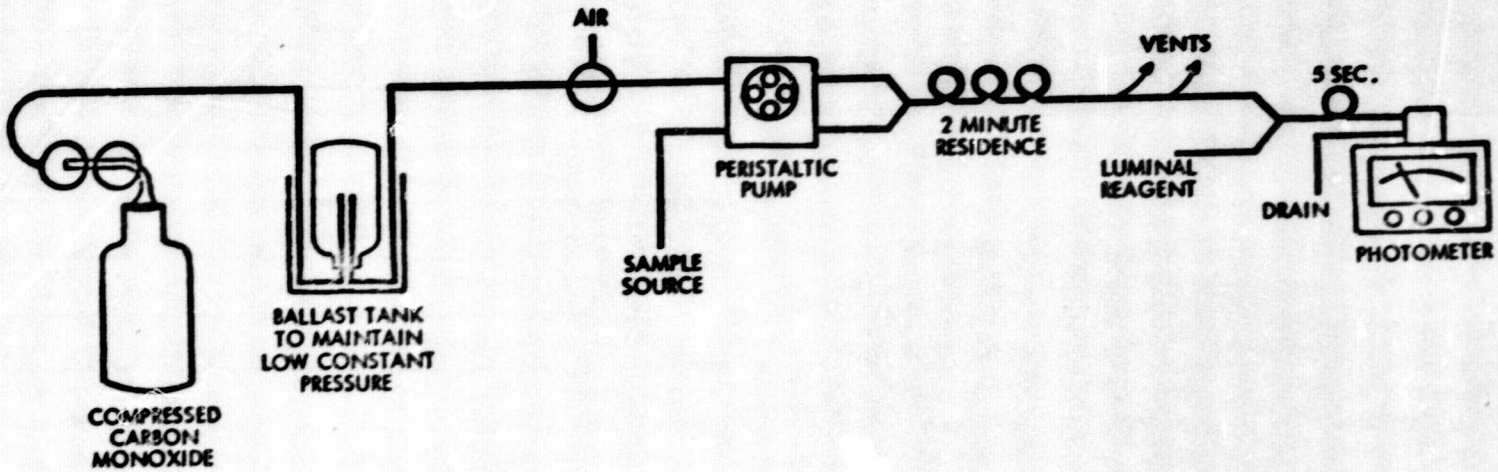

Figure 1 Initial experimental design of luminal - carbon monoxide flow system

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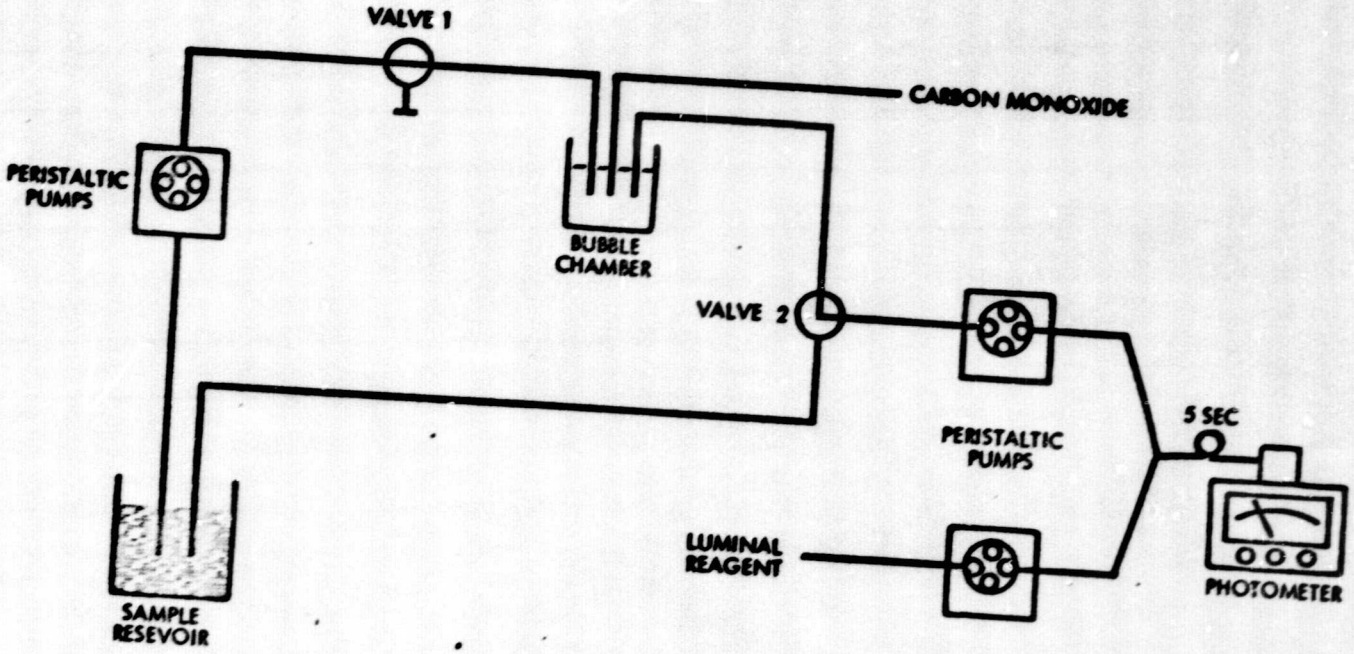

Figure 2 Second experimental design for a luminal - carbon monoxide flow system. The system was operated in the presence and absence of light.

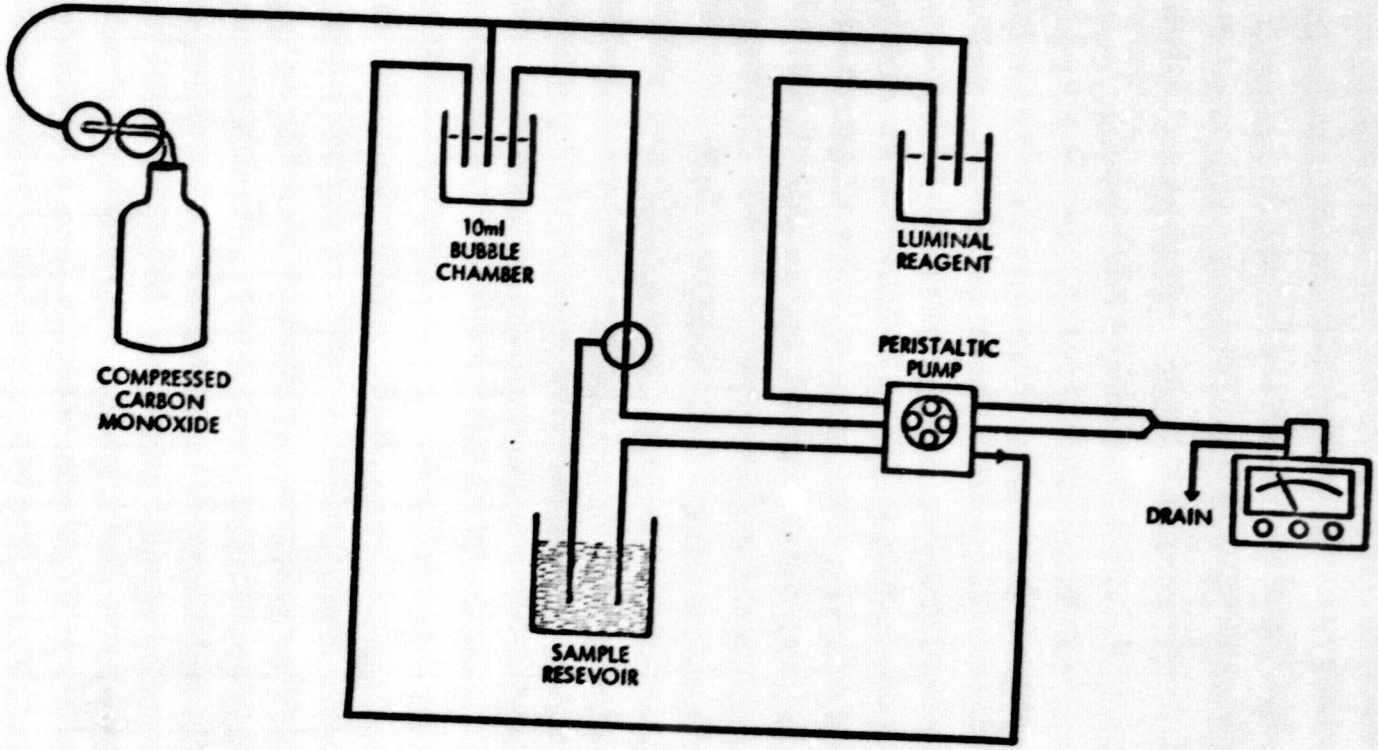

Figure 3 Experimental flow system designed to bubble carbon monoxide through sample and luminal reagent

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2. A bubble chamber with a volume over 10 ml produces better results, possibly due to flushing oxygen out of the line with the treated sample.

3. The shortest possible flow lines should be used for the carbon monoxide treated samples to prevent possible carbon monoxide porphyrin dissociation.

II. ASSOCIATION OF BACTERIA WITH LARGE PARTICLES

Examination of "real-world" wastewater effluent samples indicated that, in the case of returned sludge and mixed liquor, the majority of bacteria are associated with large particles. Studies with samples taken from a southwest Houston wastewater treatment plant indicated that 99.8% of the bacteria were associated with particles greater than 5 microns. This study warns of the potential dangers of using a 10 micron pre-filter for these types of samples.

Studies comparing the luminol-iron porphyrin measurements and Coulter electronic particle of real wastewater effluent samples are continuing to show good correlation as shown in Table 2.

III. VALENCE STATE OF LUMINOL REACTIVE IRON PORPHYRINS

Spectrophotometric measurements of cytochrome-c were conducted to support the present theory that oxidized iron porphyrins are responsible for luminol chemiluminescence. The experimental results showed that cytochrome-c, oxidized by air or a shift in pH to pH3, produced as much as 10 times the luminol chemiluminescence than samples reduced by ascorbic acid or hydrogen over platinized asbestos.

