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7/25/68

BIODEGRADATION OF ANTHRAQUINONE DISPERSE DYES

A THESIS

Presented to

The Faculty of the Graduate Division

by

Stancil Driftwood Powell

In Partial Fulfillment

of the Requirements for the Degree

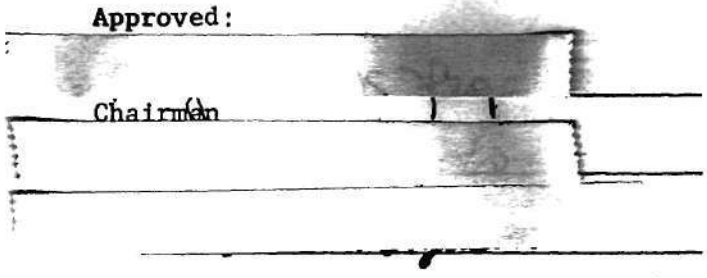
Master of Science

in the A. French Textile School

The Georgia Institute of Technology

September, 1969

BIODEGRADATION OF ANTHRAQUINONE DISPERSE DYES



Date approved by Chairman: 9-23-69

DEDICATION

To my wife, Diane, whose love and support made the completion of this thesis possible.

ACKNOWLEDGMENTS

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SUMMARY

This thesis investigation showed that three anthraquinone disperse dyes, Disperse Violet 1 (C.I. 61100), Disperse Blue 3 (C.I. 61505), and Disperse Blue 7 (C.I. 62500), were partially metabolized by bacteria normally present in domestic activated sludge. A fourth dye, Disperse Red 15 (C.I. 60710), was left unchanged by the sludge. The metabolites (isolated via thin-layer chromatography) were found to be derivatives of the original dyes. No evidence of anthraquinone ring degradation was detected. The toxic effect of the dyes on the bacteria in the sludge is discussed. Also, the effect of the low water-solubility of these dyes on the rate of metabolism is discussed. Metabolic pathways are suggested.

CHAPTER I

INTRODUCTION

Statement of the Problem

The rapid expansion of the textile dyeing and finishing industry and its concentration in specific stream basins has emphasized the importance of determining the fate of complex auxiliary chemicals and dyes used in the process, and present in the effluent, when subjected to conventional waste treatment processes. As a result of the increasing usage of hydrophobic fibers such as cellulose acetate, nylon, and polyester, that class of dyes known as disperse dyes has become of increasingly greater importance. In the dyeing process when the ratio of dye weight to fiber weight increases, the per cent dye exhausted from the dyebath decreases. Therefore, the amount of unreacted dye, discharged as waste, increases. This is generally true for most classes of dyes, but particularly true of disperse dyes. It has been estimated that 17,000 pounds per week of dyes are discharged as spent dyebaths into the streams of the Coosa River Basin alone (1). With this magnitude of unreacted dye discharged into the streams, coloration is inevitable unless preventive measures are taken.

More important than the color of the streams is the effect of the dye upon the ecology of the streams and upon the human body which may indirectly ingest these dyes. It is possible that discharged dyes may, in the presence of dissolved oxygen, sunlight, bacteria, and other

chemicals, degrade into other compounds which may, or may not, be toxic.

Disperse dyes generally fall into two chemical classifications as illustrated by the azo and the anthraquinone types. This thesis investigation is concerned principally with the anthraquinone class.

It is possible that anthraquinones might be degraded by bacteria. In a sense anthraquinones are ketones, and it is known that some aliphatic ketones are readily attacked by bacteria. Acetone, the simplest ketone, is readily digested. Some sugars are cyclic ketones and are readily digested.

If anthraquinones could likewise be degraded, they could be submitted to treatment by activated sludge in a conventional waste treatment plant before discharging into the streams. One serious drawback to this possibility may be the extremely low solubility of anthraquinone disperse dyes. Their solubility is of the order of magnitude of one milligram per liter (mg/l). This low solubility may lower the rate of degradation. It is hoped, however, that adsorption of the dyes onto the surface of the sludge might counteract this problem.

Purpose of the Research

It is the purpose of this research to determine whether the bacteria normally present in domestic sludge can effectively degrade anthraquinone disperse dyes, and if they can be degraded, how efficiently they are degraded. Also, an attempt will be made to characterize the degradation pathway and to give evidence as to what the products of degradation are.

Survey of the Literature

Certain naturally occurring anthraquinones have long been used as stimulant cathartics. This group is known as the anthracene or emodin cathartics and the principal agents of the group are cascara sagrada and senna. The major constituents are anthraquinone or emodin (1,3,8-trihydroxy-6-methyl-anthraquinone) (2). Emodin has a structure which is similar to some of the disperse dyes used in the textile industry.

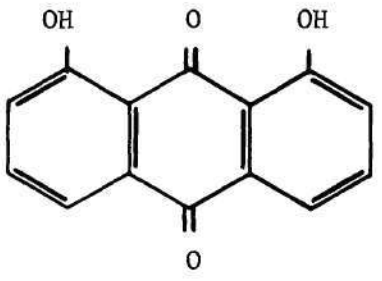
Sims (3) found that anthraquinone was metabolized by rats to 2-hydroxy-anthraquinone. This work is in agreement with work done earlier by Sato, Fukuyama, Yamada, and Suzuki (4), who administered anthraquinone orally to rats and isolated 2-hydroxy-anthraquinone and traces of 1-hydroxy-anthraquinone from the urine.

Makarenko (5) studied the effects of 1-amino-anthraquinone on rats and guinea pigs. He administered 1000 mg/kg per day for 14 days and found a host of changes including changes in behavior, weight loss, hypohemoglobinemia, increase in leukocytes in peripheal blood, increase of excitation threshold, and increase in oxygen demand.

