THE EFFECTS OF FLOW RATE-LAMP INTENSITY INTERDEPENDENCE ON DYE-SENSITIZED CONTINUOUS PHOTOCHEMICAL ANALYSIS: APPLICATIONS TO PROCAINE

A Thesis Presented to
the Faculty of the Department of Chemistry
College of Natural Sciences and Mathematics
University of Houston

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

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by
Joseph W. Mitchell
August 1975

To

Jame W. Mitchell

Father, Leader, and Friend

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ABSTRACT

The applicability of dye-sensitized continuous photochemical analysis (hereafter DCPA) to a new substrate is demonstrated. Data are presented on the conditions for the measurement of procaine using Rose Bengal as the dye-sensitizer. DCPA apparatus has been modified so that the effects of the flow rate-lamp intensity interdependence on the detector response can be measured using an intensity selection method which does not vary the filament temperature of the photolysis lamp(s). Effects of dilution on the detector response as a function of flow rate are determined. Substrates in DCPA are classified into two groups: 1. a class including nicotine, having no back reaction and being photon limited at high flow rates; 2. a class including procaine, having an interferring back reaction. Calibration curve for procaine at 50 ml/min and 750 W is linear, having a slope of 276 mAbs/m$\underline{M}$, while the calibration curve at 250 ml/min and 2250 W is nonlinear, yet having an approximate slope of 520 mAbs/m$\underline{M}$. Determination can be done from 4×10^{-5} $\underline{M}$ to 1×10^{-3} $\underline{M}$ original procaine concentration.

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CHAPTER I

INTRODUCTION

In recent years continuous analysis has become widely used and has been extensively reviewed (1,2,3,4). As indicated in the review articles, commercially available continuous analysis instruments are familiar items in clinical and analytical laboratories. In addition, the theory and application of photochemical reactions to analytical problems has grown considerably (5,6,7). Many photochemical reactions employing dyes as sensitizers are known (8,9,10). White and Fitzgerald paved the way for the work reported here by developing dye-sensitized continuous photochemical analysis (hereafter DCPA) (8,9,10). They have combined the available technology and advantages of continuous analysis systems with principles of photochemical analysis.

DCPA has been demonstrated as a technique applicable to several different compounds: ascorbic acid in orange juice (8), nicotine, caffeine, epinephrine, and indoles (9,10). In this previous work two dyes were used as sensitizers: methylene blue and Rose Bengal. In DCPA, the compound to be determined (hereafter substrate) is introduced into the flowing dye carrier stream and is then carried by the stream to the reactor zone where high intensity lamps excite the dye sensitizer. The excited state of the dye then reacts with the substrate and is reduced, producing a colorless or

leuco form of the dye and other reaction products. Under proper conditions the change in absorbance of the dye (Δ_{abs}) is proportional to the concentration of the substrate injected. Thus rapid, mechanized determination of the substrate is possible.

White and Fitzgerald have shown that the dye used, pH of the dye/substrate mixture, photochemical reactor temperature, dye/substrate reaction stoichiometry, reaction kinetics, flow rate, and lamp intensity are all variables affecting the extent of photobleaching of the dye (9). Of these variables, the number of photons available for the analytical reaction is a function of both the lamp intensity and the flow rate. The lamps used are "black body" sources, so that the wavelength of maximum emission is a function of the temperature of the lamp filament (11) which, in turn, is a function of the current passing through the filament. Varying the wattage applied to the lamp by changing the current or the voltage changes both the wavelength of maximum emission (λ_{max}) and the entire spectral emission. Thus the lamp intensity for DCPA is better adjusted by changing the number of lamps operated at fixed power rather than varying the voltage applied to one lamp. Increasing the intensity by turning on additional lamps increases the number of photons in the reaction zone without changing the spectral distribution of the exciting radiation.

The flow rate affects three things: 1. the dilution of the substrate; 2. reaction time, t_r, which is the time that the dye/substrate mixture is exposed to the light; 3. delay time, t_d, which is the time required for the dye/substrate mixture to travel from the reactor to the measurement device. Slower flow rates increase t_r and thus increase the number of photons available to excite the dye while the dye/substrate mixture is in the reactor. Thus if the reaction is limited by the concentration of the excited dye triplet, the extent of reaction would be increased by lowering the flow rate (or by increasing the lamp intensity as discussed above).

On the other hand, changing the flow rate changes the dilution of the substrate. At first glance one would expect the dilution to increase with flow rate if the syringe introduces the sample at a constant rate into the dye carrier stream. The sample would be mixed into a small length of the dye carrier stream at slow flow rates and a large length of the dye carrier stream at high flow rates. However, White and Fitzgerald found that the dilution is less at high flow rates than at low flow rates (9). There are two factors contributing to this dilution: 1. the mixing at injection; 2. diffusion broadening in the apparatus. By experimenting with the shape of the mixing "Tee", White and Fitzgerald empirically found that a "Y" shape produces

the least turbulence and dilution at injection (9) (the same mixing "Y" shape has been retained for this work). As the original dye/substrate plug moves through the reactor coils the substrate can diffuse out into the dye carrier stream, thus broadening the substrate "plug" and lowering the peak concentration. Then, as the triplet dye and the substrate react to form leuco dye, diffusion continues and lowers the peak concentration of the leuco dye. Thus by the time that the original dye/substrate mixture has reached the detector, the photolysis products have diffused and are considerably more dilute than was the substrate at injection. Empirical results show that the peak broadening due to diffusion is, indeed, greater at slow flow rates than at fast flow rates (9). This dilution then decreases the measured change in absorbance. For dye/substrate combinations there is one set of flow rate and lamp intensity values that produces the greatest photobleaching. These values represent a trade-off between the number of available photons and dilution.

A number of reactions can occur during DCPA (7,12,13); these may be generalized as:

$$D + h\gamma \xrightarrow{k_{ex}} D^*(s) \qquad 1.$$

$$D^*(s) \xrightarrow{k_{isc}} D^*(T) \qquad 2.$$

$$D^*(T) \xrightarrow{k_r} D \qquad 3.$$

$$D^*(T) + S \xrightarrow{k_{pr}} LD + \text{substrate oxid. prod.} \qquad 4.$$

$$LD + X \xrightarrow{k_b} D + \text{other products} \qquad 5.$$

$$D^*(T) + X \xrightarrow{k_p} LD + \text{other products} \qquad 6.$$

where D is the ground state dye, D*(S) is excited singlet of the dye, D*(T) is excited triplet of the dye, S is the substrate to be determined by DCPA, LD is the leuco dye (reduced form), and X is any substance present in the mixture which can react with the leuco dye to produce the colored ground state or which can react with D*(T) to yield LD. Reaction 5 is a negative interference and reaction 6 is a positive interference.

k_{ex} is the rate constant for excitation of the dye singlet, k_{isc} is the rate constant for intersystem crossing of the D*(S) to D*(T), k_r is the rate constant for the decay of D*(T) to D, k_{pr} is the rate constant for the photoredox reaction between D*(T) and S, k_b is the rate constant for the negative interference reaction, and k_p is the rate constant for the positive interference reaction.

Excitation of the dye to D*(S) is very fast; typically on the order of 10^{-15} sec (14). The quantum yield of intersystem crossing for xanthene dyes similar in structure to Rose Bengal indicate that it is favorable for fast and quantitative production of D*(T) (12,13,15). Thus the

concentration of D*(T) is a function of the number of photons available for excitation. As discussed above, this is related to both flow rate and lamp intensity.

From the reaction sequence it can be seen that the dye can be in three states at the measurement device: D, D*(T) and LD. The measured absorbance of the mixture is due to D only. When the dye solution flows through the reactor and is exposed to the light both D and D*(T) could be present at the spectrophotometer. The amount of D*(T) at the spectrophotometer is a function of flow rate, intensity, and triplet relaxation rate, k_r. At constant flow rate and intensity the concentration of D*(T) is at a steady state, being that amount which does not decay to the ground state in traveling from the reaction zone to the detector. LD is produced by reactions 4 or 6. The change in absorbance of interest is that caused by injection of S. This Δ_{abs} is due to that amount of D*(T) that is prevented from decaying to the ground state (reaction 3) by reaction 4.

From this we can see that the rates of reactions 1 through 6 can greatly affect the Δ_{abs} produced by a substrate. If reaction 4 does not go to completion within the time scale of the experiment, Δ_{abs} will be smaller. This can happen: 1. if k_{pr} is slow enough to prevent the completion of reaction, or 2. if the concentration of D*(T) is so low that the reaction can't go to completion. Reaction 3 can

affect the Δ_{abs} only if the reaction rate, k_r, is of the order of t_d, the delay time. k_r must be fast enough that D*(T) does decay prior to measurement of Δ_{abs}. Yet, if k_r is too fast, the amount of D*(T) present to react with the substrate is reduced. Thus, ideally, the concentration of D*(T) is large in the reaction zone and decreases to zero during the transportation from the reactor to the spectrophotometer. If reaction 5 proceeds to a measureable extent, it will decrease the Δ_{abs}. Thus, anything which reacts with the LD to produce D has a detrimental effect on DCPA. Conversely, if reaction 6 occurs significantly and X is contained in the dye stream the baseline absorbance will decrease. This can affect Δ_{abs} only if the concentration of D*(T) is lowered enough to slow reaction 4. If X comes from the sample, the Δ_{abs} will be increased, resulting in a positive error in the determination of substrate S.