IV. ATP METHODOLOGY CONFERENCE PRESENTATION

Appendix 1 contains the paper entitled, "Use of the Luminol Assay for the Determination of Bacterial Iron Porphyrins Flow Techniques for Wastewater Effluent" presented at the 2nd Bi-Annual ATP Methodology Conference in San Diego, California, March 1977. This paper includes the current procedures and advances for using the luminol reaction for quantitating bacteria.

V. PATENT

A patent entitled, "Rapid, Quantitative Determination of Bacteria in Water" (NASA Case No. GSC 12, 158-1) was drafted during this quarter. This patent describes the methods for rapid quantification of bacteria including procedures for concentration and analysis using the firefly luciferase-ATP reaction and the luminol-iron porphyrin reaction.

TABLE 1

COMPARISON OF LUMINESCENT SIGNAL WITH CARBON MONOXIDE

METHOD	INJECTION	FLOW
CO BUBBLED THROUGH SAMPLE ONLY	61.3%	51.4%
CO IN SAMPLE AND LUMINOL REAGENT	38.5%	44.0%

* Comparison of the reduction of luminescent signal with carbon monoxide using various techniques. Sample was calculated to contain 56% viable bacteria

TABLE 2

COMPARATIVE DATA USING THE LUMINOL -IRON PORPHYRIN REACTION

SAMPLE	COULTER COUNT	LUMINOL ANALYSIS
CLARIFIER EFF. (SW HOUSTON) (6 Apr. 77)	2.6×10^6	8.0×10^6
CHLORINATED EFF. (SW HOUSTON) (6 Apr. 77)	3.1×10^6	1.2×10^7
UNCHLORINATED EFF. (SW HOUSTON) (18 May 77)	3.34×10^6	3.98×10^6
CONCENTRATED CHLORINATED EFF. (SW HOUSTON) (18 May 77)	1.15×10^8	1.18×10^8

* Comparative data using the luminol -iron porphyrin reaction for quantitating bacteria and the Coulter electronic particle counter

APPENDIX 1

USE OF THE LUMINOL ASSAY FOR THE DETERMINATION OF BACTERIAL IRON PORPHYRINS: FLOW TECHNIQUES FOR WASTEWATER EFFLUENT

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USE OF THE LUMINOL ASSAY FOR THE DETERMINATION OF BACTERIAL IRON PORPHYRINS: FLOW TECHNIQUES FOR WASTEWATER EFFLUENT

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ABSTRACT:

A chemiluminescent method for monitoring microbial populations in wastewater effluent has been developed as part of the Johnson Space Center Water Monitoring System. An inexpensive, semi-automated flow system as well as an automated computer-operated system have been developed incorporating the alkaline luminol-hydrogen peroxide reaction with bacterial iron porphyrins. By measuring the luminescence response, an accurate indication of bacterial concentration can be determined in real time with a sensitivity limit of 10^4 cells per milliliter. Hydrogen peroxide pretreatment and reaction rate resolution prevent interference from soluble iron porphyrins and metallic ions found in wastewater effluent.

The luminol response from five pure and mixed cultures of bacteria isolated from effluent has produced linear results.

Since it appears that luminol responds to total cells, a means of differentiating live from dead cells is proposed.

INTRODUCTION:

A chemiluminescent method for monitoring microbial populations in wastewater effluent has been developed for use in the Johnson Space Center Water Monitoring System. The assay is based on the chemiluminescent reaction between luminol (5-amino-2, 3-dihydro-1, 4-phthalazinedione), hydrogen peroxide, and an activating agent in an alkaline

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aqueous solution. Ferricyanide or hypochlorite can be used as activating agents to produce chemiluminescence although systems derived from hydrogen peroxide and a transition metal such as iron, copper, or cobalt can be substituted. Slide 1 shows the basic reaction. While several mechanisms have been proposed, some mechanisms involving free radicals (1) or a one electron oxidation of the luminol dianion (2, 3, 4), no single mechanism completely explains the reaction for all activating agents. Slide 2 gives some indication of the relative intensities of the chemiluminescent signals from various luminol activating agents.

The chemiluminescent system used for monitoring microbial levels involves the reaction between luminol and most likely iron porphyrins such as cytochromes, catalase, or peroxidase as the activating and probably oxidizing agents. Many investigators have proposed using the luminol reaction for monitoring bacteria (5, 6, 7). The system used by NASA incorporates similar features of these tests with some unique features. Our system is automatable with special techniques for eliminating possible sources of interference, such as soluble iron porphyrins and metallic ions.

The chemiluminescent assay for bacteria requires that the sample contain more than 10^4 cells per milliliter for accurate quantification. If the sample contains less than 10^4 cells per milliliter some means of concentration is necessary. Concentration by the Amicon Hollow Fiber Dialyzer/Concentrator has been primarily used in our system; however, concentration methods such as centrifugation and membrane filtration have also been developed. Procedure and results with the Amicon Hollow Fiber unit will be presented by Eldon Jeffers in a later talk. Concentration by centrifugation is perhaps the simplest and most efficient means of concentrating a sample; however, the least automatable. Another method which has been developed is concentration on a 0.2 micron Gelman acropor membrane filter. This filter was selected since it is one of the few membrane filters which is resistant to the necessary extractant, 0.1N sodium hydroxide - 50% ethanol. The filter is made of a copolymer of acrylonitrile and polyvinyl chloride on a nylon substrate. The procedure involves collecting bacteria on the membrane filter and then extracting the iron porphyrins with the 0.1N NaOH - 50% EtOH mixture. Other extracting agents such as nitric acid have been tested; however, only the 0.1N NaOH - 50% EtOH extracts the porphyrins and allows them to pass through the filter.

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In addition, the mixture stabilizes the porphyrins once they are in solution. Problems associated with this technique are those common to all membrane filters, such as clogging and slow flow rates.

Since many compounds besides intra-cellular iron porphyrins produce a chemiluminescent response several techniques have been incorporated into the assay to eliminate false signals. A simplified assay of a concentrated intact microbial sample would include the following steps:

1) Pre-incubation of the sample with a dilute concentration of hydrogen peroxide. This step eliminates any soluble iron porphyrins present.

2) If chlorine is present in the sample such as in chlorinated wastewater, sodium thiosulfate can be used to reduce chlorine to the non-reactive chloride.

3) The pretreated sample is then injected into the luminol reagent. This step extracts the iron porphyrins from the bacteria and the resulting chemiluminescence can be recorded using a photometer.

4) The light response is then measured 5 seconds after the initial reaction. This step eliminates the false signals from the short-lived light responses from transition metals such as iron, cobalt, and copper, and much of the signal from ferricyanide.

Hydrogen peroxide pretreatment can be used to eliminate soluble porphyrins which would otherwise produce a false signal. In hydrogen peroxide oxidation as in natural degradation of iron porphyrins a bridge-carbon atom is eliminated from the porphyrin nucleus (8). Very little chemiluminescent response is observed from the resulting dissociated iron atom. This reduction occurs even in the case of catalase interference. The result is that the signal from soluble iron porphyrins is eliminated but porphyrins within intact bacteria are protected for the subsequent assay.

Slide 3 shows the effect of hydrogen peroxide pretreatment concentration with time on a 10^{-7}M catalase sample. The greatest reduction of signal occurs in the first five minutes. Slide 4 shows the effect of hydrogen peroxide

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pretreatment concentration on actual bacteria sample, Escherichia coli. At final concentrations less than 1.5% peroxide no significant loss of signal is observed with pretreated E. coli. A final concentration of 0.5% hydrogen peroxide for 2 minutes was selected as the optimum concentration and time for effective reduction of soluble porphyrins and little loss of signal from the bacteria sample.

Slide 5 shows the effect of 0.5% hydrogen peroxide for 5 minutes on three species of bacteria, Escherichia coli, Bacillus subtillis; and Pseudomonas aeruginosa. It is apparent that the growth stage of some species of bacteria does influence the susceptibility of the bacteria to the hydrogen peroxide pretreatment. While some loss of signal is observed with pretreated bacteria, the loss is not significant compared to the loss of signal from other pretreated materials. Slide 6 shows the effect of 0.5% hydrogen peroxide pretreatment on a number of compounds capable of stimulating a luminol light response. Over 90% of the interference due to porphyrin material can be eliminated using this technique.

However, since some loss of bacteria can occur with the hydrogen peroxide pretreatment, this step should only be used if a problem with soluble iron porphyrin is expected. In the case where a sample is concentrated by centrifugation very little soluble porphyrins would be expected in the pellet. Since the Amicon Hollow Fiber unit (50,000 Mol. Wt. cut-off) concentrates only the bacteria and very little soluble porphyrins, the signal from soluble porphyrins will probably not be significant thus not requiring hydrogen peroxide pretreatment. If bacteria were concentrated on a membrane filter probably very little iron porphyrins would be present; however, the filter could be washed with the 0.5% hydrogen peroxide prior to extraction. Obviously, once the porphyrins are extracted with 0.1N sodium hydroxide - 50% ethanol no hydrogen peroxide should be added.

Chlorine has already been mentioned as a luminol chemiluminescent activating agent. Adding sodium thiosulfate to the sample to a final concentration of 50 mg/l effectively reduces all the chlorine to the non-reactive chloride. No effect on bacteria or luminol activity has been noticed with this treatment. The reaction rate resolution described later will also eliminate false signals from chlorine.

As was noticed in Slide 6 the hydrogen peroxide pretreatment did little to eliminate the false signals from inorganics such as the transition metals. To eliminate the effects of inorganic interference we have made use of the different reaction rates of the various luminol activating agents. Slide 7 illustrates how the reaction rates of potassium ferricyanide and ferrous sulfate differ from iron porphyrins such as catalase and the bacterium, E. coli. These reaction rate curves are specific for 0.1% hydrogen peroxide, different peroxide concentrations showing different shaped curves. What can be seen in Slide 7 is that if the light measurement is recorded at a point 5 seconds after the initial mixing of sample and luminol reagent, only the catalase and E. coli response would be observed. When coupled with the hydrogen peroxide pretreatment, the reaction rate resolution method makes the luminol system quite specific for bacteria.