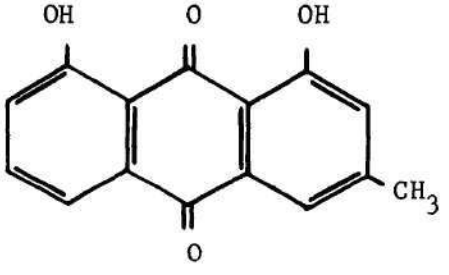
Anthraquinones have been studied for their medicinal value other than for cathartics. Sery and Furgiuele (6) tested a range of chemicals for inactivation of the herpes simplex virus and found the derivatives of quinones to be the most effective. Adamov (7) discovered that naphthoquinone, anthraquinone, alizarine, and 1-amino-anthraquinone were capable of absorbing immune antibodies and resisted desorption by weak acid and alkali. Saki, Minoda, Saito, and Fukuoka (8) tested a variety of chemicals in the treatment of tumors in mice. They tested 1,2,3,5,6,7-hexahydroxy-anthraquinone at a concentration of 0.05 per cent and found

it ineffective. Wittoesch et al. (9) in a general review and study of over 800 cases observed a melanotic pigmentation of the colonic mucosa where anthraquinone cathartics have been taken over extended periods of time. They also noted that the pigmentation was benign and reversible after a period of 4 to 12 months. Thirty new workers in the chemical industry were skin tested for latent sensitivity to anthraquinone and other chemicals by Lutowiecki (10). Six showed latent sensitivity. Examinations one year later for toxic damage or allergic eczema showed six severe and four mild cases.

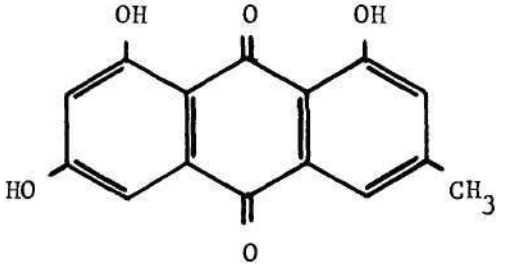
An interesting characteristic of anthraquinones is that they have been found to complex with deoxyribonecleic acid (DNA) of the cell. Swanbeck (11) noted that chrysizan, chrysophanic acid, dithranol, and emodin all complex with DNA in solution by hydrophobic bonding as indicated by a 30-40 mu shift to the red in the visible spectrum (See Figure 1). Later Waring (12) found that certain anthraquinones combined with DNA of the cell disrupting the helical structure of the molecule. This finding has been a basis for a theory for mechanisms of drug actions.



Chrysazin (1,8 - dihydroxy-anthraquinone)



Chrysophanic Acid (3-Methyl-1,8-dihydroxy-anthraquinone)



Emodin (3-Methyl-1,6,8-trihydroxy-anthraquinone)

Figure 1. Structure of Some Naturally Occurring Anthraquinones

CHAPTER II

INSTRUMENTATION AND EQUIPMENT

Activated sludge was used as the medium for biological degradation. Domestic sludge was collected from the aeration tanks at the Atlanta South River Treatment Plant.

A static system in which the sludge was contained in one gallon jars was chosen rather than a continuous system to avoid working with large volumes of effluent. A stream of air was introduced at the bottom of the jar to supply oxygen and keep the sludge in continual motion.

The visible spectra of the effluent, initial dyes, and isolated metabolites were monitored on a Beckman DB-G Grating Spectrophotometer with recorder.

Thin layer chromatography was used to isolate metabolites. Plates were prepared using a Desaga spreader.

Infrared spectra of the original dyes and metabolites were obtained using a Beckman IR-10 recording spectrophotometer. Samples were run in potassium bromide pellets.

Measurements of pH were made using a Corning Model 12 pH meter.

CHAPTER III

PROCEDURE

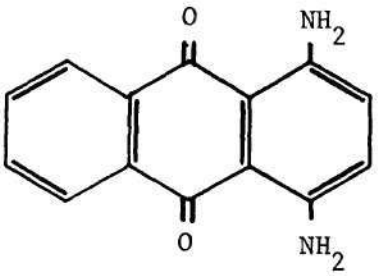
Choice of Dyes

The dyes chosen for this study were selected for three reasons. They have known chemical structures, are of commercial importance, and together, represent a good range of colors. They are Disperse Red 15 (C.I. 60710), Disperse Violet 1 (C.E. 61100), Disperse Blue 3 (C.I. 61505), and Disperse Blue 7 (C.I. 62500). Their chemical structures are shown in Figure 2. These dyes were originally developed for dyeing cellulose acetate. They are also used on polyester and other fibers. It can be seen that they are all either amines or amine derivatives of anthraquinone. The amine group is not normally found on naturally occurring anthraquinones and may therefore be potentially toxic to bacteria.

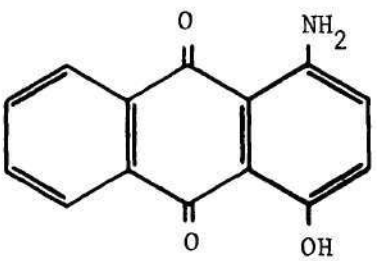
Separation of Dyes from Dispersants

Because of their very low water solubility disperse dyes as marketed contain up to 50 per cent of a dispersing agent to hold them in suspension. The dispersing agent is usually a long chain molecule with one end having an affinity for water and the other having an affinity for the dye. It was felt that to study the effect of bacteria on these dyes, more exactly, the dispersants should be removed.