The conditions to produce the largest Δ_{abs} for a given amount of sample are: concentration of D*(T) is sufficient to react with all of the substrate, k_{pr} is fast enough to insure complete reaction, k_r is just fast enough to insure that all unreacted D*(T) relaxes to D before reaching the detector, and k_b and k_p are as small as possible (preferably zero). Under these conditions Δ_{abs} depends on the stoichiometry of reaction 4. Flow rate and intensity are critical

variables because they control the concentration of D*(T) as discussed earlier.

The best DCPA results are obtained when two criteria are met (8,9,10): 1. instrument parameters are optimized to produce the maximum Δ_{abs} for a given sample concentration; and 2. Δ_{abs} varies linearly with the concentration of the sample injected. The variation of DCPA instrument parameters has the purpose of locating the values which produce the best analytical results. DCPA has been shown to be effective in the determination of nicotine, ascorbic acid, caffeine, epinephrine and indoles (8,9,10). The purpose of this work is to extend DCPA methods to other compounds, and, in the process of so doing, further characterize the influence of the instrumental parameters on the Δ_{abs} and sensitivity (slope of calibration curve). To this end several compounds are examined: tryptophan, procaine, and nicotine; and Rose Bengal is the dye-sensitizer used. Nicotine (9) and tryptophan (10) are included to provide a comparison with previous work. One goal is to identify the set of solution and instrumental conditions for the most sensitive measurement of these three substrates by DCPA. Also, it is planned to examine flow rate-intensity interdependence as mirrored in the Δ_{abs}, by using an intensity selection method which does not change the lamp(s) filament temperature.

CHAPTER II

EXPERIMENTAL

Apparatus

The apparatus used in this work is the same as that previously described (9,10) with several modifications designed to facilitate studies of flow rate-intensity interdependence. The flow rate of solutions through the system is controlled by a needle valve and measured by a "flotation ball" meter as previously described (8,9). The apparatus is improved for this work by pressure-sealing all connections so that flow rates from 30 ml/min to 360 ml/min can be routinely used. Previously the flow rate range was limited to between 75 ml/min and 250 ml/min. Use of higher flow rates increases the possibility of damaging the flow-through pH electrode (9). The present apparatus is constructed so that the electrode is removed when not in use. The photochemical reactor is similar to those used previously (8,9). However, there are two significant modifications in 1. the shape of the glass spirals through which the dye/substrate mixture flows; and 2. the number and placement of photolysis lamps. The current reactor, shown in Figure 1, consists of two concentric glass spirals constructed of 4 mm i.d. tubing. The dye/substrate mixture flows through the inner coil first, then passes to the outer coil. The total volume of both coils is 84 ml. The length of time required for the

dye/substrate mixture to travel through the reactor coils varies from 168 sec at a flow rate of 30 ml/min to 17 sec at 300 ml/min. The spirals are encased in a cylindrical glass water jacket through which water is passed. This serves to thermostat the reactor spirals at any temperature between 20°C and 50°C. Temperature control is ± 2°C.

The light sources are three Westinghouse DEC 750 W projector lamps, clamped base up, around the outside of the water jacket (see Figure 1). The lamps are positioned so that the intensity of each on the reactor coils is the same. In this work the wattage applied to the lamps is not changed; rather the number of lamps is switch selectable. This allows the selection of 750, 1500 or 2250 W with no change of the spectral emission of the lamps (11). The use of higher wattages dictates increased forced air cooling of the lamps. Two identical blowers (200 cfm) are used rather than only one used previously (9). The entire reaction zone is covered by a reflective sheet metal housing. This serves to channel air over the lamps, increase the intensity of light in the spirals via reflections, and protect the operator if a lamp shatters. A black cloth overcovering shades the operator from the intense light.

As in previous work (9,10) the detector is a Bausch and Lomb Spectronic 100 with digital readout to ± 0.001 A units (hereafter 1 mAbs).

FIGURE 1

TOP VIEW OF PHOTOCHEMICAL REACTOR

FOR DCPA

I: Inner spiral; 6.5 cm dia.

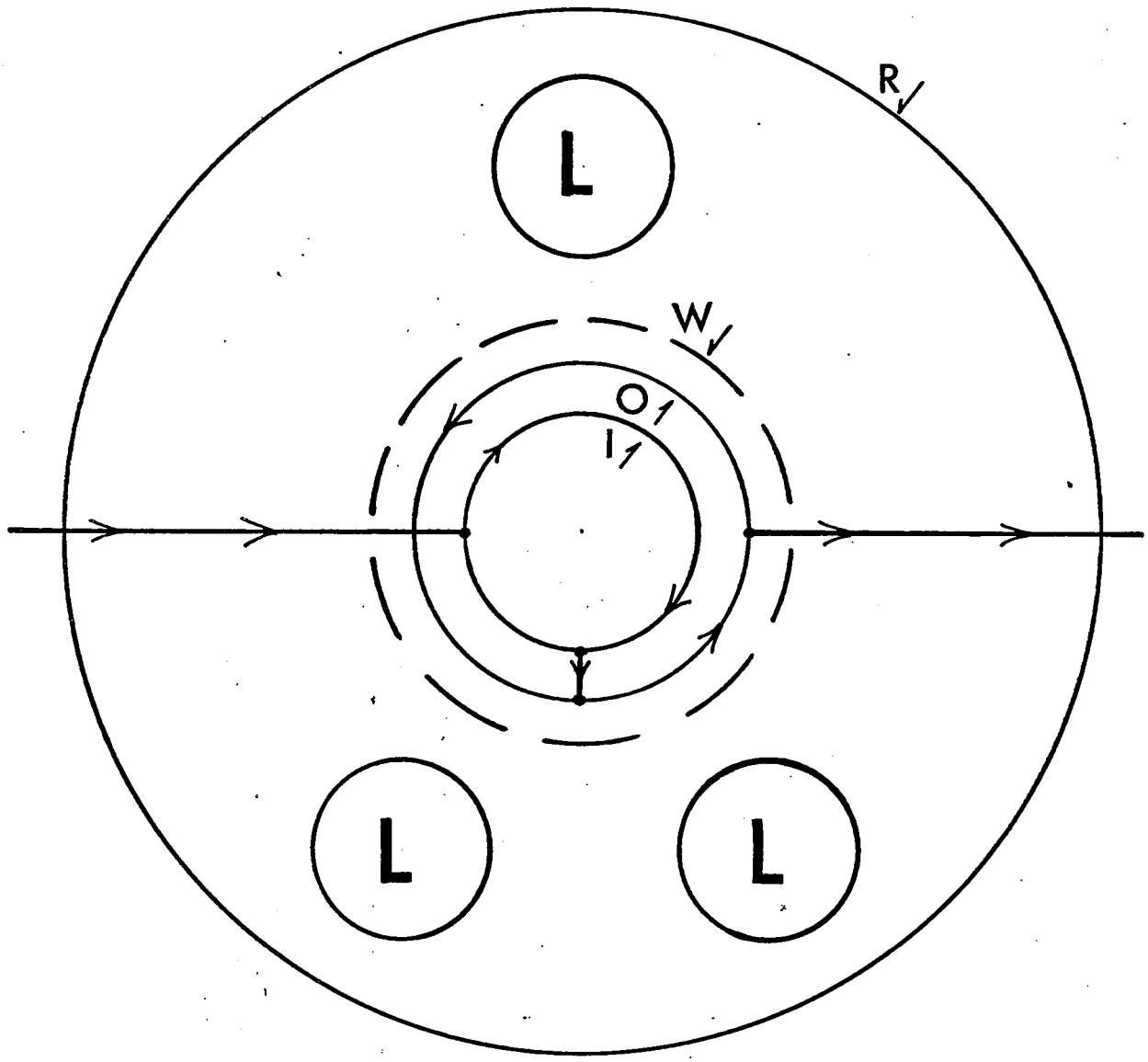
O: Outer spiral; 9.5 cm dia.

Volume of both coils: 84 ml. Arrows indicate direction of flow. Analyte enters top of inner coil, transfers to outer coil at bottom, passes to top of outer coil and exits water jacket. Photons transmitted by partially reacted solution in outer coil can pass into inner coil where analyte mixture has higher absorbance.

W: Cylindrical glass water jacket; 12 cm dia. x 13 cm. tall; water circulated through connectors not shown in diagram.