The luminol reagent used in the assay consists of 2.5×10^{-4}M luminol, 0.1% hydrogen peroxide, 6.33×10^{-3}M ethylenediame tetraacetic acid (EDTA) and 0.75N sodium hydroxide. This solution has been determined as optimum in terms of:

1) maximum signal to noise ratio - luminol concentration

2) elimination of interference by peroxide pretreatment and reaction rate resolution - hydrogen peroxide concentration

3) elimination of chemiluminescent inhibition - EDTA concentration

4) optimum pH - sodium hydroxide concentration.

A volume ratio of 1:1, sample to luminol reagent, has been used in all these studies.

The optimum luminol concentration was determined in terms of lowest blank and highest light response for a 4×10^7 Escherichia coli/ml sample. Slide 8 illustrates the sample response, blank response and endogenous light associated with increasing luminol concentrations.

A plot of the signal to noise ratio for the different luminol concentration can be found in Slide 9. Self-quenching of the reaction apparently becomes important when the

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luminol concentration is greater than 5×10^{-4}M (9). The optimum luminol concentration was thus determined to be 2.5×10^{-4}M.

Two phenomenon are dependent on the hydrogen peroxide concentration; 1) peak light response, and 2) the related reaction rate curves. Slide 10 illustrates how the peak light response varies with different hydrogen peroxide concentrations in the luminol reagent for a bacteria sample. Slide 11 shows how the reaction rate curves change with those same hydrogen peroxide concentrations. 0.1% hydrogen peroxide was chosen as the optimum concentration due to the highest light output and yet sufficient reaction rate resolution of the interference signals from bacteria signals.

The sodium hydroxide in the luminol reagent also serves two functions: 1) as an extractant to rupture the cells and release the iron porphyrins for the reaction, and 2) to provide an alkaline solution for the chemiluminescent reaction.

The optimum pH was the criteria for selecting the sodium hydroxide concentration. Luminol chemiluminescence with transition metals such as iron and cobalt (II) show a maximum around pH 10.9 (10, 11) while the maximum chemiluminescence for E. coli is exhibited around pH 12.4 (conversation with Dr. Rudy Moyer). 0.75N sodium hydroxide which has a pH of 12.4 is therefore used as the basis for the luminol reagent. Sodium hydroxide extraction is comparable in efficiency to the 0.1N nitric acid used in the ATP assay.

Certain wastewater effluents contain some materials which appear to inhibit luminol chemiluminescence. It is known for instance that some amino acids, in particular amino acids containing sulfhydryl groups such as cysteine and thiourea inhibit the luminol reaction. It has been found that 6.33×10^{-3}M EDTA eliminates the inhibition phenomenon found in the effluent at the Johnson Space Center site. It is speculated in this particular instance the EDTA may be chelating some metallic ion; however, the actual source and site of the inhibition is not yet known.

The luminol procedure described up to this point is adequate for a single sample or multiple sample assay; however, for continuous monitoring an automatable flow system is much more advantageous. Such a system has been

constructed by NASA for the purpose of continuously monitoring wastewater effluent. The schematic diagram in Slide 12 shows the flow system which incorporates all the steps which have been outlined above. The sample mixes with 3% hydrogen peroxide for a final concentration of 0.5% with a 2 minute residence time; this is the pretreatment step which eliminates the soluble porphyrins. The hydrogen peroxide pretreated sample then mixes 1:1 with the luminol reagent. The 5 second delay of the solution before entering the photometer eliminates the short-lived inorganic interferences and only the response from the iron porphyrins is measured.

Slide 13 shows the actual laboratory setup. The apparatus shown are; 1) a Buchler peristaltic pump which proportions samples and reagents, and 2) the Aminco Chem-Glow photometer. The apparatus is fairly inexpensive and reliable requiring only periodic replacement of pump tubing.

The automated chemiluminescent wastewater monitoring system at Johnson Space Center is similar to the laboratory setup; however, it is controlled and operated by a small computer and a series of solenoid actuated valves. The light units are directly converted to equivalent bacteria per milliliter as computed by the computer according to a standard calibration curve.

In both the automated and semi-automated luminol systems, some means of calibration must be employed. Isolated iron porphyrins can be used as standards; however, they must be used in 50% ethanol mixtures since porphyrins rapidly decompose in aqueous solutions. The luminol response for most bacteria is fairly constant and correlation can be made between the amount of iron porphyrin per bacteria cell. It might be mentioned here that all the aerobes this laboratory has tested have produced a fairly constant luminol response; however, Streptococus faecalis, a facul tative anaerobe produces very little response. We suspect that this may be true of all anaerobes either because they do not contain the "normal" cytochrome respiratory chain or all the iron porphyrins are in the reduced state.

The best method for calibrating the bacteria sensor appears to be assaying known quantities of cultured bacteria. By establishing the light response for known concentrations of bacteria a standard curve can be produced.

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All calibrations are performed using cultured bacteria with known total counts. Slide 14 is a calibration curve performed with the semi-automated system using known concentration of E. coli.

The automated system calibration was performed using the 5 prevalent bacteria species found in Johnson Spacecraft Center wastewater. The wastewater sample was plated, colonies that represented the most prevalent morphological types were subcultured and these pure species were mixed in various percentages and dilutions in deionized water for a total of 120 samples. The bacteria were identified as Klebsiella pneumoniae, Pseudomonas putrifaciens, E. coli, another Pseudomonas species, and gram negative rods. The results, Figure 15, illustrate the relationship between the chemi-luminescent light unit and microbial count by spread plate method. It was found that the slope of the calibration curve was 1 and that a luminol light unit was equivalent to 2×10^6 cells per ml, independent of bacteria species. The lower limit of sensitivity for the automated flow system as tested was 2×10^5 cells per ml.

Slide 16 illustrates the performance of the interference elimination methods with a tap water sample. The hydrogen peroxide pretreatment and reaction rate resolution methods combine to eliminate 99% of the signals attributed to non-bacterial sources. Little effect on a bacteria sample, E. coli response is observed.

The interference elimination methods as illustrated in the somewhat challenging example above can eliminate most of the false signals from samples. The system has limits and can be saturated, swamped by very high concentrations of interfering materials. Practical experience has demonstrated that even though common interferences have been minimized, specific environments such as industrial sites may present specific problems. Domestic wastewater which may be of a fairly constant composition should be of less concern.

The luminol reaction system is a detection system for total bacteria, live as well as dead cells. A method is now being developed which will allow the luminol system to differentiate live from dead cells. This method simply

involves bubbling carbon monoxide through a bacterial suspensions for 15 minutes which results in a loss of luminol signal from the viable bacteria. A direct correlation exists between the percent reduction of luminol response after carbon monoxide pretreatment and the percent viable cells present in the sample.

Slide 17 illustrates the relationship between the percent reduction of luminol response with carbon monoxide treatment, and the percent viable bacteria in the sample for several species. Since the luminol response from the untreated sample is a measure of the total number of cells and the percent luminol reduction is an indication of the percent viable cells, an accurate count (with 10%) of the actual numbers of dead and live cells can be made.

The model we have constructed to explain this phenomenon is based on several assumptions: 1) luminol reacts with the oxidized iron porphyrins in bacterial cells, 2) the cytochromes in dead cells are mainly in the oxidized state as a result of the equilibrium of the reaction favoring that species and, 3) the carbon monoxide complexes with the reduced cytochromes (12, 13) (found in the live cells) and inactivates them with the chemiluminescent reaction.

Slide 18 depicts our model cells. The cytochromes of actively metabolizing viable cells are undergoing constant oxidation and reduction while in dead cells most of the cytochromes are oxidized. Carbon monoxide complexes with the reduced cytochromes of the live cells and inhibits any chemiluminescent response with luminol. Since the cytochromes in the dead cells are mostly oxidized no complexes are formed and chemiluminescence can be observed with the dead cells.

This method for differentiating live from dead cells produces accurate results using a simple injection type system. Work is currently being undertaken to adapt this system to a continuous type flow system.

In conclusion, the luminol system has several advantages over the firefly luciferase - ATP assay. In our studies, luminol system is generally ten times more sensitive to most bacteria excluding anaerobes and certainly less expensive. Most interfering agents likely to produce false signals can be eliminated and lastly, the carbon monoxide procedure for differentiating live from dead cells makes this assay extremely versatile.

Not only is this assay well suited for continuous monitoring of wastewater effluent, but it could have clinical uses as well as uses in basic metabolic research.

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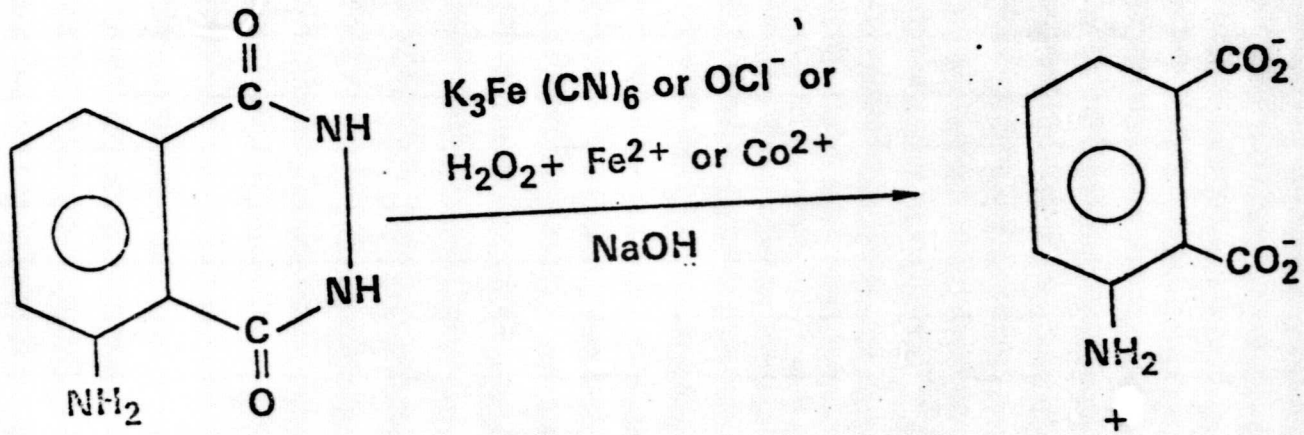
BASIC LUMINOL REACTION