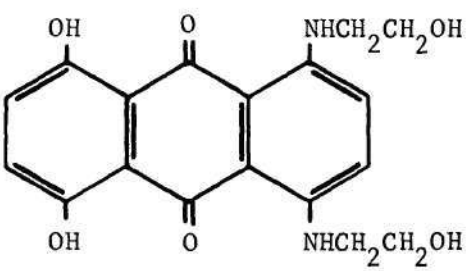
Five grams (gm) of the commercial dye were dissolved by boiling in 500 milliliters (ml) of 95 per cent ethyl alcohol in a 1000 ml



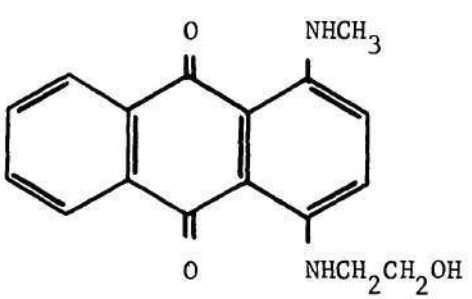
Disperse Violet 1 (C.I. 61100)



Disperse Red 15 (C.I. 60710)



Disperse Blue 7 (C.I. 62500)



Disperse Blue 3 (C.I. 61505)

Figure 2. Structures of Dyes Submitted to Degradation

Erlenmeyer flask. The hot liquor was filtered immediately through a Buchner funnel. This removed the dispersing agents and any fillers present. The ethyl alcohol was then evaporated on a steam bath using a Rotavapor vacuum distillation aparatus. The dye was redissolved in 750 ml of methanol, transferred to a four liter Erlenmeyer flask, and boiled one minute with one and a half liters of distilled water. Distilled water was added slowly until precipitation occurred. The flask was allowed to cool overnight wrapped in tinfoil in the dark. Later, the crystals were collected on a Buchner funnel and washed once with distilled water. This recrystallization should have removed any lingering fillers and dispersing agents as well as any other impurities (13).

Chromatography of Dyes and Metabolites

It was realized that commercial dyes, even when separated from the fillers and dispersants, still are not pure compounds. The starting materials used in synthesizing dyes are not pure, and even if they were, few organic reactions yield only one product.

It was decided that before the dyes were submitted to biological treatment they need not be pure compounds. However, it was also decided that each component be characterized, if not identified. Thin-layer chromatography was chosen as the tool for this work. With this technique the number of components could be established, as well as, individual colors, relative amounts, and an infrared spectra of the major components. Each component's R_f value, which is the ratio of distance traveled to distance developed in a particular solvent, is also of great usefulness in characterizing a component.

Both 5 x 20 cm and 20 x 20 cm plates were prepared using a Desaga spreader. Thirty grams of E. R. Merck Silica gel G were thoroughly mixed with 60 ml of distilled water and spread at a thickness of 250 microns. The plates were allowed to air dry for one hour and then heated at 130°C. for one hour. Plates were stored under vacuum in a desiccator. Samples were spotted on the plates 1.5 cm from the bottom in an area of 20-50 mm^2 with a micro capillary tube. Chromatograms were developed 15 cm. Mixtures of chloroform and acetone were found to give the best separations of components. Normally 90 per cent chloroform and 10 per cent acetone was used. Thin-layer data for the purified dyes and metabolites will be found in the Appendix in Table 1.

Procedure for Degradation Experiments

Many papers have been written on the treatment of particular industrial wastes. From a study of the literature, it appears that there is approximately a 50-50 split as to whether static systems or continuous systems are better for laboratory study. After considering the advantages and disadvantages of each, the author chose to use a modified static system. The large volumes involved with continuous systems were felt to be a major disadvantage. The major disadvantage of static systems discussed by many writers and commonly called "shock loading" was not felt to be a major disadvantage by the author. In fact, it was thought to be rather realistic.

Domestic sludge collected from the Atlanta South River Treatment Plant was acclimated to laboratory conditions by feeding one gm/liter of sodium benzoate each day. The sludge was aerated twenty-two hours and

allowed to settle. At the end of one hour's settling, the per cent settled sludge was recorded. The effluent was removed and replaced with tap water containing the daily feeding of sodium benzoate. Earlier experiments using peptone as the acclimation food showed no final changes in the dye molecule. Therefore, a food more similar in molecular structure to the dyes being studied was investigated. Since the dyes had some aromatic character in their structure, an aromatic compound which was readily digestible, was felt to be needed. For this reason, sodium benzoate was used as the acclimation food.

Initially the settled sludge volume was adjusted to 25 per cent. At the end of a week's acclimation the volume had grown to 33 per cent. Before feeding of the dyes the sludge was transferred to one gallon jars marked off in 200 ml graduations. Each jar contained 3000 ml of sludge at 33 per cent settled solids (one hour).

Analytical methods used to monitor the tanks were chemical oxygen demand (COD), bacterial counts, pH, temperature, per cent sludge (one hour's settling), and visible spectra. COD was valuable during acclimation, but showed very little after dyes alone were fed because the concentration of dye fed was below the limits of accuracy of the test.

At the end of one week's acclimation the feeding of dyes was begun. As usual, 1500 ml of effluent was removed from the system after one hour's settling. It was replaced with 1500 ml of tap water which contained 10 mg of dye dispersed by boiling 15 minutes, and the normal amount of sodium benzoate. This procedure was repeated over a five-day period adding 10 mg of dye to the system each day, but reducing the amount of sodium benzoate by one-fifth each day until only dye was fed. After

sodium benzoate feeding was discontinued, dye was fed one more day. The system was then allowed to aerate, settling only once daily for testing, for 15 days. It was found that the dyes were rapidly adsorbed to the surface of the sludge or absorbed by the sludge. This allowed an estimated build-up in dye concentration to approximately 25 mg/l. Two blanks were run, also, which were treated the same except for feeding of dye.

Isolation of Metabolites

Since it was discovered that the dyes were absorbed by the sludge, it was felt that any metabolites that may have been produced might also be absorbed. It was decided that the entire sludge should be extracted rather than just the effluent. Direct extraction of the sludge with ether and chloroform proved to be unfeasible because separation into layers was hindered. An alternate method, however, was developed.

The sludge was diluted first to 50 per cent by volume with acetone to solubilize the dyes and metabolites. The mixture was stirred and heated to 45°C. for one hour and then allowed to settle. The supernatant liquid was poured off and filtered using No. 1 Watman paper in a Buchner funnel. Next, the acetone was evaporated on a steam bath using a Rotavapor vacuum distillation apparatus. Now the mixture could be readily extracted with ethyl ether. The ether extracts were concentrated under vacuum to approximately 10 ml. One of the blanks was extracted in the same manner. The other was divided into four equal portions, to each of which was added 25 mg/l of one of the dyes in acetone. These blanks were treated in the manner described above. The blanks were used to distinguish between metabolites and compounds already present.