R: Reactor shield; reflective sheet metal.

L: 750 W lamps; one or any combination may be operated; air blowers below lamps not shown.


R
L
W
O
I
L
L

Reagents

Commercially available technical grade Rose Bengal (color index 45440; Eastman Organic Chemicals, lot 721-1UR) is used without further purification (9).

Dye concentration is about 3×10^{-5} M or 30 mg/liter. Dye reservoir pH is adjusted with NaOH or HCl as desired. The optimum pH for all substrates used here is 10.5 ± 0.3 and this pH is used for all work reported here. N_2 saturation of the dye solution is performed by passing compressed gas through it for at least one hour prior to use. The absorbance of the dye is approximately 1.5 with small variations from batch to batch and is near the upper limit of linearity for the spectrophotometer (9).

Substrates for this work are commercially available: nicotine (practical grade) from Eastman Organic Chemicals, procaine hydrochloride (lot 21C-1100) from Sigma Chemical Company, and DL-tryptophan from Eastman Organic Chemicals. All solutions are prepared in distilled water rather than deionized water for reasons previously discussed (9). 1.00×10^{-2} M stock solutions are prepared by weight and are known to be stable for long periods of time (16). Lower concentrations are prepared by quantitative dilution as needed. These are pH adjusted and N_2 purged for 45 min prior to use.

Procedures

Dye Photolysis. Measurement of change in baseline due to dye decomposition (Reaction 6) is performed as follows. The reservoir of dye is prepared at pH 10.2 and purged with N_2. The reaction zone temperature is held between 40°C and 43°C. The absorbance of the dye solution is measured at 0, 750, 1500 and 2250 W of lamp power at various flow rates, ranging from 30 ml/min to 300 ml/min in approximately 30 ml/min intervals. At each combination of flow rate and intensity the percent photolysis is calculated by the equation:

$$\% \text{ photolysis}_{@W} = \frac{Abs_{0W} - Abs_{@W}}{Abs_{0W}} \times 100\% \qquad 7.$$

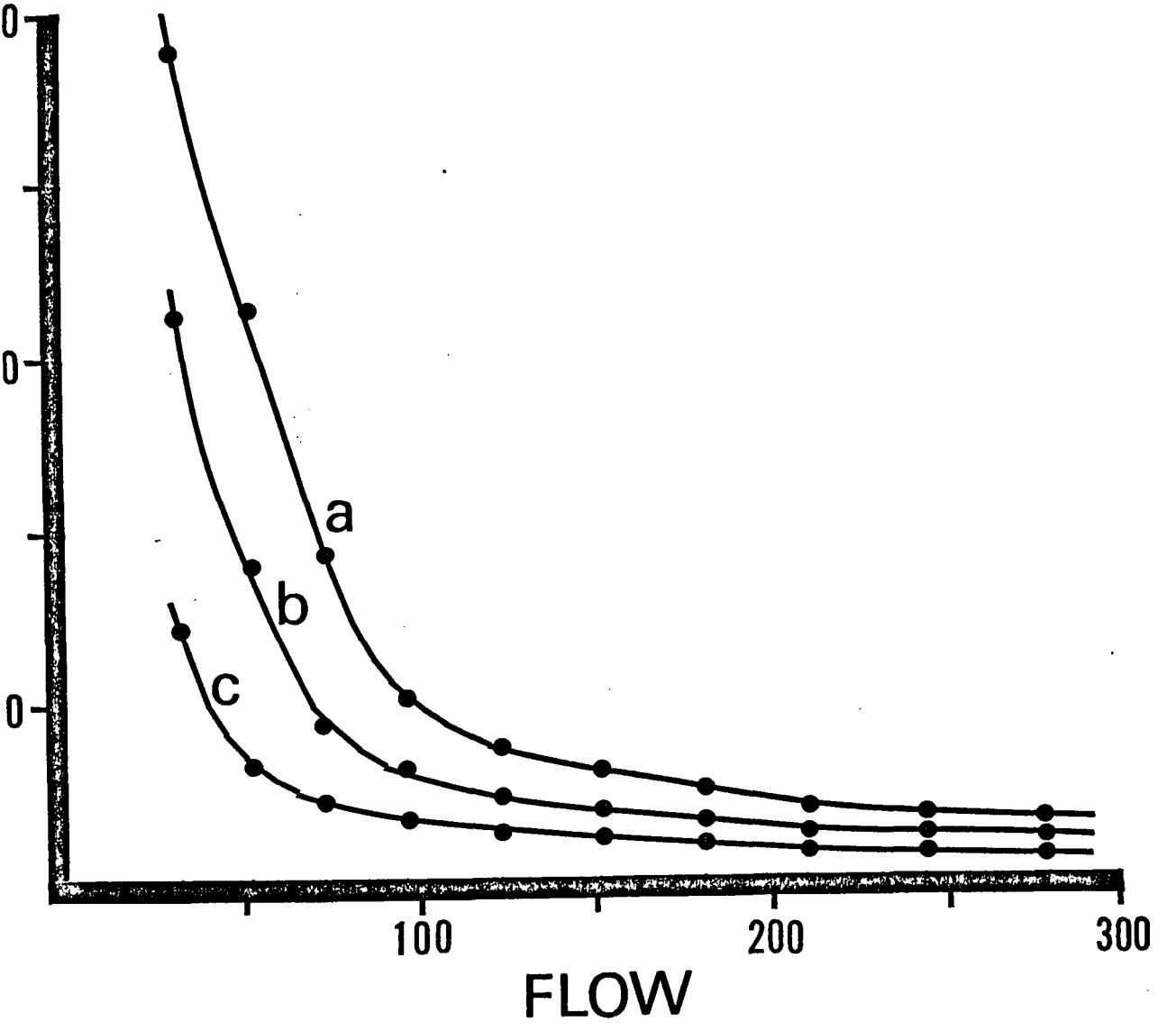
Figure 2 is a plot of percent photolysis versus flow rate at each of the three wattages.

Dilution Effects. The extent of substrate dilution is a function of the dye carrier flow rate (9). The dilution factors are measured as follows. All solutions are prepared in pH 9.6 ammonia-ammonium chloride buffer to insure constant ε of the dye. Stock dye solutions of 150 mg/liter and 75 mg/liter are prepared by weight. (Dye concentration used in DCPA is normally ca. 30 mg/liter.) These are quantitatively diluted to produce a series of known concentrations from 0.300 to 30.0 mg/liter. The static absorbance of these

FIGURE 2

PHOTOLYSIS OF ROSE BENGAL DYE CARRIER STREAM AS A FUNCTION OF FLOW RATE AND LAMP POWER

% Phot.:	Percent photolysis of dye; calculated using equation 7; see text for details
Flow:	Flow rate; in ml/min
Lamp Power:	a, 2250 W; b, 1500 W; c, 750 W


0
0
0
a
b
c
100
200
300
FLOW

of these solutions is measured to provide data for a Beer's Law calibration of the spectrophotometer. This Beer's Law plot is linear from 1.5 mg/liter (A = 0.044) through 30.0 mg/liter (A = 1.429) with a slope of 49.1 (mAbs-liter)/mg.

For the measurement of the dilution factors, the reservoir is filled with buffer and the spectrophotometer Abs set to 0.000. Then the stock dye solutions of 150 mg/liter and 75 mg/liter are injected into the flowing buffer stream. The peak Abs of the injected dye is measured at each flow rate and the dye concentration at the peak calculated from the previous Beer's Law calibration. From this data one can extract a dilution factor defined as:

$$\frac{1}{\text{dilution}} = \frac{\text{conc. dye injected}}{\text{conc. dye peak at spectrophotometer}} \qquad 8.$$

These measurements have been performed on two separate occasions and the averaged results are presented in Figure 3.

Flow Rate Intensity Δ_{abs} Profiles. 5.00×10^{-4} M nicotine, 1.00×10^{-3} M procaine, and 5×10^{-4} M tryptophan are prepared from stock solution and nitrogen purged. The optimum pH range for the four substrates used in this study, determined as previously described (9), is 10.5 ± 0.3. All solutions for this study are prepared in this range. Temperatures used for the reaction zone are 45-50°C for nicotine, 42-45°C for procaine, and 39-41°C for tryptophan.