Luminol

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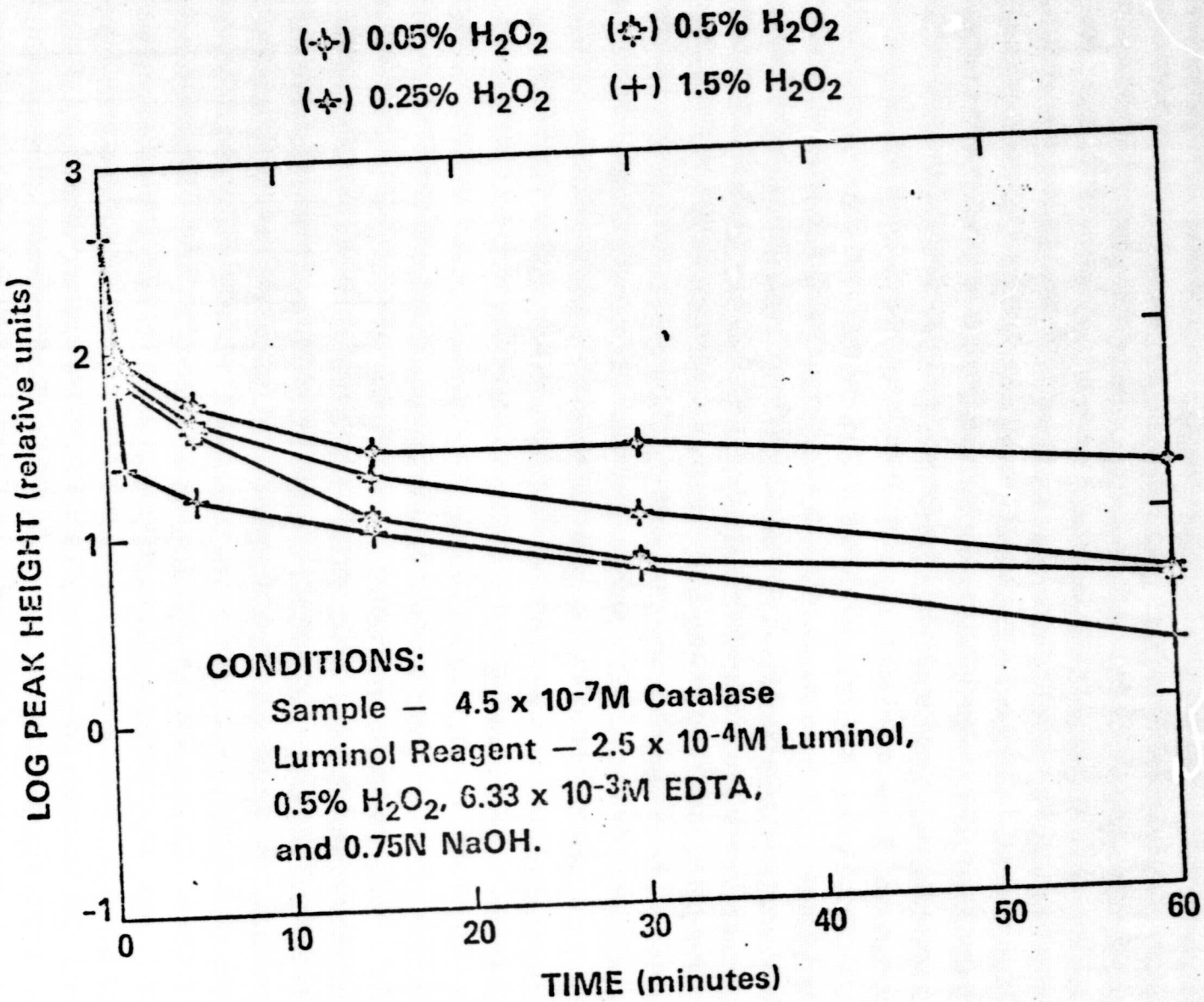
RELATIVE LUMINESCENCE RESPONSES OF LUMINOL ACTIVATORS

Compound	Concentration	Response	Fe Atoms/ Molecule
Catalase	1×10^{-7}M	9490	4
Hemoglobin	1×10^{-7}M	6500	4
Cytochrome c	1×10^{-7}M	25	1
Ferricyanide	1×10^{-7}M	5	1
Ferrous Sulfate	1×10^{-7}M	0.03	1
Cobaltous Chloride	1×10^{-7}M	2.6	0
Cl_2	1×10^{-7}M	0.03	0

CHEMILUMINESCENT RESPONSE OF HYDROGEN PEROXIDE PRETREATED CATALASE



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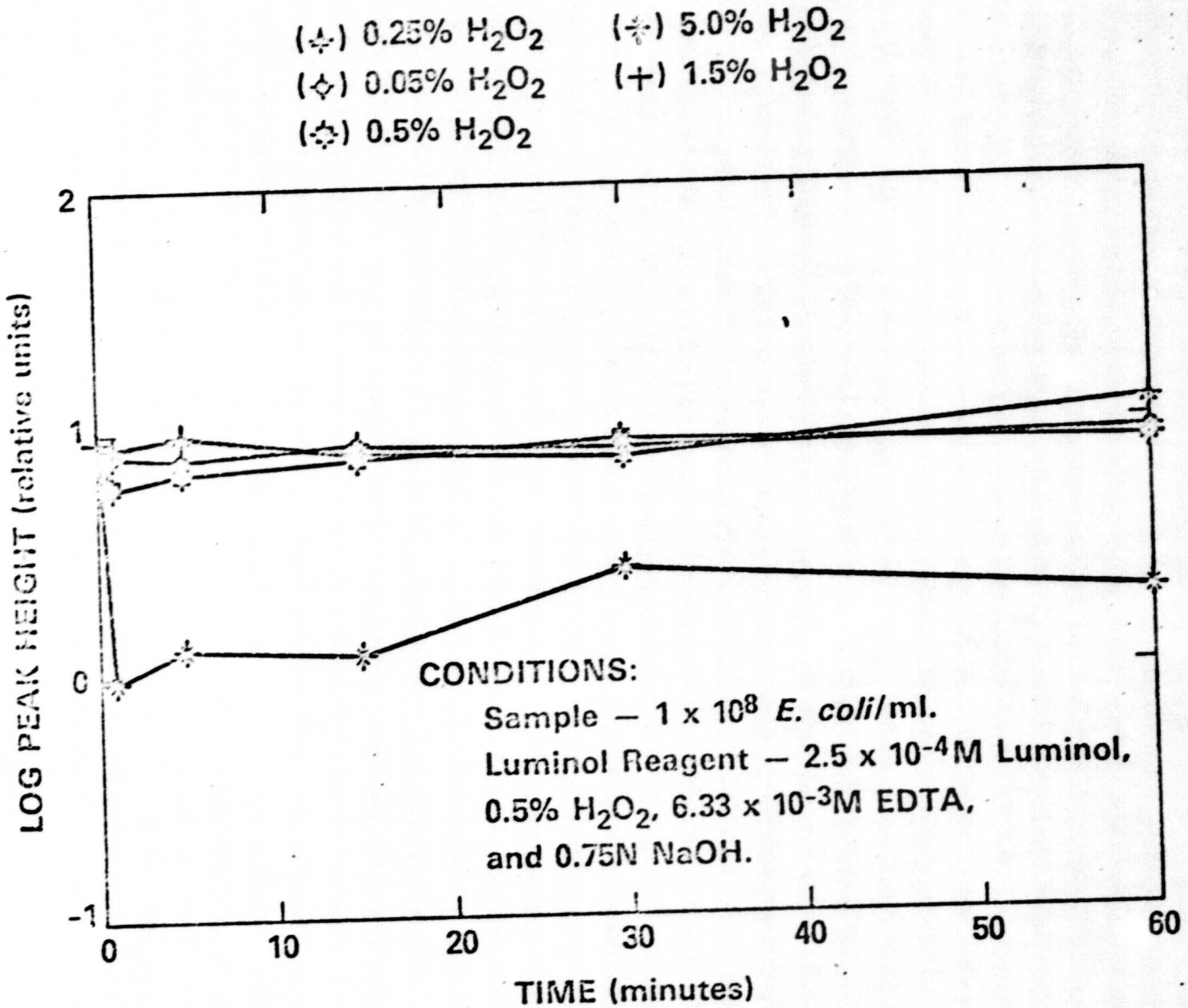
CHEMILUMINESCENT RESPONSE OF HYDROGEN PEROXIDE PRETREATED *E. COLI*



SPECIES	REDUCTION OF SIGNAL	
	GROWTH PHASE	
	STATIONARY	LOGARITHMIC
ESCHERICHIA COLI	8%	24%
BACILLUS SUBTILIS	16%	36%
PSEUDOMONAS AERUGINOSA	43%	40%