The concentrated extracts including blanks were spotted on thin-layer plates and developed in a mixture of 90 per cent chloroform and 10 per cent acetone. The chromatograms of the degraded samples were compared with the chromatograms of the blanks, and spots not common to both were considered to be metabolites.

The spots of metabolites were removed from the plates and extracted from the Silica Gel G with acetone. The necessary quantity of potassium bromide required to make a pellet was added to the acetone extract. Then the acetone was initially dried in a forced air oven at 80°C. and further dried in a vacuum oven at 70°C. Pellets were made using vacuum and 10 tons pressure for three minutes. An infrared spectrum was obtained from the pellet.

CHAPTER IV

DISCUSSION OF RESULTS

Visible spectra of the effluent for each dye were taken after one, three, five and fifteen days. A sample of effluent was removed from the aeration tank and extracted for three hours in an ether extractor. The extracts were concentrated to a few milliliters and a visible spectrum taken. As a blank determination, dyes were aerated at the same concentration as in the sludge for one week in tap water with no change noted in the visible spectrum. Also, no change was noted after 30 minutes aeration in sludge.

After one day of aeration all of the dyes began to show the appearance of a yellow compound at 410-425 mu. The compound was not stable and disappeared shortly after removal from the sludge by extraction. It could only be extracted at a neutral pH; otherwise it was water-soluble. Chemical reduction of each of the dyes with sodium hydrosulfite yielded compounds which absorbed in the same region and were rapidly converted back to the original dye on exposure to air. The yellow compounds produced by bacterial action were believed to be the same as those produced chemically. This conversion to the "leuco" form seems to precede any further degradation. Sometimes certain anthraquinone dyes are produced in the leuco form and then oxidized to the more highly colored form because the reaction proceeds faster.

In the case of Disperse Red 15, conversion to the leuco form

appeared to be the only extent to which it degraded. No new spots on the thin-layer plates could be found visually, by fluorescence, or by charring with sulfuric acid.

Metabolism of Violet 1

After one day of aeration the leuco form of Violet 1 began to appear and reached its maximum concentration after five days. This maximum correlated roughly with an increase in COD to a maximum. Approximately one week after feeding of dye was discontinued, pH began to drop from 8.0 to 7.0. This correlated with a decrease in sludge volume and decrease in COD. The visible spectrum taken on the final day of aeration shows a broadening of the area of maximum abosrbance into one single broad peak. It is believed that this broadening was caused by the appearance of a metabolite that absorbed at nearly the same wavelength. This metabolite had an R_f value of 0.85, which is considerably higher than Violet 1. The more non-polar character of the metabolite indicates that Violet 1 may have been converted to a nitro derivative by oxidation of one of the amine groups or may have had an amine group acylated.

Violet 1 was metabolized approximately 10 per cent over a period of 15 days. Bacteria counts run at 10^{-3}, 10^{-4}, and 10^{-5} dilutions remained relatively constant throughout the entire aeration period. The plates at 10^{-4} dilution showed two to four times the number of colonies expected by comparison with the 10^{-3} dilutions. This may indicate a slight degree of toxicity since further dilution to 10^{-5} gives the expected number of colonies from the 10^{-4} dilution.

Metabolism of Blue 3

After one day of aeration, the appearance of the leuco form of Blue 3 was noted, as well as a broadening of the area of maximum absorbance. Again the leuco form appeared to reach a maximum in concentration after approximately five days, although it could not be detected in as great a quantity as in Violet 1. Five days after feeding of dye was discontinued a decrease in pH and sludge volume occurred which correlated with a decrease in COD and presence of the leuco form.

Two metabolites were isolated from the sludge. A violet compound whose R_f value was 0.55, and a blue compound whose R_f value was 0.90, were obtained from thin-layer plates. The metabolite with $R_f = 0.55$ had an infrared spectra very similar to Violet 1 ($R_f = 0.55$). The metabolite with $R_f = 0.85$ bore strong resemblance to the blue metabolite ($R_f = 0.85$) of Violet 1 in that their R_f values, colors, and infrared spectra were all the same.

Bacteria counts showed that the dye was relatively non-toxic. Counts remained nearly constant throughout the aeration period. Dilution brought no increase in the relative number of colonies.

The degree of degradation was estimated to be approximately 10 per cent.

Metabolism of Blue 7

The visible spectrum of Blue 7 after one day of aeration showed the presence of the leuco form of the dye just the same as in the previous instances. Also, it showed the beginnings of a broadening process and a shift to the blue. After 15 days it was evident that two compounds

were present, as the doublet had separated into a triplet. The maximum in COD occurred within two days after dye feeding had been discontinued, much earlier than in the case of Violet 1 and Blue 3. There was a lag of five days, however, before pH began to drop. Per cent sludge remained relatively constant until the pH began to drop, then it also dropped.

Thin-layers of the sludge extract showed the dye to be about 60 per cent degraded into another dye which is more non-polar than Blue 7. The R_f value of the violet metabolite (blue in solution) is 0.45, whereas, the R_f value of the original dye was 0.10. It was evident that the metabolite was not just the de-alkylated amine, since that compound would have been expected to be more polar than the original (migrates faster in non-polar solvent than original dye). The infrared spectrum showed the appearance of a weak band in the carbonyl region (1700 cm^{-1}), suggesting the possible formation of an acetanilide.

Blue 7 seemed to be the least toxic of the dyes studied. The relative number of colonies was the same at all dilutions and the number of colonies continued to increase through the entire aeration period.

Data recorded daily during the aeration of each dye can be found in the Appendix in Tables 2, 3, 4, and 5. Visible spectra of the effluents are given in Figures 3, 4, 5, and 6. Plots of COD versus Time are found in Figure 7. Suggested degradation pathways are shown in Figures 8, 9, 10, and 11. The infrared spectra of the dyes studied are found in Figures 12, 13, 14, and 15.

Anaerobic Studies

Disperse Blue 3 and Disperse Violet 1 were studied under anaerobic

conditions for the purpose of determining color abatement in the absence of air. It was felt that unless color abatement occurred, significant degradation would not have occurred, as had been observed in the aerobic studies. Therefore, only visual monitoring was done.