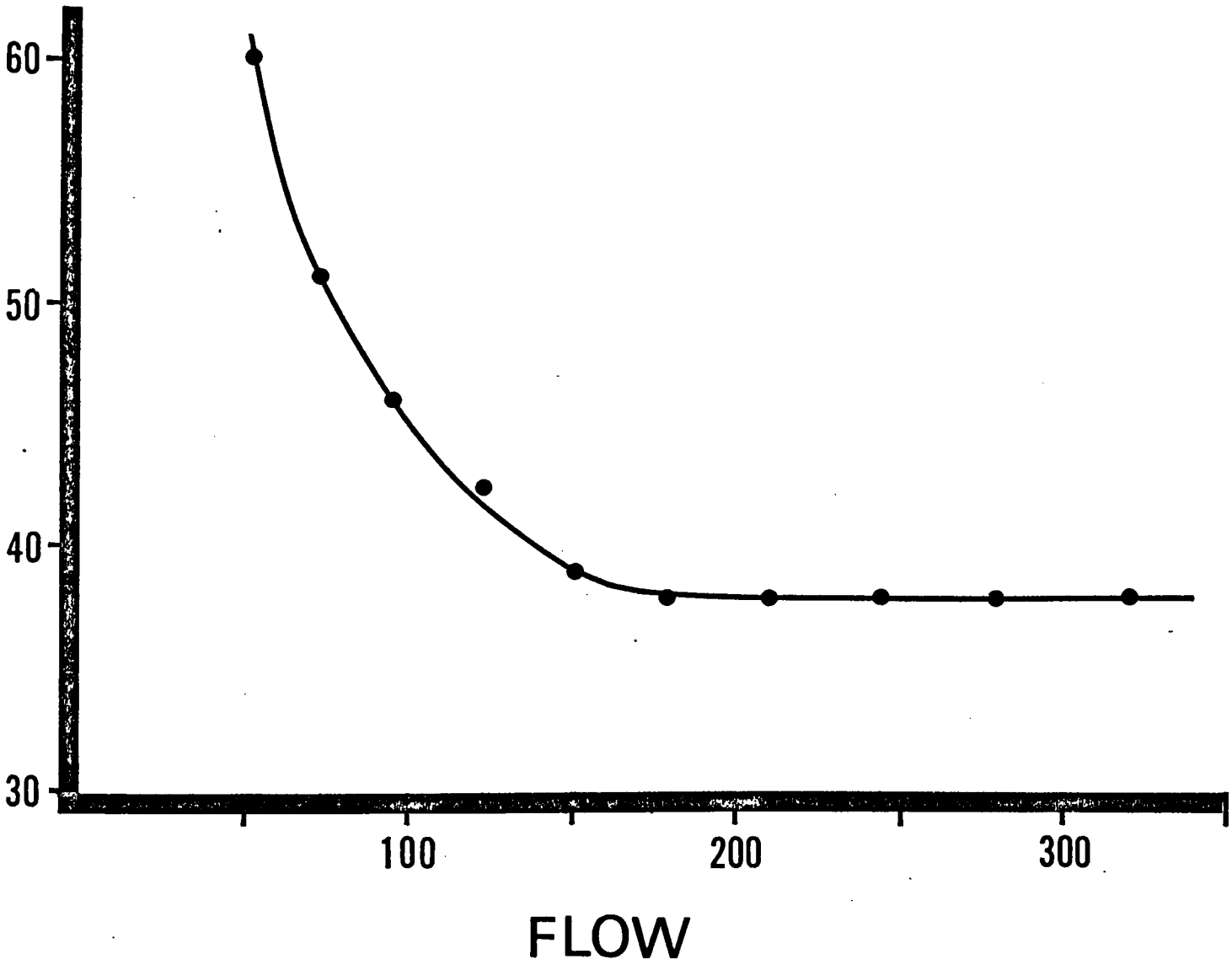
FIGURE 3

OBSERVED DILUTION OF INJECTED SAMPLE AS FUNCTION OF FLOW RATE

$(DIL)^{-1}$: reciprocal of dilution; calculated by equation 8; see text for details.

Flow: flow rate; in ml/min

Note that there is no change in sample dilution when injected into stream flowing at 175 ml/min or faster.


60
50
40
30
100
200
300
FLOW

Δ_{abs} is the absolute value of the difference between the absorbance of dye carrier stream and the absorbance of the reacted dye/substrate mixture, expressed in units of mAbs (9). Δ_{abs} is measured at a given flow rate with all three lamps operating (2250 W), next with two lamps operating (1500 W), and, finally, with one lamp operating (750 W). Then the next flow rate is set by adjusting the needle valve. The flow rate is varied from 30 ml/min to 300 ml/min in approximately 30 ml/min intervals. Figures 4 and 5 are plots of Δ_{abs} for nicotine and procaine versus flow rate at the three lamp intensities. Tryptophan data yields plots very similar to nicotine. They have the same shape. Maximum Δ_{abs} (260) for tryptophan occurs at 120 ml/min and 2250 W.

Temperature Dependence of Procaine. Previous work has shown that the temperature of the reaction zone has an effect on the Δ_{abs} produced from the reaction of the dye and the substrate (9). Conditions for the study of temperature effects are: 240 ml/min; 2250 W, based on the information in Figure 5. The Δ_{abs} produced by injecting 1.00×10^{-3} M procaine into the dye carrier stream is measured as a function of temperature from 28°C to 52°C (approximately 5°C intervals). Then Δ_{abs} at a given temperature divided by Δ_{abs} at 28°C is calculated. These vary smoothly from 1.00 at 28°C to 1.23 at 52°C, indicating that higher temperature favors reaction

FIGURE 4

NICOTINE-ROSE BENGAL PHOTOBLEACHING AS FUNCTION OF FLOW RATE AND LAMP INTENSITY

Δ_{abs}: Absolute value of the difference between the "baseline" absorbance of the dye carrier stream alone and the peak absorbance of the photoreacted dye/substrate mixture in mAbs units; see text for concentrations and conditions.

Flow: Flow rate of Rose Bengal carrier stream in ml/min

Symbols used for lamp power:

● , 2250 W; ■, 1500 W; ▲, 750 W

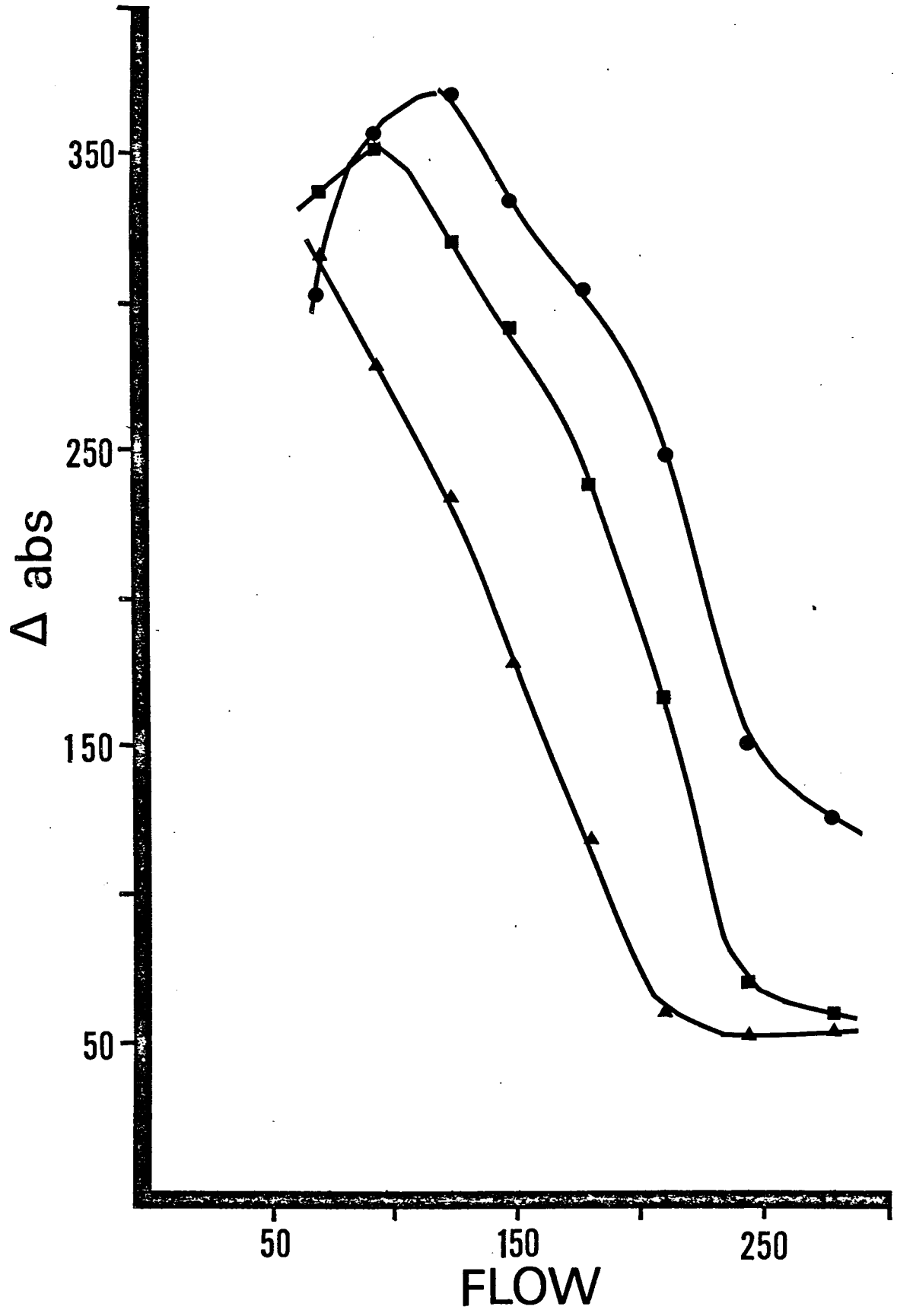
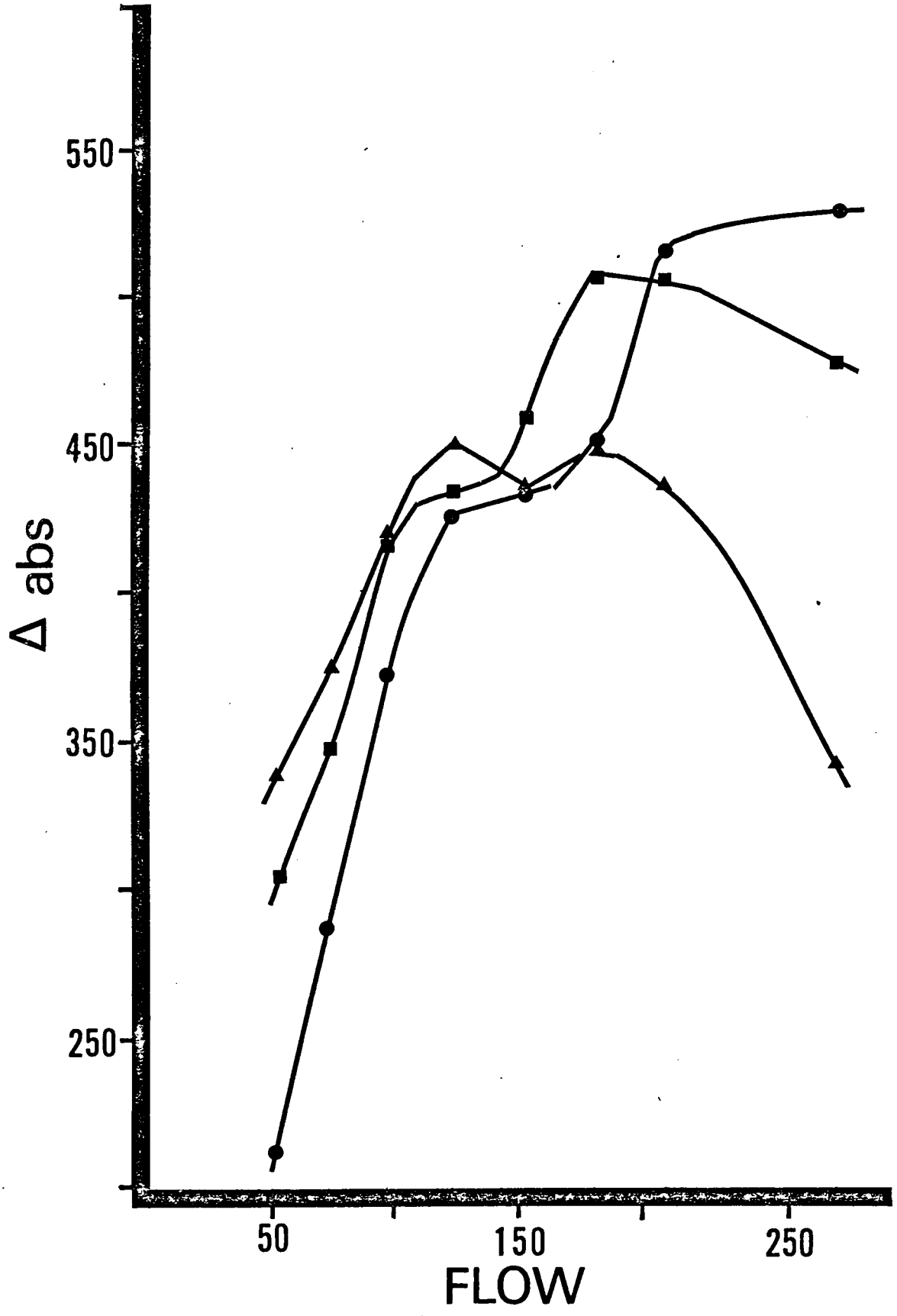

350
250
150
50
Δ abs
50
150
250
FLOW

FIGURE 5

PROCAINE-ROSE BENGAL PHOTOBLEACHING AS FUNCTION OF FLOW RATE AND LAMP INTENSITY

See Figure 4 for definitions and symbols. See text for concentrations and conditions.


550
450
350
250
Δ abs
50
150
250
FLOW

between the dye and the substrate to produce greater Δ_{abs}. The temperature dependence of procaine is thus similar to, but less than, that previously found for nicotine (Figure 2 reference 9). Nicotine data were rerun for the present study and previous results (9) were confirmed.

Calibration Curves. Calibration curves are prepared for nicotine and procaine as follows. The dye reservoir is prepared as described above. The procedures for obtaining the data for the calibration curve are the same as previously described (9). The Δ_{abs} is then plotted vs. the concentration of the substrate. The slope and statistical information are determined by least-squares for those substrates that are linear functions. Limits of detection are also determined.

Stop Flow Experiments. Post-photolysis reactions in the dye/substrate mixture can be measured by stopping the dye carrier stream while the mixture peak is in the spectrophotometer cell. This is accomplished by closing the flow rate control valve, which is located downstream from the spectrophotometer, and the valve at the dye reservoir when the dye/substrate mixture peak reaches the spectrophotometer. This traps the mixture there. When no substrate has been injected, the absorbance of the dye carrier remains constant when flow is stopped. When 5×10^{-4} M nicotine has been injected, the absorbance of the dye/substrate mixture decreases, causing an increase of approximately 10% of the Δ_{abs} produced

by nicotine, at the optimum conditions of analysis. For 1×10^{-3} M procaine, the absorbance of the dye/substrate mixture at the spectrophotometer increases, causing a decrease of the Δ_{abs} produced by about 7% at the optimum conditions for analysis. These findings will be discussed further in the next chapter.

CHAPTER III

RESULTS AND DISCUSSION

Apparatus

The improvements in the apparatus provide greater ability to evaluate the characteristics of the photochemical reactions. The use of three 750 W lamps instead of one 1000 W lamp (9,10) has the advantages of high total intensity and constant λ_{max} at all intensities. The reaction spiral now is longer and provides longer exposure, t_r, to the light. The wider range of available flow rates adds versatility. It is now possible to identify contributing factors to the behavior of DCPA under the various conditions now available with these instrumental improvements.

Flow Rate-Intensity Effects on Dye Carrier Stream

The concentration of D*(T) in the reaction zone is critical to the analysis of the substrate. If it is not sufficiently high, there will be incomplete reaction of the dye and the substrate, and Δ_{abs} will be too low. It is well established that excitation from ground state to the first excited singlet is rapid for molecules (14). While there is no published value for the fluorescence lifetime of Rose Bengal, eosin and fluorescein, which are similar in structure, have lifetimes of 4.7 and 6 nsec respectively (17). Thus it is expected that rose bengal has a fluorescence lifetime in the same range. Phosphorescence lifetimes have

not been found in the literature for any of the xanthene dyes. The time delay, t_d, between the reaction zone and the spectrophotometer is 7 sec at 280 ml/min. and our stop-flow experiments clearly show that there is no detectable triplet state of Rose Bengal reaching the spectrophotometer at this flow rate. Thus the triplet relaxation lifetime of Rose Bengal is less than 7 sec. However, it is observed that the light of excitation does cause decomposition of the dye. Apparently either an excited form of the dye or some impurity can decompose to decrease the measured absorbance. Figure 2 shows that this decomposition is directly propotional to the intensity of the light, which is a function of the number of lamps operating, and t_r, which is a function of the flow rate. Decomposition of Rose Bengal to the leuco form lowers the amount of dye triplet available to react with the substrate. This effect is most significant at slow flow rates and high lamp intensities and will be discussed further in conjunction with the corrected flow rate-intensity profile for nicotine.

Flow Rate-Intensity Effects on Nicotine and Similar Substrates

The flow rate-intensity profiles obtained for substrates (Figure 4 and 5) are biased by the fact that dilution is greater at slow flow rates, as shown by Figure 3. Hence the Δ_{abs} at slow flow rates does not accurately indicate the extent of photochemical reaction between the substrate and the

dye. Figure 3 is plotted in such a way as to emphasize that as the flow rate increases, the effect of dilution decreases. From 180 ml/min to 360 ml/min the dilution factor remains constant. Analytical sensitivity is inevitably lost for substrates measured at flow rates below 180 ml/min. By normalizing the dilution at each flow rate to the dilution at 360 ml/min, the flow rate intensity profile can be modified so that the data are corrected for the greater dilution at slow flow rates. All normalization factors have been calculated by:

$$N.F. = \frac{(\text{Dilution})^{-1}_{\text{at flow rate}}}{(\text{Dilution})^{-1}_{\text{at 360 ml/min}}} \qquad 9.$$

Because the dilution is constant above 180 ml/min, only the data below this flow rate require correction.