EFFECT OF 0.5% HYDROGEN PEROXIDE PRETREATMENT ON BACTERIA

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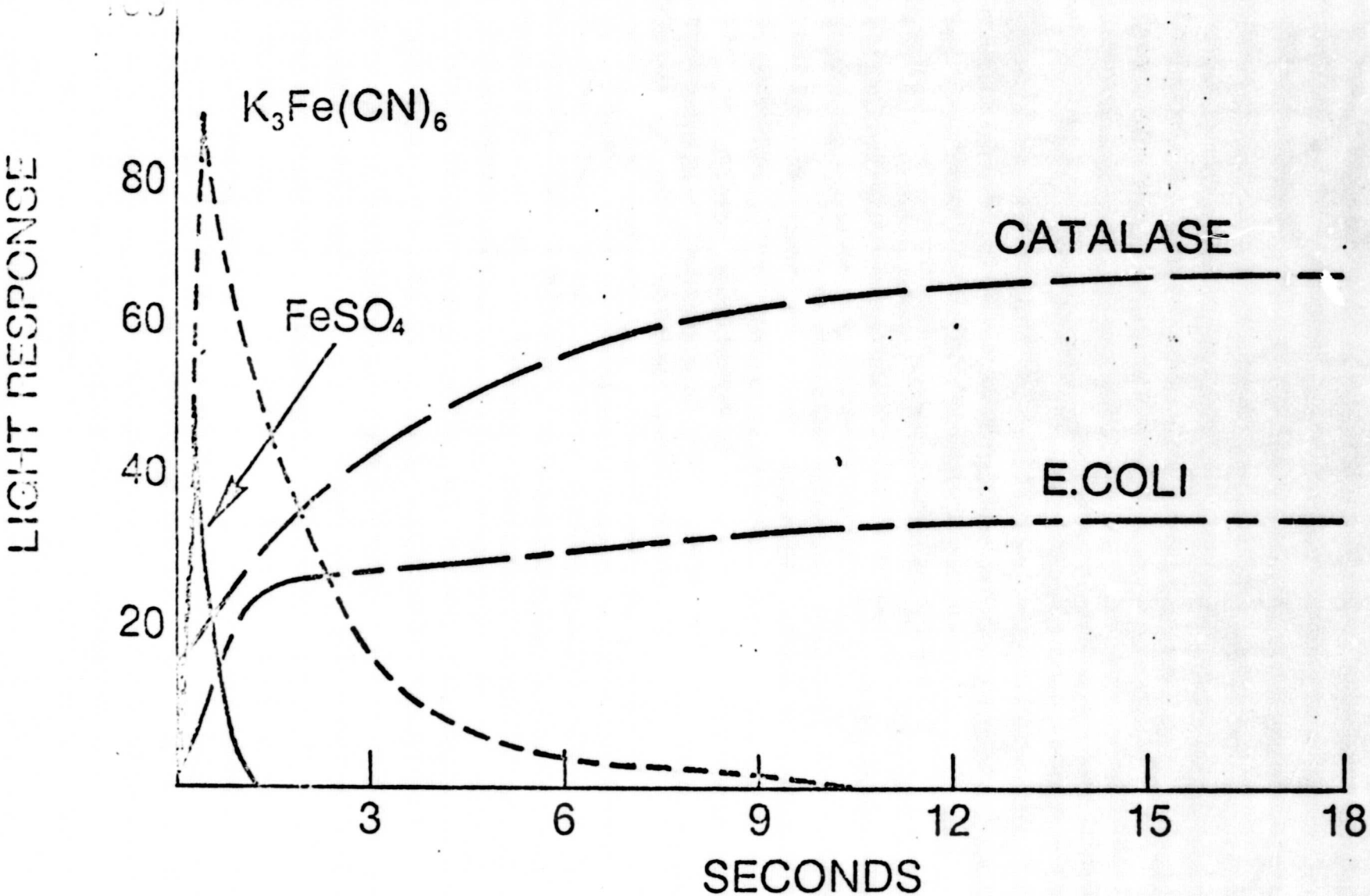
OXIDIZING AGENT	REDUCTION OF SIGNAL
CATALASE	94%
HEMOGLOBIN	95%
EXTRACTED BACTERIAL PORPHYRINS (NaOH - EtOH)	97%
POTASSIUM FERRICYANIDE	50%
COBALT(OUS) CHLORIDE	20%
FERROUS SULFATE	0

EFFECT OF 0.5% HYDROGEN PEROXIDE PRETREATMENT ON SOME LUMINOL OXIDIZING AGENTS



REACTION RATE CURVES FOR VARIOUS LUMINOL OXIDIZING AGENTS (ARBITRARY CONCENTRATIONS).

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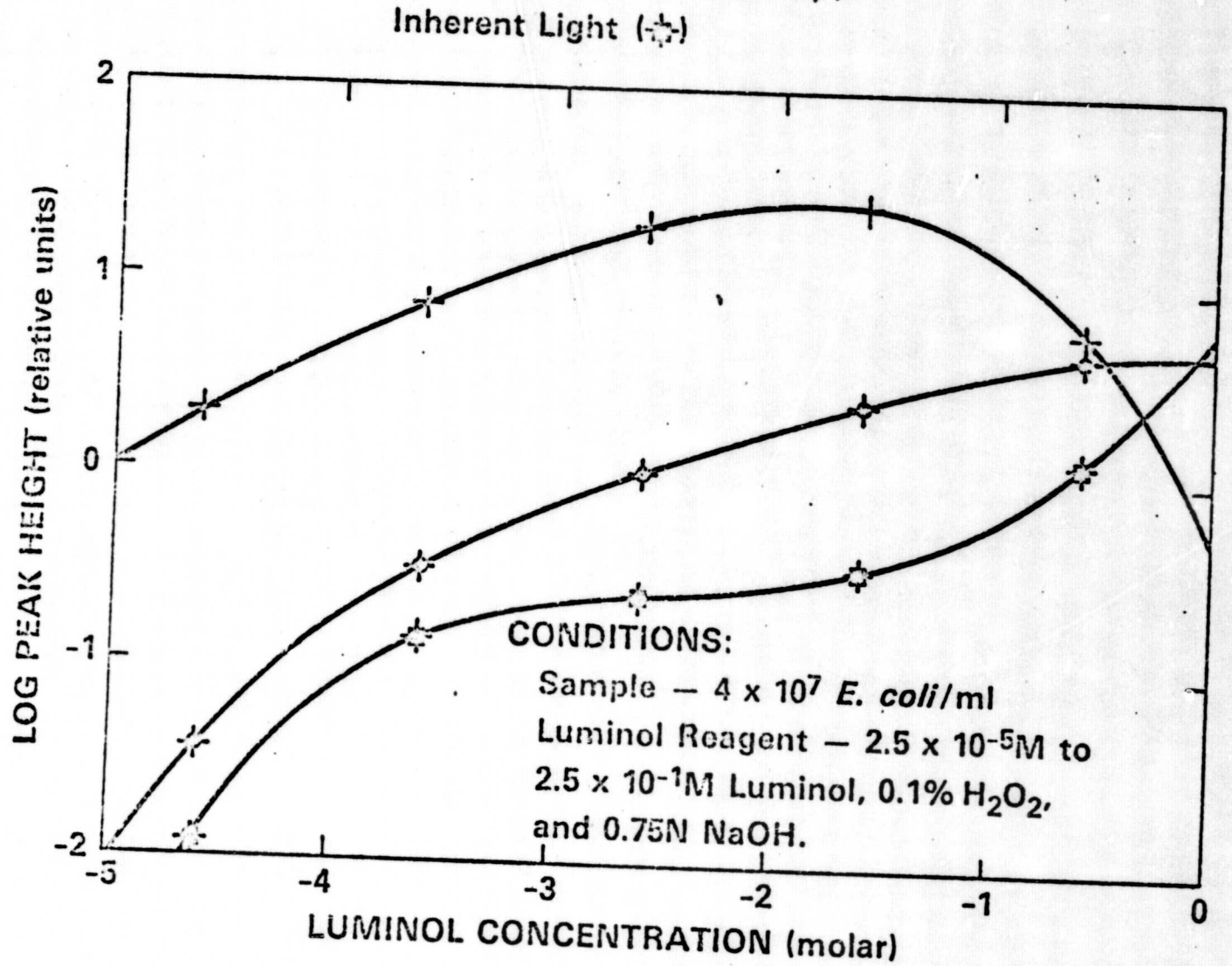
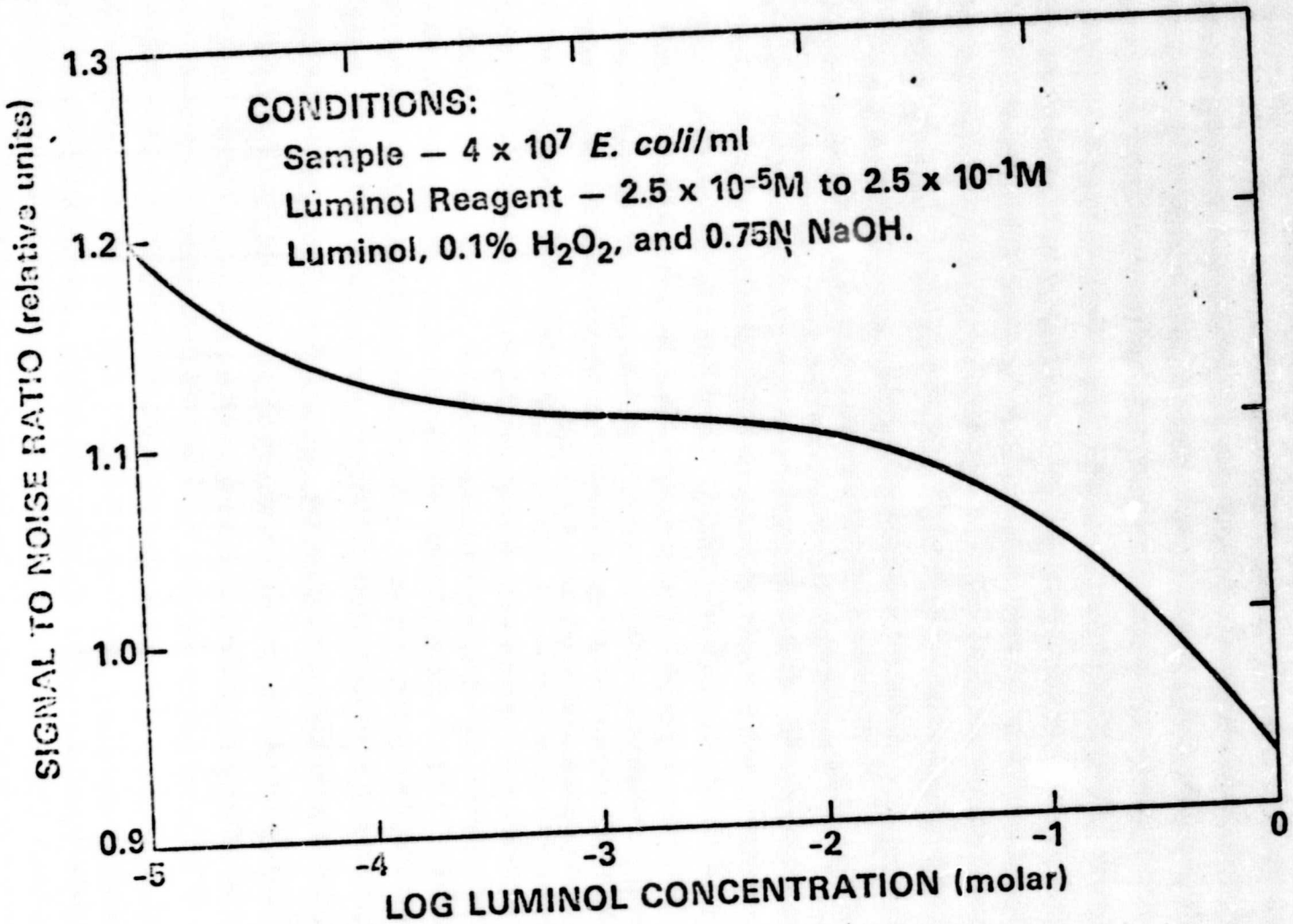
CHEMILUMINESCENT RESPONSE TO *E. COLI* WITH VARYING LUMINOL CONCENTRATIONS