One-gallon bottles containing 3000 ml of sludge acclimated with sodium benzoate (1 gm/l/day) were fed 50 mg/l of the dye in acetone. The bottles were capped tightly with tinfoil and set aside for 30 days.

After 30 days it was evident that some of the dye had been absorbed by the sludge (sludge became tinted); however, no significant reduction in effluent color had taken place.

The author realized that there are more precise methods for making anaerobic studies, but felt this technique would show the advisability of conducting further research on anaerobic digestion of anthraquinone disperse dyes.

CHAPTER V

CONCLUSIONS

This thesis investigation showed that aeration of certain anthraquinone disperse dyes produced only partial degradation of the dye molecule after 15 days. Therefore, it was decided that these dyes can not be effectively treated in conventional waste disposal systems.

The nature of the metabolites produced showed that the dyes had not actually been degraded, but merely converted to derivatives of the original dyes. The metabolites appeared to be as highly colored as the dye from which they originated. There was no evidence that the anthraquinone ring system, or backbone of the molecule, had been degraded.

Disperse Red 15, Disperse Blue 3, and Disperse Violet 1 all had similar water-solubilities and showed little or no tendency to degrade. Disperse Blue 7, having more water-solubilizing groups in its structure, was metabolized to another highly colored compound by approximately 60 per cent.

Although the dyes proved to be only slightly toxic to the bacteria (as shown by bacteria counts), it is important that one realize that color abatement should not be of highest priority in dye effluent disposal, but rather that metabolic fate of the dyes is of utmost importance.

CHAPTER VI

RECOMMENDATIONS

This study showed that certain commercially important anthraquinone disperse dyes are partially metabolized by bacteria normally present in domestic activated sludge. It is suggested that these compounds and others similar to them be investigated with respect to their therapeutic effects. There is strong evidence that chemical compounds comparable in molecular structure to the dyes studied adversely affect test animals (5).

The results of this thesis investigation add support to the beliefs that non-aqueous dyeing systems with solvent recovery and reuse may provide an acceptable answer to pollution problems resulting from the use of aqueous dyeing systems. With solvent recovery, dyes and auxiliaries can be disposed of as solid waste.

It is the belief of the author that the textile industry may not need to change completely to non-aqueous systems, but that economical techniques will be found for the recovery and reuse of water as a solvent. There are certain advantages to using water as a solvent that may never be overcome by non-aqueous systems. In any case, waste disposal methods by means other than biological techniques must be found.

APPENDIX

Table 1. Thin-Layer Data for Sludge Extracts

	Color, Description	R_f Value*	Percentages**
	Disperse Red 15		
1.	Red, Dye Component	0.78	80
2.	Red, Dye Component	0.88	10
3.	Yellow, Dye Component, Fluorescent	0.92	10
	Disperse Violet 1		
1.	Violet, Dye Component	0.55	80
2.	Red, Dye Component	0.80	10
3.	Blue, Metabolite	0.85	10
	Disperse Blue 3		
1.	Blue, Dye Component	0.35	50
2.	Violet, Metabolite	0.55	10
3.	Blue, Metabolite	0.80	10
4.	Blue, Dye Component	0.90	30
	Disperse Blue 7		
1.	Blue, Dye Component	0.10	40
2.	Violet, Metabolite	0.45	60

* Developed in 90 per cent $CHCl_3$ - 10 per cent Acetone

** Estimated from spot size and color intensity

Table 2. Disperse Blue 7 Daily Data

Day No.	Effl. Color	pH*		Temp. $^{o}C.$	% Sludge	mg of Dye Fed	mg/l COD	** Visible Spectra
1	Yellow	7.3	7.0	26	30	0	45	-
2	"	8.0	7.5	24	30	0	40	-
3	"	8.4	7.8	24	33	0	28	-
4	"	8.5	8.0	24	33	10	24	-
5	Bluish Yellow	8.2	8.1	24	33	10	31	X
6	"	8.2	8.1	24	33	10	60	-
7	"	8.2	8.0	24	33	10	64	X
8	"	8.2	8.1	23	30	10	96	-
9	"	8.1		24	33	0	135	X
10	"	7.8		24	33	0	115	-
11	"	7.9		24	36	0	79	-
12	"	8.1		24	36	0	59	-
13	Green	8.0		24	36	0	56	-
14	"	7.8		24	36	0	63	-
15	Light Green	6.9		23	32	0	63	-
16	"	7.0		23	36	0	60	-
17	"	6.9		24	32	0	55	-
18	"	6.9		24	32	0	50	X

* pH before and after feeding
** Visible spectrum monitored on days marked X

Table 3. Disperse Violet 1 Daily Data

Day No.	Effl. Color	pH*		Temp. °C.	% Sludge	mg of Dye Fed	COD	** Visible Spectra
1	Yellow	7.3	7.0	26	30	0	49	-
2	"	8.0	7.5	24	30	0	46	-
3	"	8.4	7.7	24	33	0	48	-
4	"	8.5	8.0	24	33	10	57	-
5	Red Violet	8.2	8.1	24	33	10	116	X
6	"	8.4	8.2	24	33	10	48	-
7	"	8.2	8.0	24	33	10	60	X
8	"	8.2	8.1	23	33	10	80	-
9	"	8.0		24	33	0	75	X
10	"	8.4		24	33	0	190	-
11	"	8.2		24	36	0	186	-
12	"	8.1		24	36	0	91	-
13	"	8.1		24	36	0	124	
14	"	8.0		24	36	0	82	-
15	"	7.0		24	32	0	55	-
16	"	7.1		24	32	0	49	-
17	"	7.0		24	32	0	59	-
18	"	6.9		24	32	0	45	X