The compounds studied can be organized into two groups based on their flow rate-intensity profiles. Nicotine and tryptophan show the same behavior; while procaine is different from these. Nicotine represents this first group in Figure 4. Tryptophan produces very similar data but is not quite as sensitive. Data were given in the Experimental section. Examination of Figure 4 shows that at fast flow rates the reaction between the dye and nicotine is photon limited. At 280 ml/min, 750 W and 1500 W of light do not

excite enough dye to cause much reaction. 2250 W does produce a moderate Δ_{abs}. As the flow rate is decreased, the Δ_{abs} produced at each intensity increases. At these lower flow rates the dye/substrate mixture has a longer time of exposure to the light, t_r. Thus there are dye triplets to react with the substrate and more time is available for the reaction to proceed. Figure 4 clearly shows that flow rates above 120 ml/min would produce greater Δ_{abs} if more photons were available.

At flow rates below 180 ml/min the dilution increases dramatically, as shown in Figure 3. This manifests itself in Figure 4 where the decreases in Δ_{abs} at slow flow rates is due to dilution rather than a lack of photons. By applying the dilution normalization factor (Equation 9) to the data in Figure 4, one produces the corrected flow rate-intensity profile given in Figure 6. Figure 6 again shows that the reaction is photon limited at high flow rates. However, the greatest amount of photolysis now occurs at 70 ml/min and 1500 W, and it appears that slower flow rates would produce greater Δ_{abs} for the lower lamp intensities. This same phenomenon is observed when the tryptophan flow rate-intensity profile is corrected for dilution.

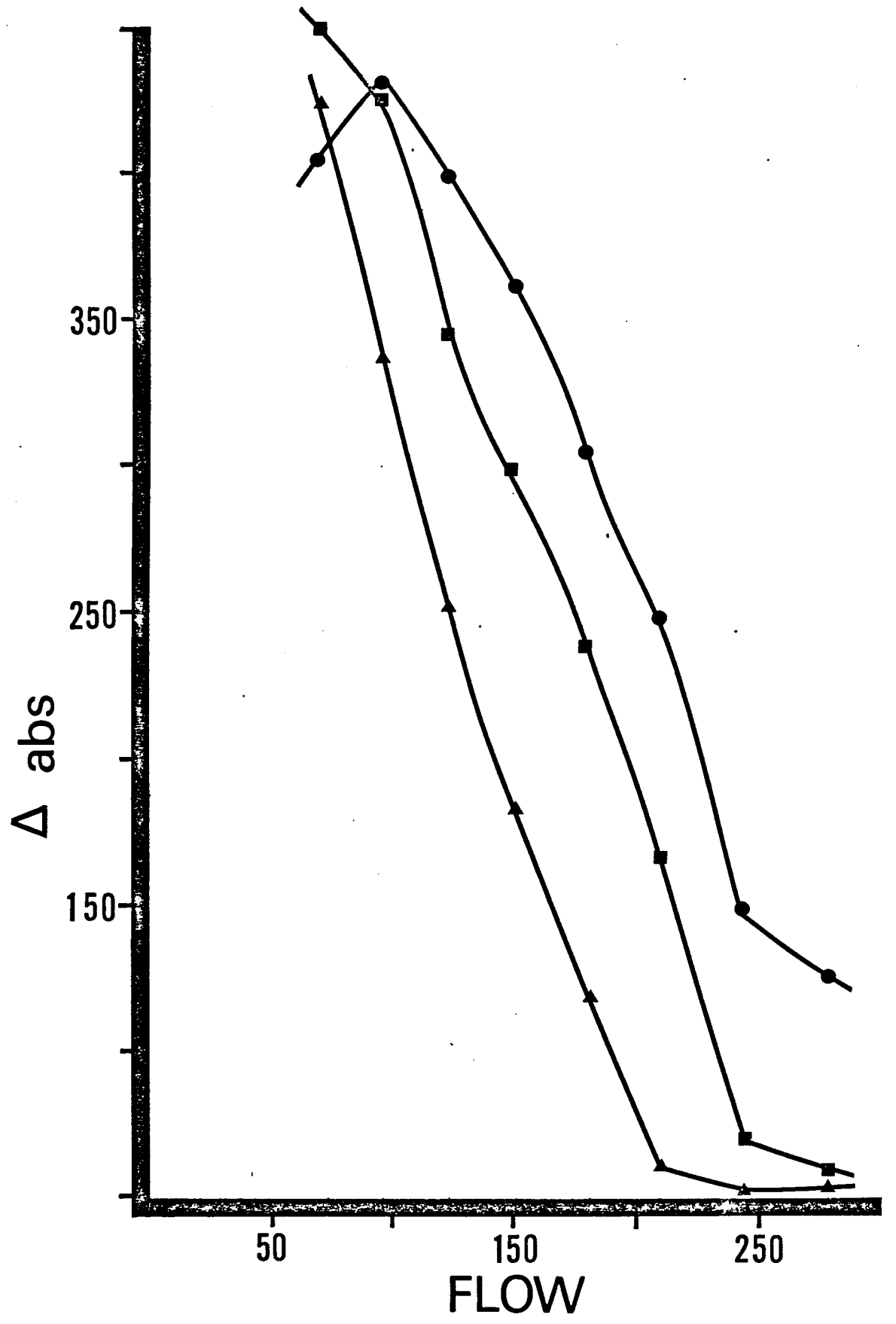
Note that at 70 ml/min in Figure 6, 2250 W produces a smaller Δ_{abs} than 750 and 1500 W. This phenomenon is due to

FIGURE 6

NICOTINE-ROSE BENGAL FLOW RATE-LAMP INTENSITY PROFILE NORMALIZED FOR DILUTION

Experimental photobleaching data from Figure 4; multiplied by appropriate normalization factor (Equation 9); see text for details.

See Figure 4 for other definitions.


350
250
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Δ abs
50
150
250
FLOW

the percent of the dye that is decomposed by the light. As shown by Figure 2, the percent of the dye decomposed at 2250 W and 70 ml/min is about 20%, which is at least twice as much as it is at 750 and 1500 W. This high decomposition of the dye lowers the concentration of D*(T) sufficiently so that the nicotine cannot react effectively. Then, the combination of lower lamp intensity and very slow flow rate provides enough photons for reaction of the dye/substrate mixture without the detrimental decomposition of a large portion of the dye.

While this normalized flow rate-intensity profile has provided information about the reaction between rose bengal and nicotine, the conditions that produce the best analytical sensitivity for nicotine are those that produce the greatest detector response and should be taken from the uncorrected flow rate-intensity profile. From the data in Figure 4, the greatest detector response for nicotine and tryptophan occurs at the tradeoff point between dilution of the dye/substrate mixture and number of available photons. Greater detector response could be obtained at higher flow rates if more lamp intensity were available.

Flow Rate-Intensity Effects on Procaine

The greatest photobleaching for procaine occurs at pH 10.5 $\pm$ 0.3 and reactor zone temperatures above 40^{o}C. In these respects it behaves similarly to nicotine. However,

the flow rate-intensity profile in Figure 5 is very different from nicotine and the other substrates. Maximum Δ_{abs} occurs from 200 to 300 ml/min and at 2250 W. Analytical determinations at flow rates faster than 250 ml/min are impractical because the dye/substrate mixture passes through the spectrophotometer too rapidly for the operator to read the display accurately. At slower flow rates the photobleaching drops off considerably, and the effect of lamp intensity becomes more complicated. At flow rates below 150 ml/min lower lamp intensities produce greater Δ_{abs} than higher lamp intensity.

At flow rates above 200 ml/min t_r is less than 20 sec. Under these conditions even the highest available lamp intensity does not excite enough of the dye to react with all of the nicotine present at 5×10^{-4} M. The fact that procaine requires such fast flow rates and high lamp intensities for maximum Δ_{abs} indicates that there is a complicating reaction that converts leuco dye back to ground state dye. The back reaction may even be photochemical since the lower lamp intensities produce greater photobleaching at slower flow rates.