SIGNAL TO NOISE RATIO FOR VARIOUS LUMINOL CONCENTRATIONS



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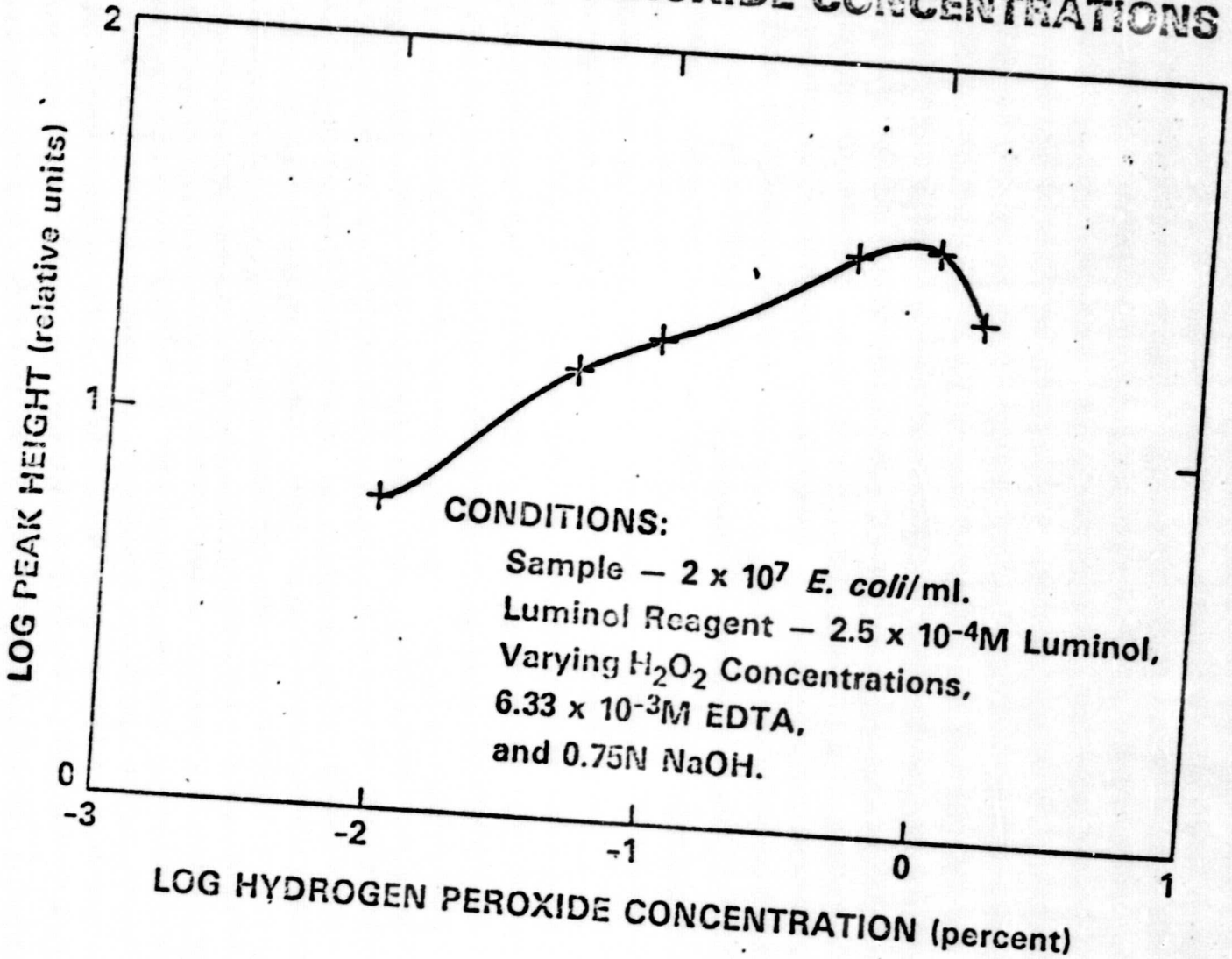
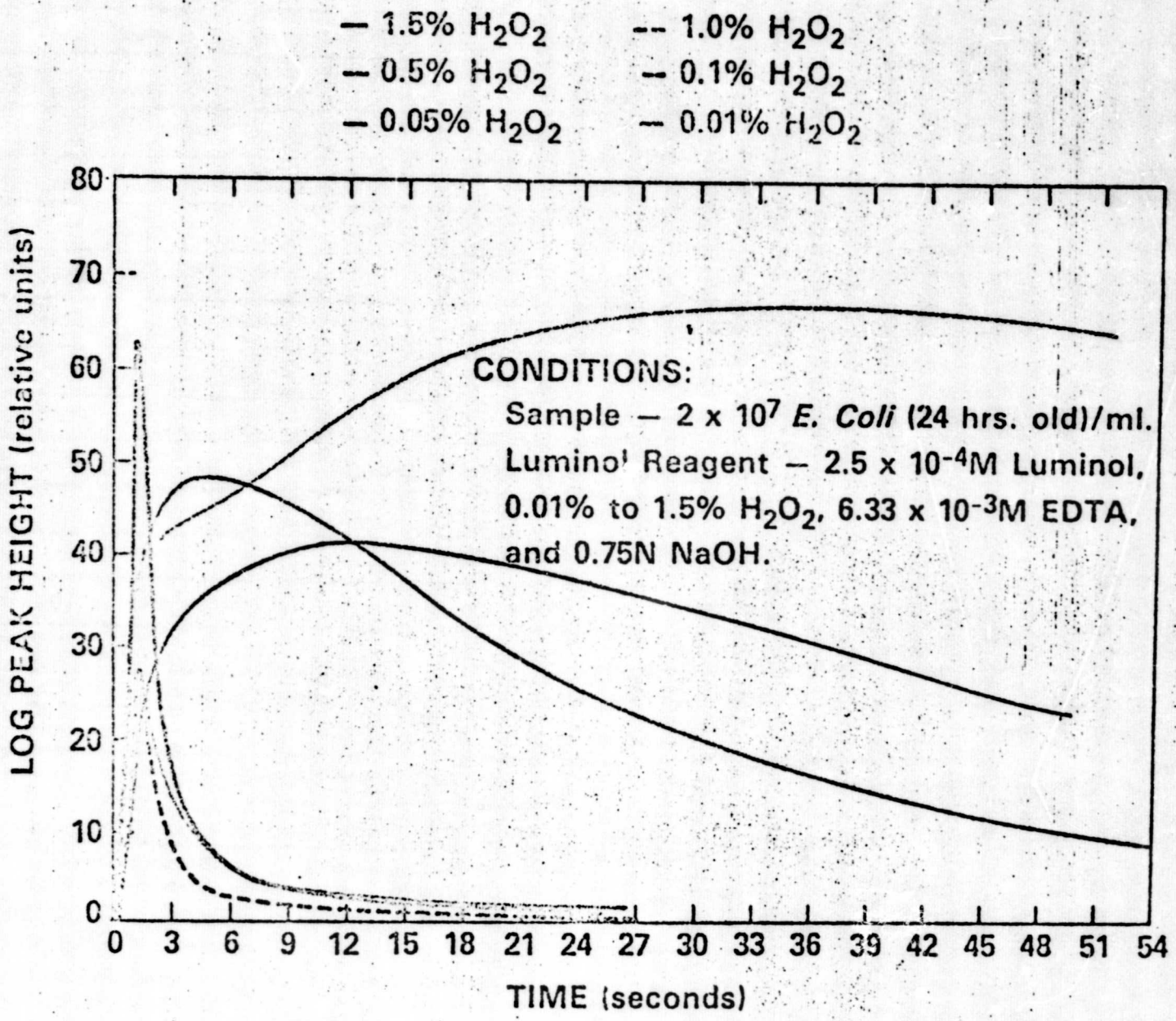
CHEMILUMINESCENT RESPONSE OF *E. COLI* WITH VARYING HYDROGEN PEROXIDE CONCENTRATIONS