* pH before and after feeding
** Visible spectrum monitored on days marked X

Table 4. Disperse Red 15 Daily Data

Day No.	Effl. Color	pH*		Temp. $^{o}C.$	% Sludge	mg of Dye Fed	mg/l COD	** Visible Spectra
1	Yellow	7.4	7.2	26	30	0	41	-
2	"	8.0	7.5	24	30	0	30	-
3	"	8.4	7.6	24	33	0	44	-
4	"	8.5	8.3	24	33	10	60	-
5	Reddish Yellow	8.0	8.1	24	33	10	149	X
6	"	8.3	8.2	24	33	10	72	-
7	"	8.2	8.1	24	33	10	60	X
8	"	8.4	8.2	24	40	10	100	-
9	"	8.2		24	40	0	115	X
10	"	8.2		24	40	0	147	-
11	"	8.3		24	36	0	127	-
12	"	8.1		24	40	0	79	-
13	Yellow	7.9		24	40	0	76	-
14	"	7.7		24	36	0	51	-
15	"	7.1		25	32	0	47	-
16	"	7.1		25	32	0	55	-
17	"	7.0		24	32	0	45	-
18	"	7.0		24	35	0	48	X

* pH before and after feeding
** Visible spectrum monitored on days marked X

Table 5. Disperse Blue 3 Daily Data

Day No.	Effl. Color	pH*		Temp. °C.	% Sludge	mg of Dye Fed	mg/l COD	** Visible Spectra
1	Yellow	8.0	7.5	25	30	0	53	-
2	"	8.0	7.6	24	30	0	41	-
3	"	8.4	7.5	24	30	0	47	-
4	"	8.5	8.0	24	33	10	65	-
5	Blue	8.2	8.0	24	33	10	45	X
6	"	8.2	8.1	24	33	10	52	-
7	"	8.2	8.0	24	33	10	56	X
8	"	8.0	7.9	23	37	10	108	-
9	"	8.2		24	37	0	95	X
10	"	8.2		24	37	0	123	-
11	Gray Blue	8.4		23	37	0	103	-
12	"	8.1		24	37	0	55	-
13	Gray	8.2		23	37	0	48	-
14	"	7.8		23	40	0	59	-
15	Light Gray	7.1		24	35	0	78	-
16	"	7.0		24	35	0	65	-
17	"	7.0		24	35	0	60	-
18	"	7.1		24	35	0	55	X