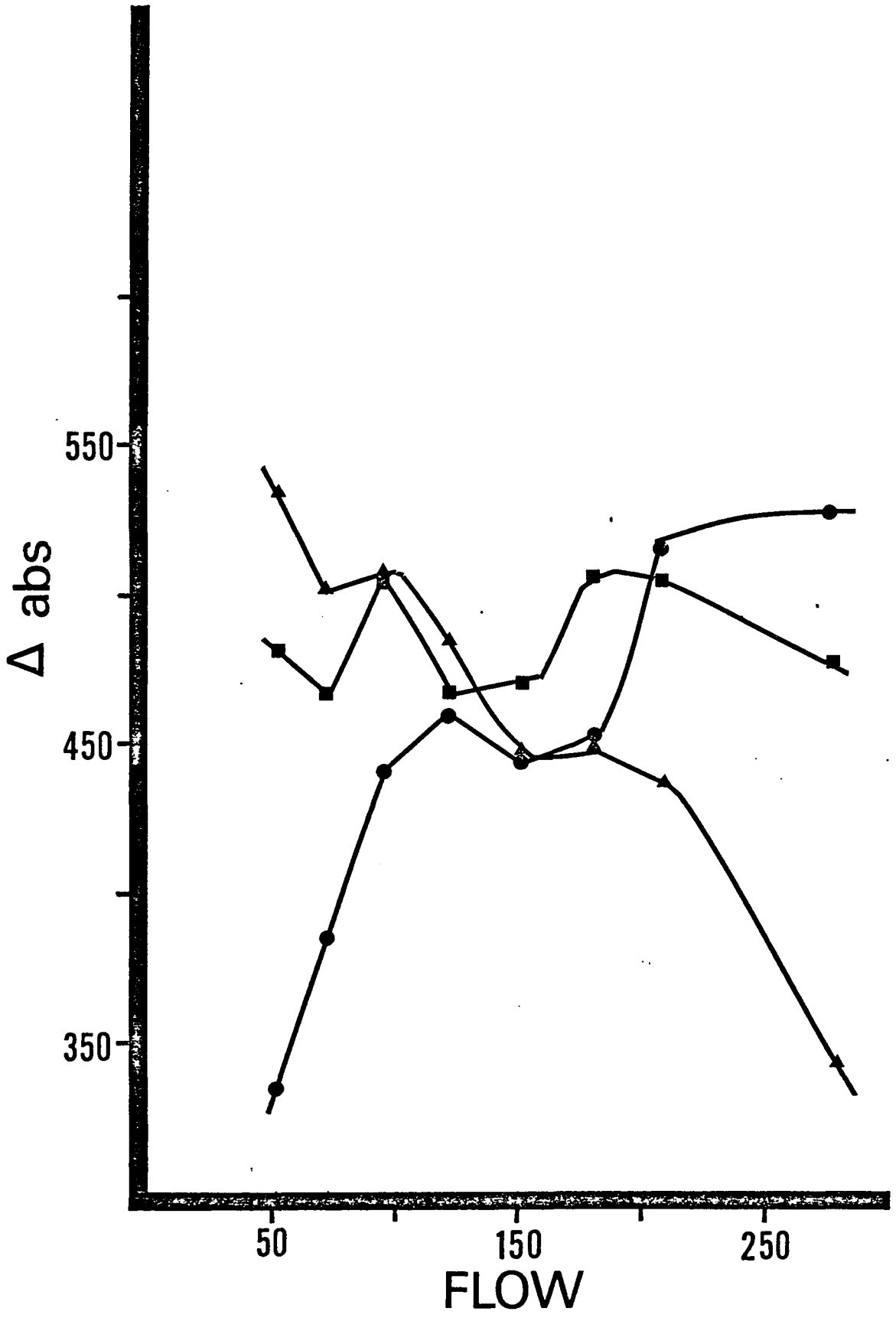
The photochemical back reaction is further substantiated by the data in the flow rate-intensity profile of procaine normalized for dilution (Figure 7). Photobleaching at 750 W is photon limited because the Δ_{abs} decreases with increasing

FIGURE 7

PROCAINE-ROSE BENGAL FLOW RATE-LAMP INTENSITY PROFILE NORMALIZED FOR DILUTION

Experimental photobleaching data from Figure 5; normalized in the same manner as Figure 6.

See Figure 4 for other definitions.


Δ abs
550
450
350
50
150
250
FLOW

flow. On the other hand at 2250 W increasing the flow rate increases the Δ_{abs}. It appears that, at slow flow rates, the highest lamp intensity causes the back reaction to proceed to a greater extent than does the low lamp intensity. Note that the photobleaching (corrected for dilution) at 750 W and slow flow rate is about the same as that at 2250 W and high flow rates. It should be emphasized again that the best analytical conditions are taken from Figure 5 and not Figure 7.

The existance of a procaine back reaction is also evinced in the stop-flow experiments, described in the Experimental section. When the Rose Bengal/procaine mixture peak is stopped in the cell of the spectrophotometer, the absorbance gradually rises (7%). This has the effect of decreasing the Δ_{abs} produced by the procaine. This should be contrasted with the behavior of nicotine, which shows a gradual decrease (10%) in absorbance when the dye/substrate mixture is stopped in the spectrophotometer cell. When the stop flow experiment is performed without procaine or nicotine present, the absorbance of the dye in the spectrophotometer remains constant. The interference reaction follows the reaction between D*(T) and procaine (Equation 4) and is represented by Equation 5 in the Introduction. The greatest sensitivity, i.e. largest Δ_{abs} for procaine, occurs under the conditions where Reaction 4 is maximized and

Reaction 5 is minimized. From the flow rate-intensity profile (Figure 5) it is seen that the best sensitivity is obtained at flow rates above 200 ml/min and at 2250 W. The dye/procaine mixture is rushed through the reactor as fast as possible to minimize the back reaction and requires maximum available light to excite the dye for the desired reaction.

Analytical Methods

Analytical results for nicotine by DCPA have been published previously (9). The conditions for determination reported previously are comparable to the best conditions for determination with the present apparatus. The greatest sensitivity for nicotine presently is obtained at: flow rate 150 ml/min, temperature above 40^{o}C, 2250 W of lamp power, and pH 10.5. The flow rate here is faster than the 91 ml/min reported by White and Fitzgerald (9). This is attributed to the fact that much more lamp intensity is available in the present apparatus than previously. Thus the reaction becomes photon limited at high flow rate now than in earlier experiments. The slope of the calibration line is 473 mAbs/mM. This compares very well to the results published previously (9).

Tryptophan, which behaves like nicotine, has also been determined by DCPA. The instrumental conditions and statistical data for the analysis of tryptophan as well

as other indoles have been reported previously (10). The fairly small slope limits the sensitivity for analysis.

Procaine measurement is not so straightforward as nicotine and tryptophan due to the interference back reaction. From the data in Figure 5, we predicted that the sensitivity of the calibration for procaine at high flow rate would increase with increase in lamp wattage. On the other hand, the sensitivity at low flow rate is predicted to be higher for low lamp intensity than high lamp intensity. It is gratifying that these predictions are born out by the data in Figure 8. The greatest sensitivity is obtained at 250 ml/min and 2250 W. However, this is not a straight line, even though it is a useful working curve with a limit of detection (10) of 4.00×10^{-5} M.

The shapes of the curves at 250 ml/min indicate that the higher concentration samples do not react completely with the dye. This phenomenon may be due to one or both of two factors. First, the flow rate is very high and the dye/procaine mixture for high concentrations may not have enough time ($t_r + t_d$) to react completely before reaching the spectrophotometer. Thus Δ_{abs} would be lower than expected for the high concentrations. Second, by Le Chatelier's principle, the interference back reaction producing ground state dye from leuco dye (Reaction 5) proceeds faster at high procaine concentration simply because greater amounts of