REACTION RATE CURVES FOR *E. COLI* WITH HYDROGEN PEROXIDE CONCENTRATIONS



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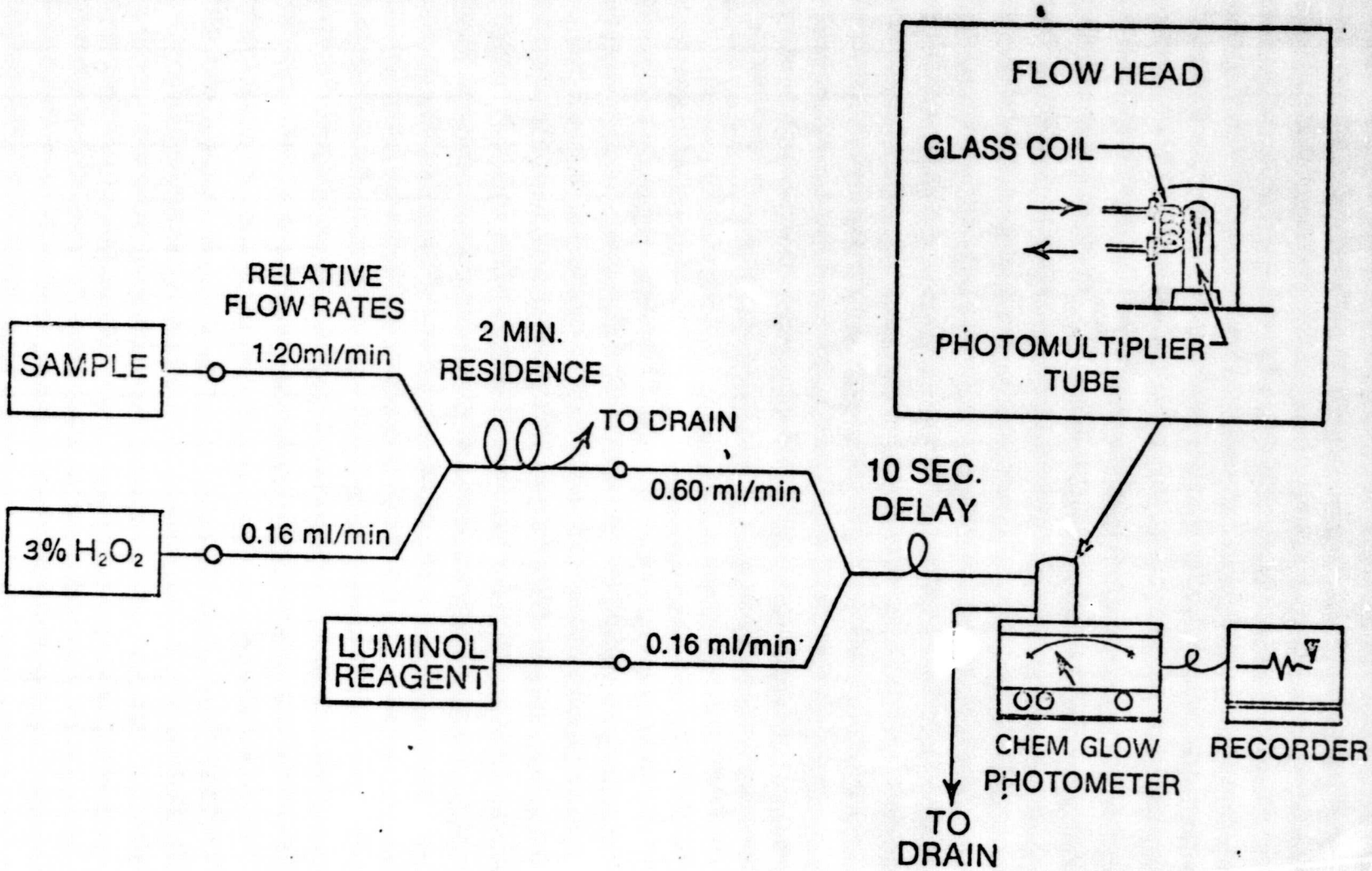
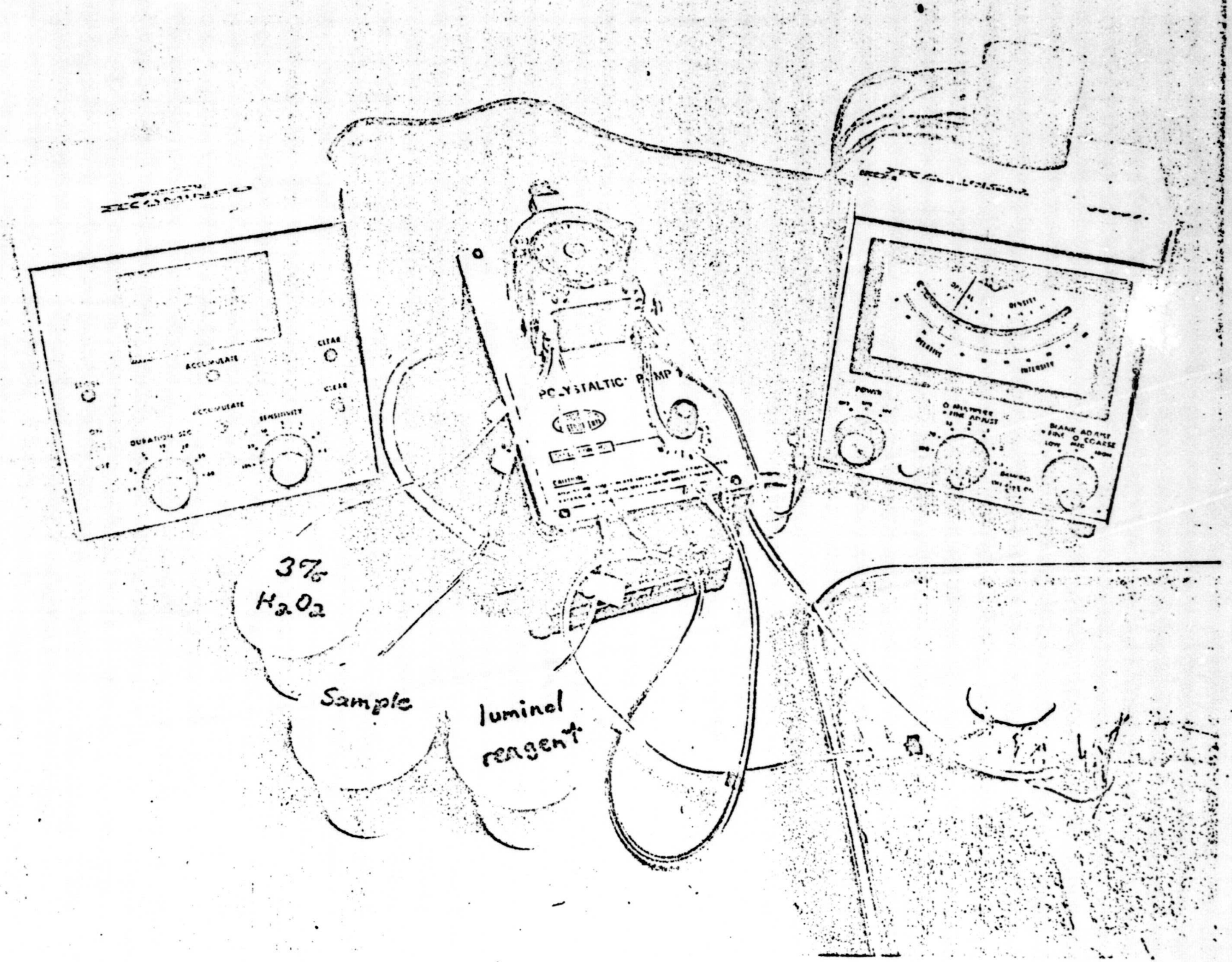


AUTOMATED LUMINOL FLOW SYSTEM INCORPORATING HYDROGEN PEROXIDE PRETREATMENT AND REACTION RATE RESOLUTION FOR ELIMINATING INTERFERENCE.


3%
H_2O_2
Sample
luminol
reagent
POLYSTALTIC PUMP
ACCUMULATE
CLEAR
DURATION SEC
SENSITIVITY
POWER
DENSITY
INTENSITY
BLANK ADJUST
FINE
COARSE