* pH before and after feeding
** Visible spectrum monitored on days marked X

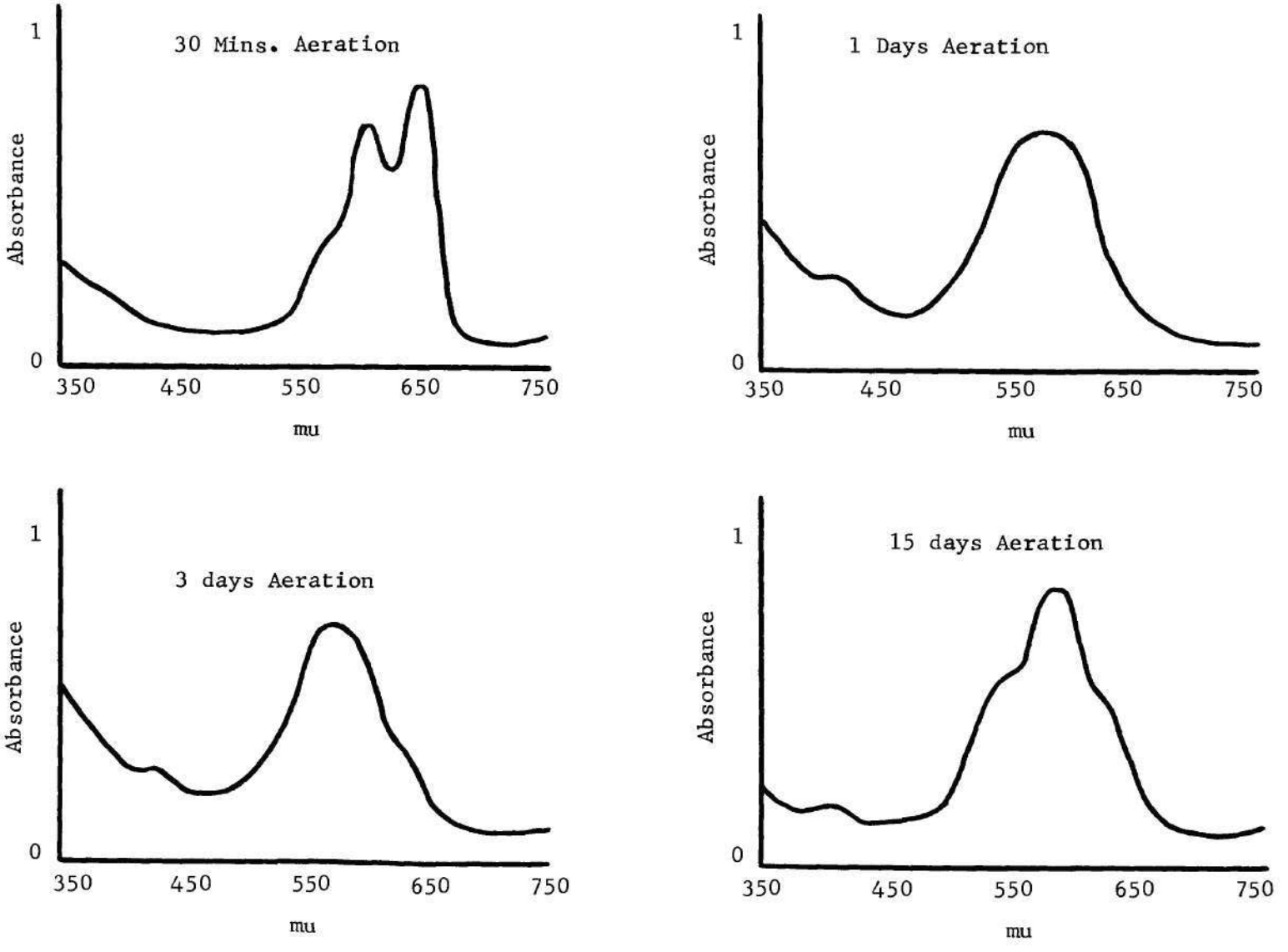


Figure 3. Visible Spectra of Disperse Blue 3 Effluents

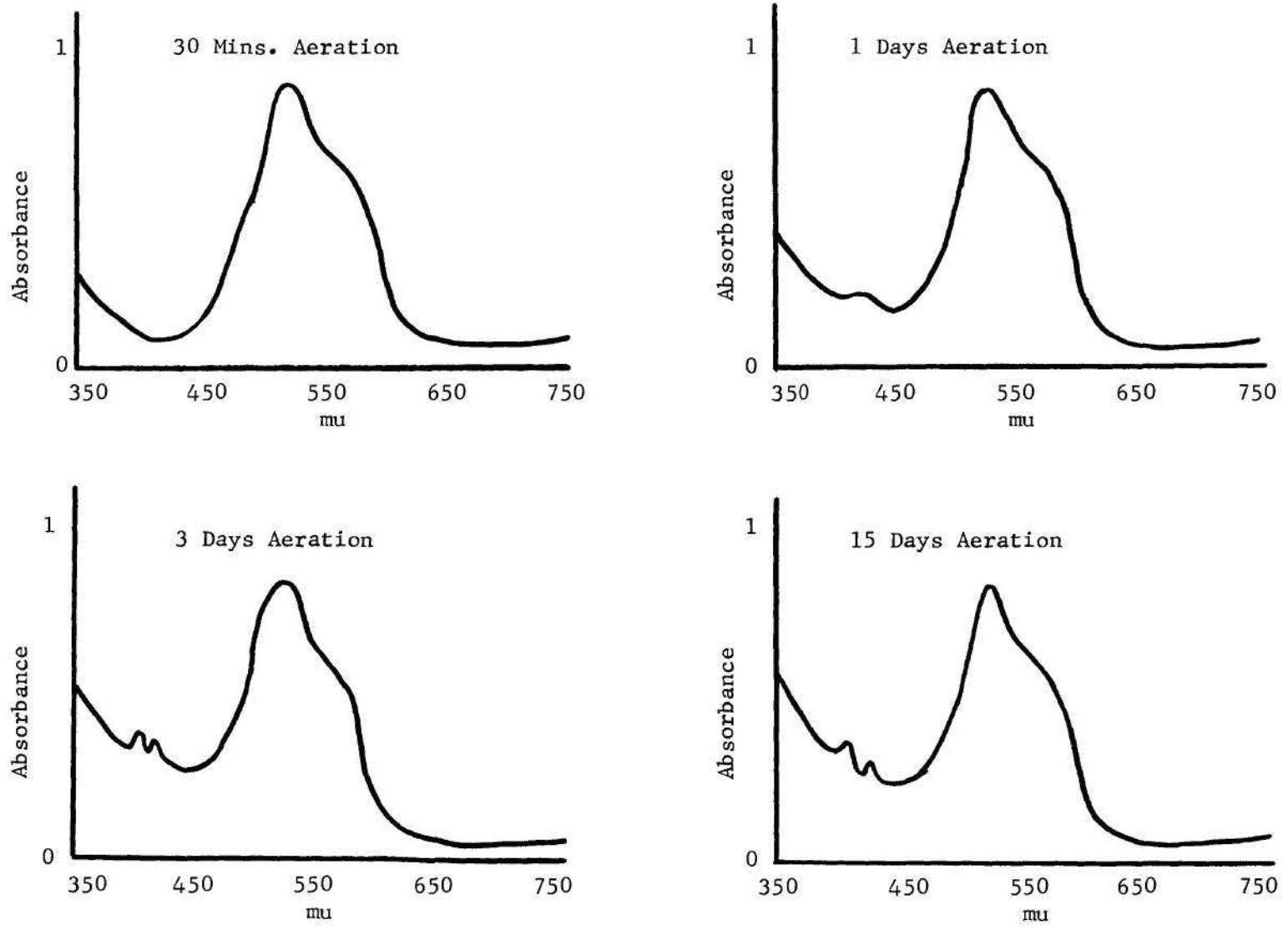