FIGURE 8

PROCAINE DCPA CALIBRATION CURVES

Δ_{abs}: See Figure 4

Conc.: Molarity of injected procaine x 10^4

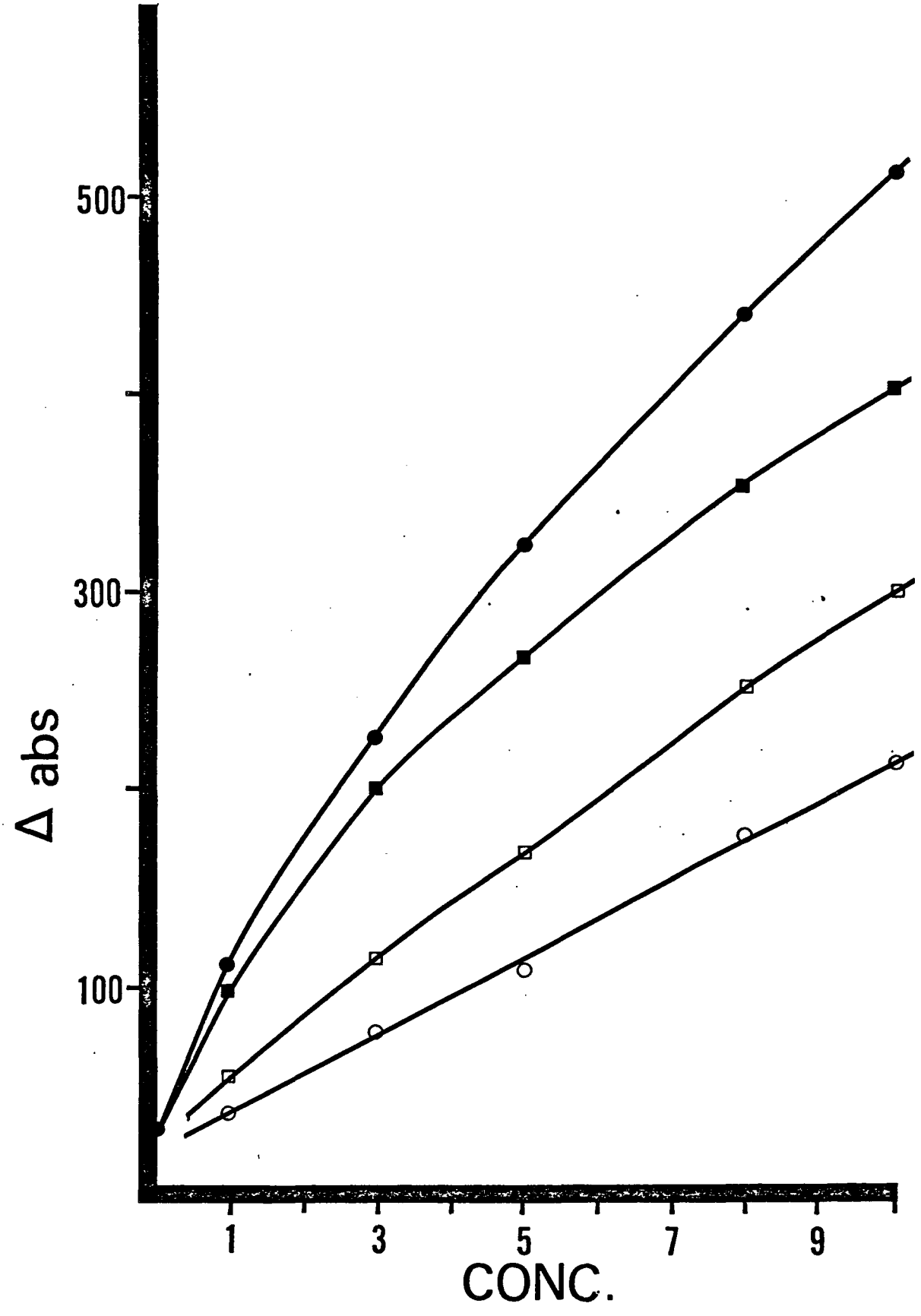
Data obtained at following conditions:

●, 2250 W @ 250 ml/min

■, 750 W @ 250 ml/min

□, 750 W @ 50 ml/min

○, 2250 W @ 50 ml/min


500
300
100
Δ abs
1
3
5
7
9
CONC.

leuco dye are produced (Reaction 4). The calibration curves at low flow rate (50 ml/min) are more nearly linear than the calibration curves at high flow rate (250 ml/min). As predicted by Figure 5 the 750 W data has a greater slope than the 2250 W data at slow flow rate and both have a slope considerably lower than the high flow rate curves. Thus sensitivity is lost but linearity is gained by operating at low flow rates. The linear regression analysis of the calibrations at slow flow rates are:

1. 2250 W: slope is 198 mAbs/m$\underline{M}$, standard error the estimate is 3.1 mAbs, correlation coefficient is 0.9986;
2. 750 W: slope is 276 mAbs/m$\underline{M}$, standard error of the estimate is 2.6 mAbs, correlation coefficient is 0.9996.

CHAPTER IV

CONCLUSIONS

This work has demonstrated the applicability of DCPA to a new substrate. Procaine can be added to the list of those previously reported (8,9,10). The apparatus modifications have increased the ranges of lamp intensity and flow rate available. The effect of lamp intensity on DCPA is better measured with three independent sources, rather than varying the power applied to one source (9). In this work the flow rate-intensity profiles of nicotine and procaine have proved valuable for characterizing the nature of the photochemical DCPA reaction. To date, two classes of behavior have been identified for the substrates. The first class, represented by nicotine in this work, has a straightforward reaction scheme, and greatest sensitivity is obtained at the trade-off of flow rate-intensity conditions where the dilution is minimized and the number of photons is maximized. The second class is represented in this work by procaine. Examination of previous DCPA data indicates that Methylene Blue/caffeine (9) and Rose Bengal/5-hydroxy-indoleacetic acid (10) can also be included. This class has a more complicated DCPA reaction scheme that includes a reaction converting leuco dye back to ground state dye. In this case, greatest sensitivity at a given flow rate is obtained with intensity such that the reaction to produce

the leuco dye is maximized and the back reaction is minimized, i.e. for procaine, high intensity at fast flow and low intensity at slow flow. However, if a means can be found to block or inhibit the interfering back reaction chemically, then even greater sensitivity for this class of compounds should be obtained.

The dependence of dilution on flow rate complicates determinations. This is particularly evident with substrates like nicotine where sensitivity is lost at slow flow due to dilution. Replacement of the present motor-driven syringe injector with a segmented sample carrier system such as used in commercial automatic analyzers (4, pp. 117-123) would allow the dye/substrate mixture to pass through the reactor with a minimum of dilution whose value is independent of flow rate. Sensitivity could be increased about fifty fold by such a modification.

The power requirements of the light source have reached a practical limit at the 2250 W presently used. Further increasing the wattage is not feasible for several reasons: 1) there physically is not room for more lamps; 2) the cooling capacity for the lamps is also limited by space, and the blowers cannot handle an increase in wattage; 3) such high power usage is uneconomical. It actually would be advantageous to reduce the power requirements of the photolysis source to increase the practical utility of the

apparatus. To this end the segmented sample injector would, again, be helpful.

Further improvement of the apparatus can occur if the reactor spirals are lengthened and if the time delay (t_d) from the reactor zone to the spectrophotometer can be varied without increasing diffusion broadening of the substrate. Lengthening the reactor spirals would provide more exposure to the light and increase the amount of D*(T) present to react with the substrate. Substrates like nicotine which are photon limited at fast flow rates could be exposed to the light longer, and thus measurement could be done at faster overall flow rates. If the distance from the reactor zone to the spectrophotometer were lengthened for nicotine to allow for complete reaction, or shortened for procaine to minimize the interference back reaction, greater sensitivity would be obtained. Thus a variable distance, which would vary t_d, from the reactor to the spectrophotometer is desirable.

The decomposition of Rose Bengal when exposed to the lamps is a disadvantage. Rose Bengal is not available in high purity and cannot be easily purified (9). It is uncertain at present whether the decomposition of the dye is due to impurities present or is a property of the dye itself. As indicated by Herkstroeter, et al. (7), the photochemical

stability of the dye used as a sensitizer is critical. Rose Bengal has limited usefulness because of its photochemical decomposition. In future DCPA experiments dyes without this drawback should be investigated.

It is hoped that this work adds to the knowledge of the utility of photochemical methods of analysis and encourages their further applications to the solution of problems in both clinical and analytical chemistry.

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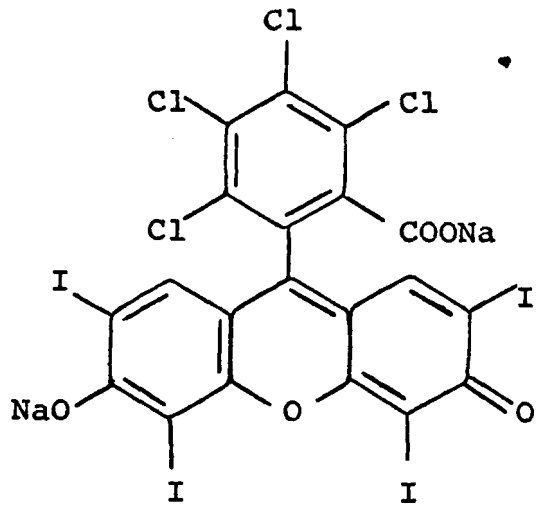
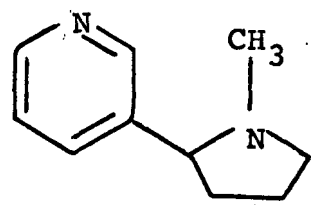
APPENDIX

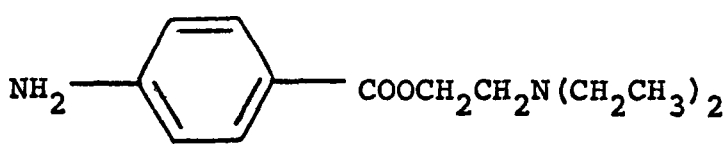
STRUCTURES OF COMPOUNDS



ROSE BENGAL



NICOTINE



PROCAINE