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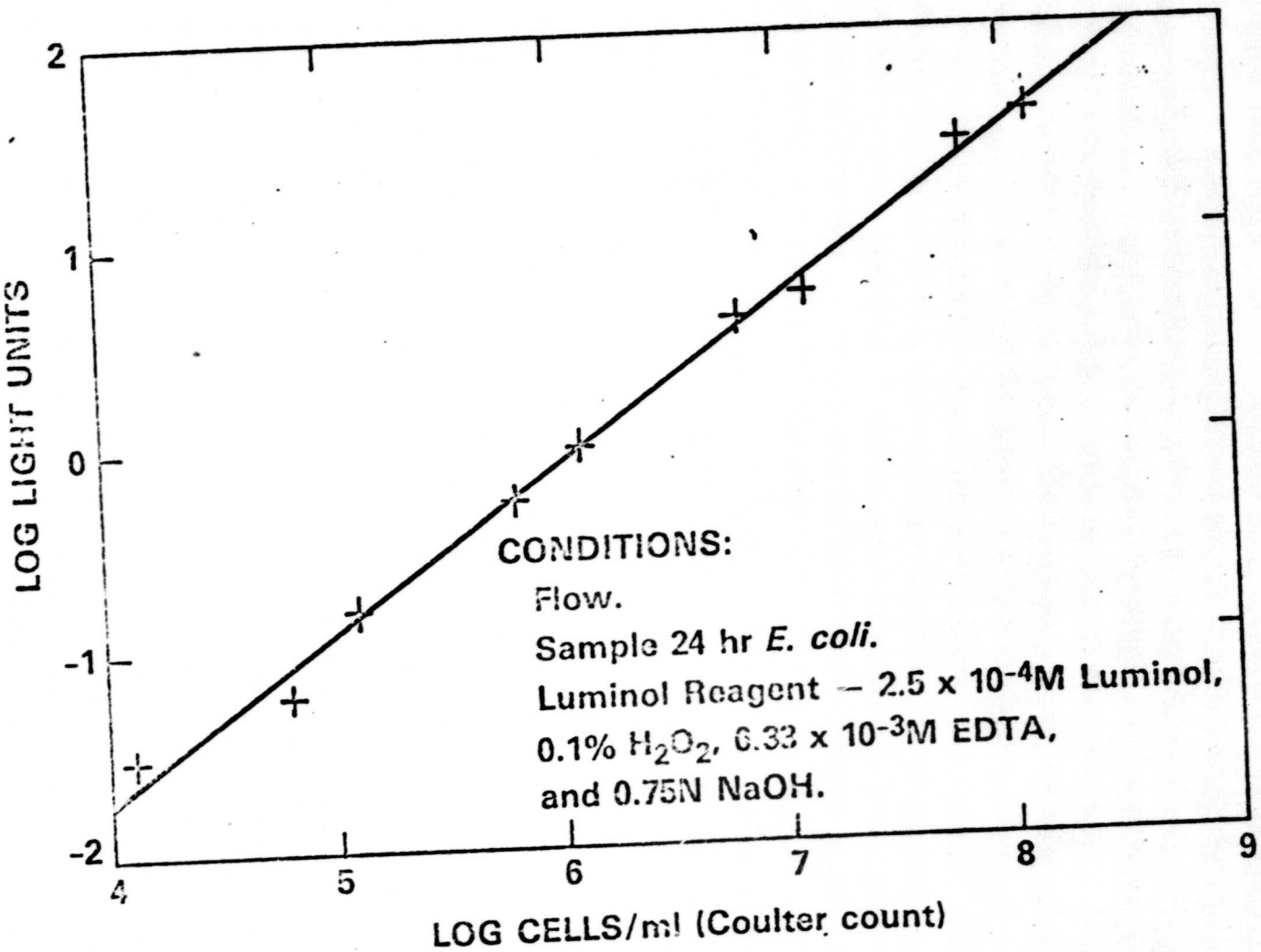
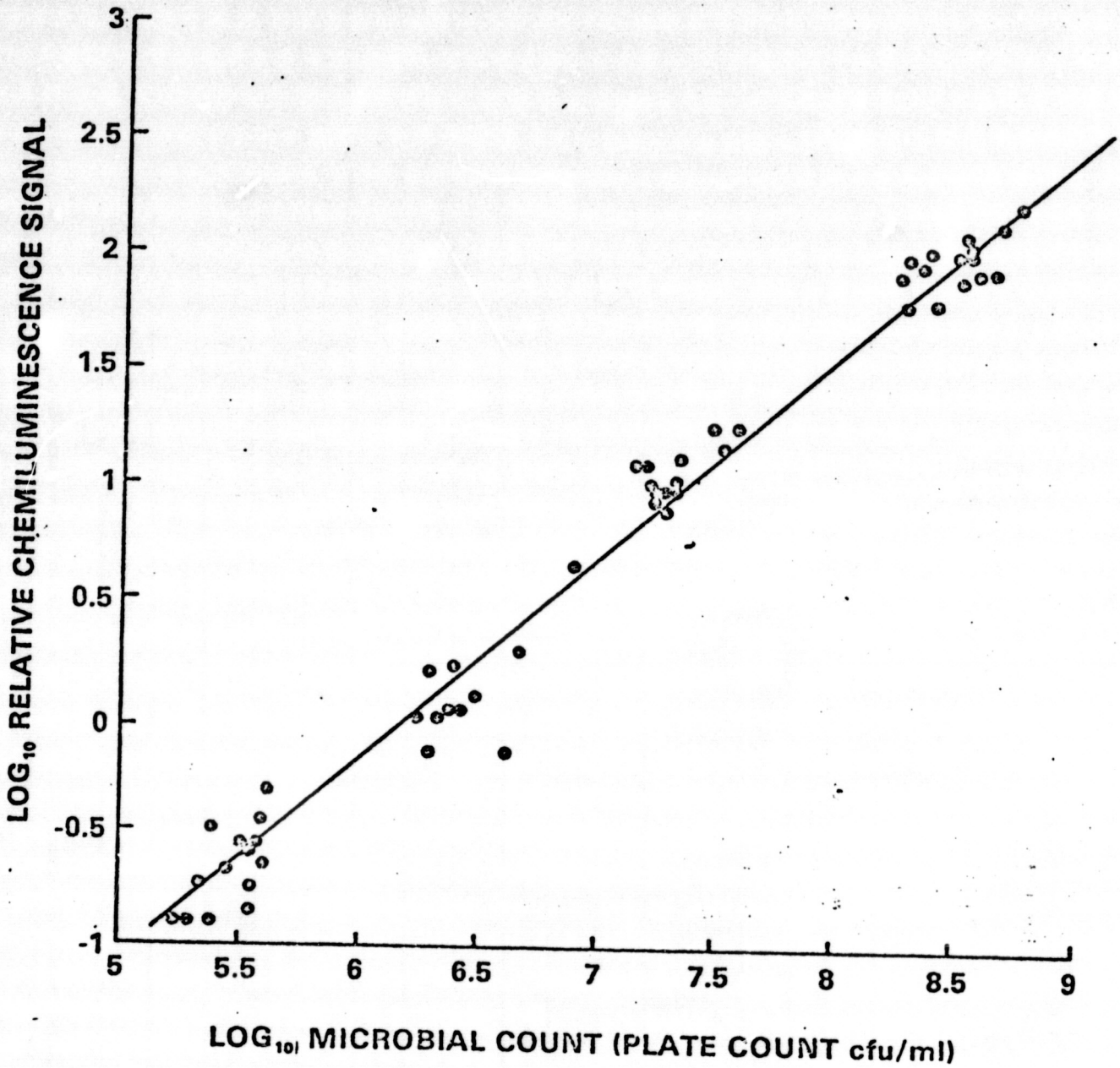
LUMINOL RESPONSE OF *E. COLI* IN TAP WATER VS. TOTAL CELL COUNT (COULTER PARTICLE COUNT)



Chemiluminescence Response vs. Plate Counts of Bacteria Isolated from Secondary Treated Wastewater



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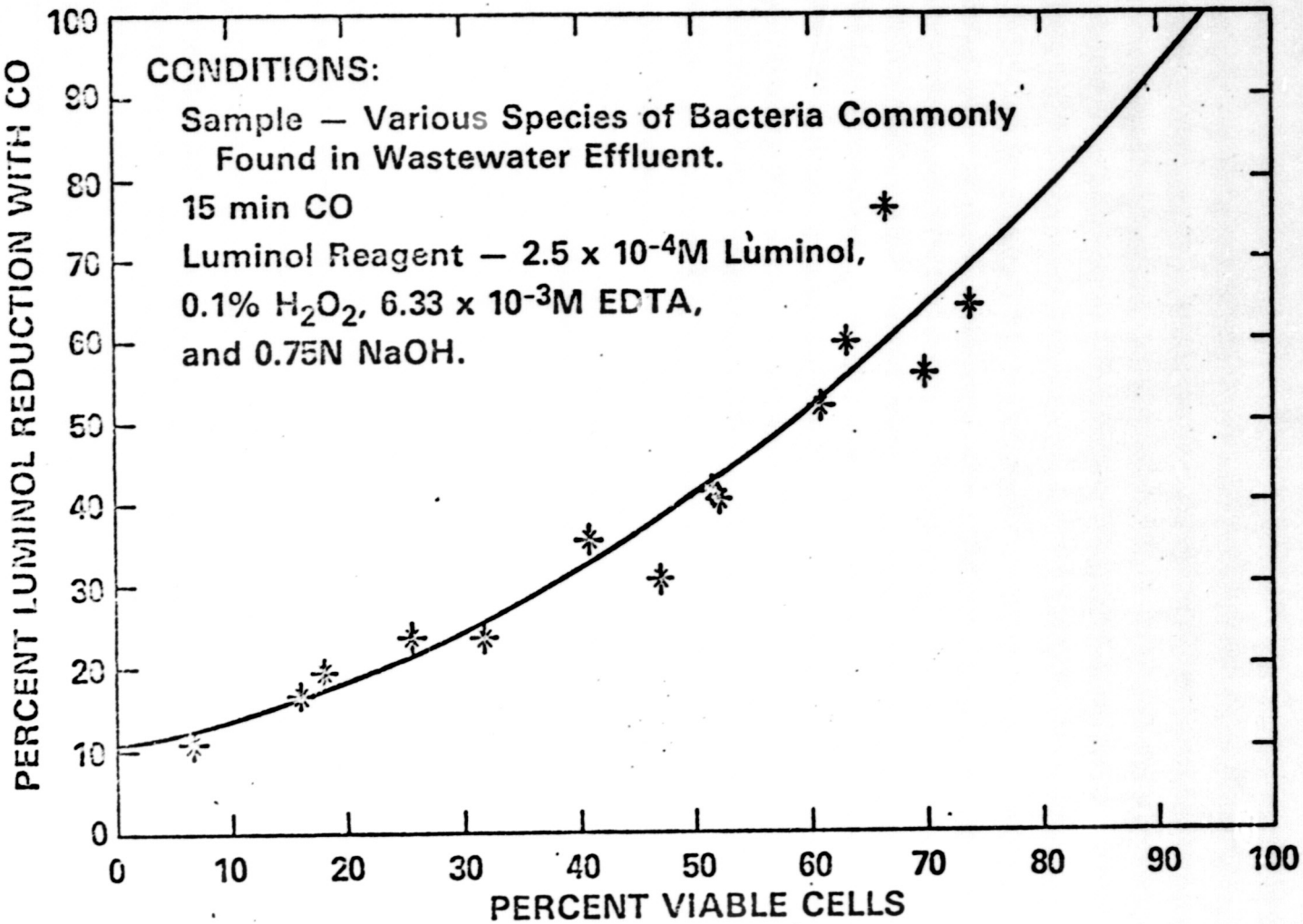
PERFORMANCE OF LUMINOL INTERFERENCE ELIMINATION METHODS

SAMPLE	CONDUCTIVITY, μMHO	UNTREATED SAMPLE, L.U.	H_2O_2 PRETREATMENT ONLY, L.U.	REACTION RATE ONLY, L.H.	BOTH LIGHT UNITS	BOTH PERCENT REDUCTION
TAP WATER	1000	41	3.5	2.4	0.7	98
REVERSE OSMOSIS EFFLUENT (TAP WATER SUPPLY)	100	17	2.1	0.8	0.3	98
DEIONIZED WATER	0.1	0.5	0.4	0.2	0.2	60
E. COLI IN DEIONIZED WATER	0.1	70	65	88	73	0

PERCENT LUMINOL REDUCTION WITH CARBON MONOXIDE VS. PERCENT VIABLE CELLS



MODEL CELLS AS THE BASIS FOR THE THEORY OF CARBON MONOXIDE REDUCTION OF LUMINOL RESPONSE FROM LIVE CELLS

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(All iron)

LIVE CELL

$Fe^{2+} \rightleftarrows Fe^{3+} + e^-$

reversible

Fe^{2+} $Fe^{3+} + e^-$

CO $Fe^{2+} \cdot CO$

$Fe^{2+} \cdot CO + \text{Luminol} \not\rightarrow h\nu$

DEAD CELL

$Fe^{2+} \rightarrow Fe^{3+} + e^-$

static (Fe^{3+} predominate species)

Fe^{2+} $Fe^{3+} + e^-$

CO $Fe^{3+} + CO$

$Fe^{3+} + CO + \text{Luminol} \rightarrow h\nu$