Figure 4. Visible Spectra of Disperse Red 15 Effluents

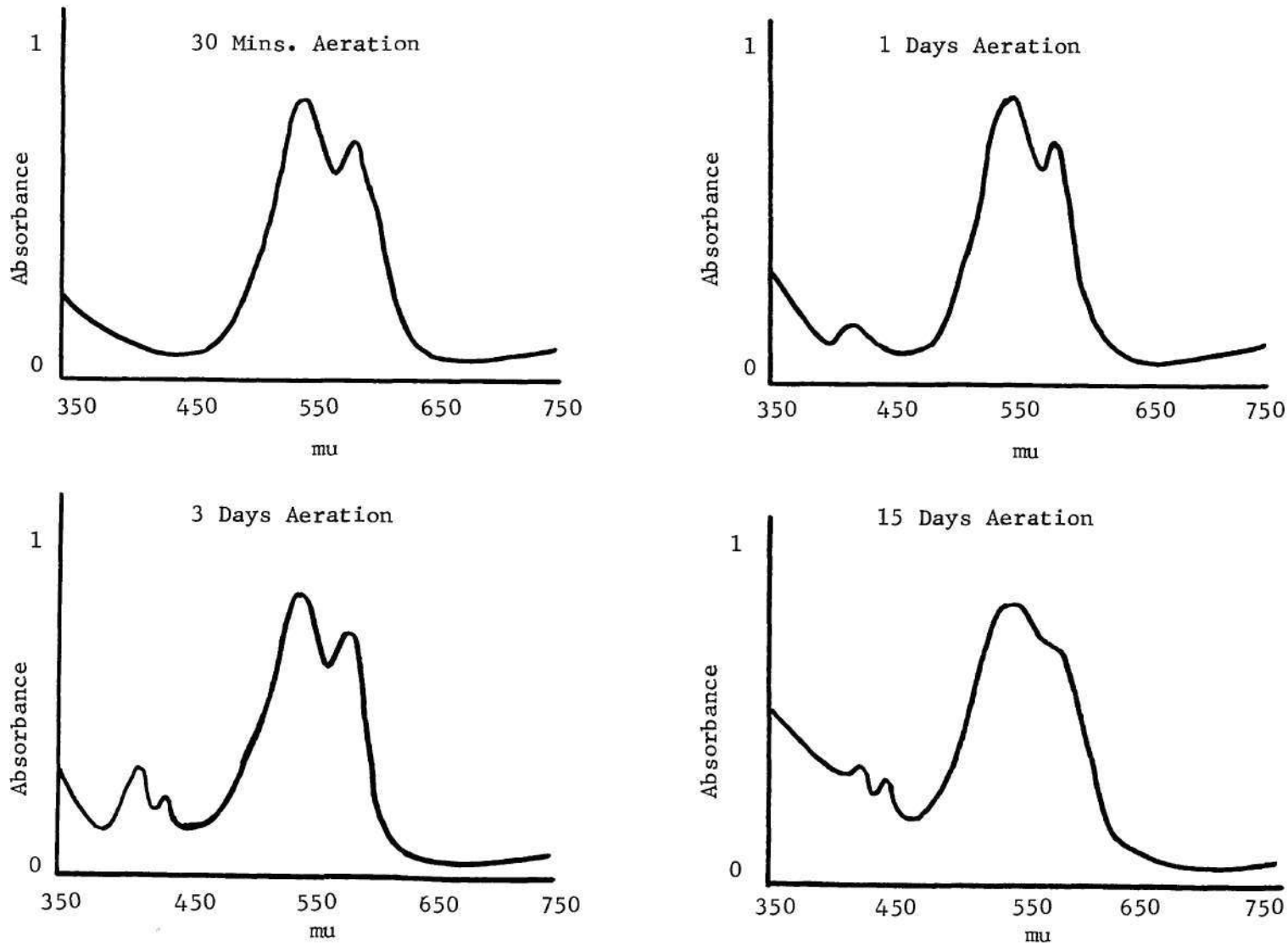


Figure 5. Visible Spectra of Disperse Violet 1 Effluents

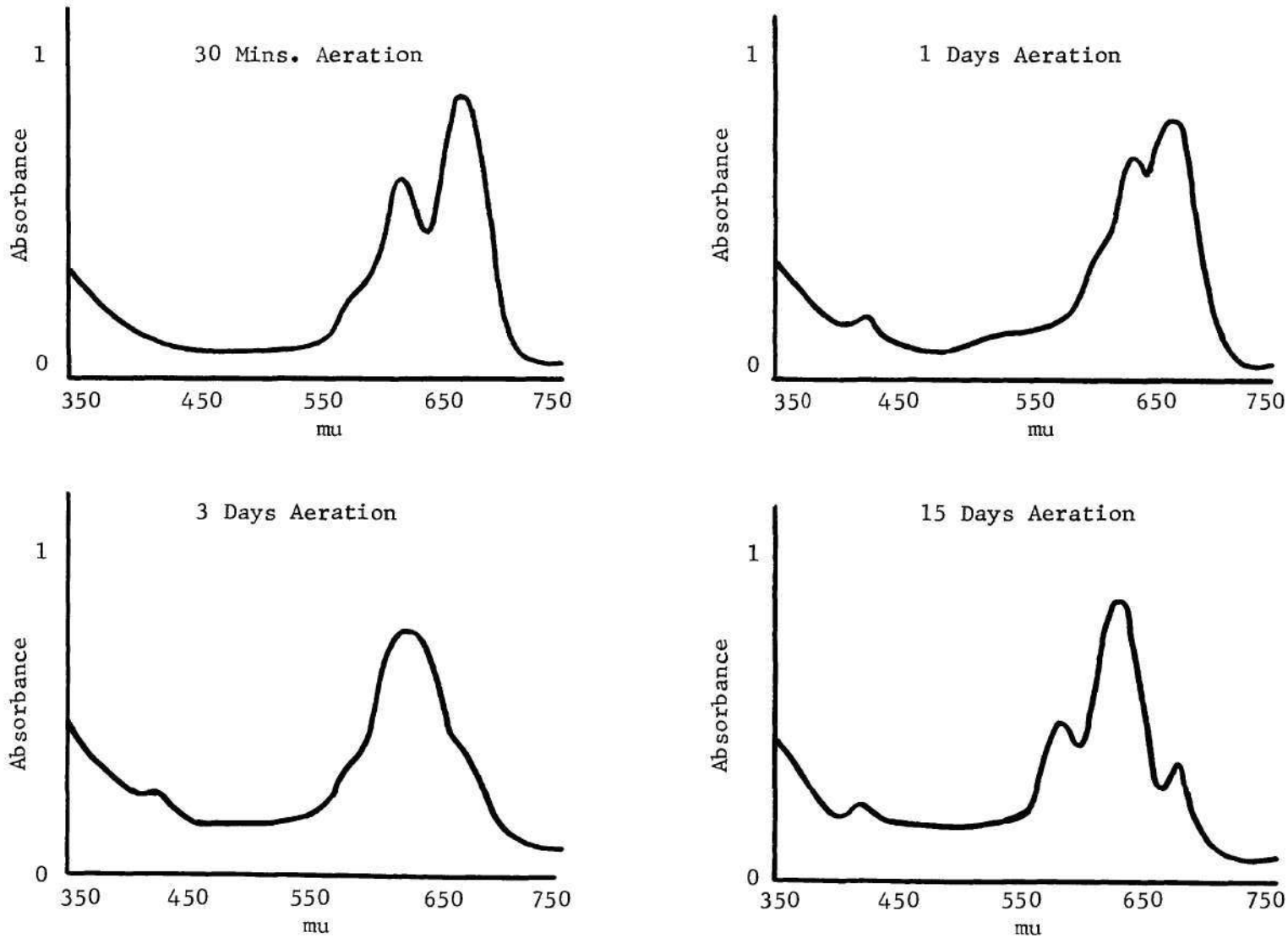


Figure 6. The Visible Spectra of Disperse Blue 7 Effluents

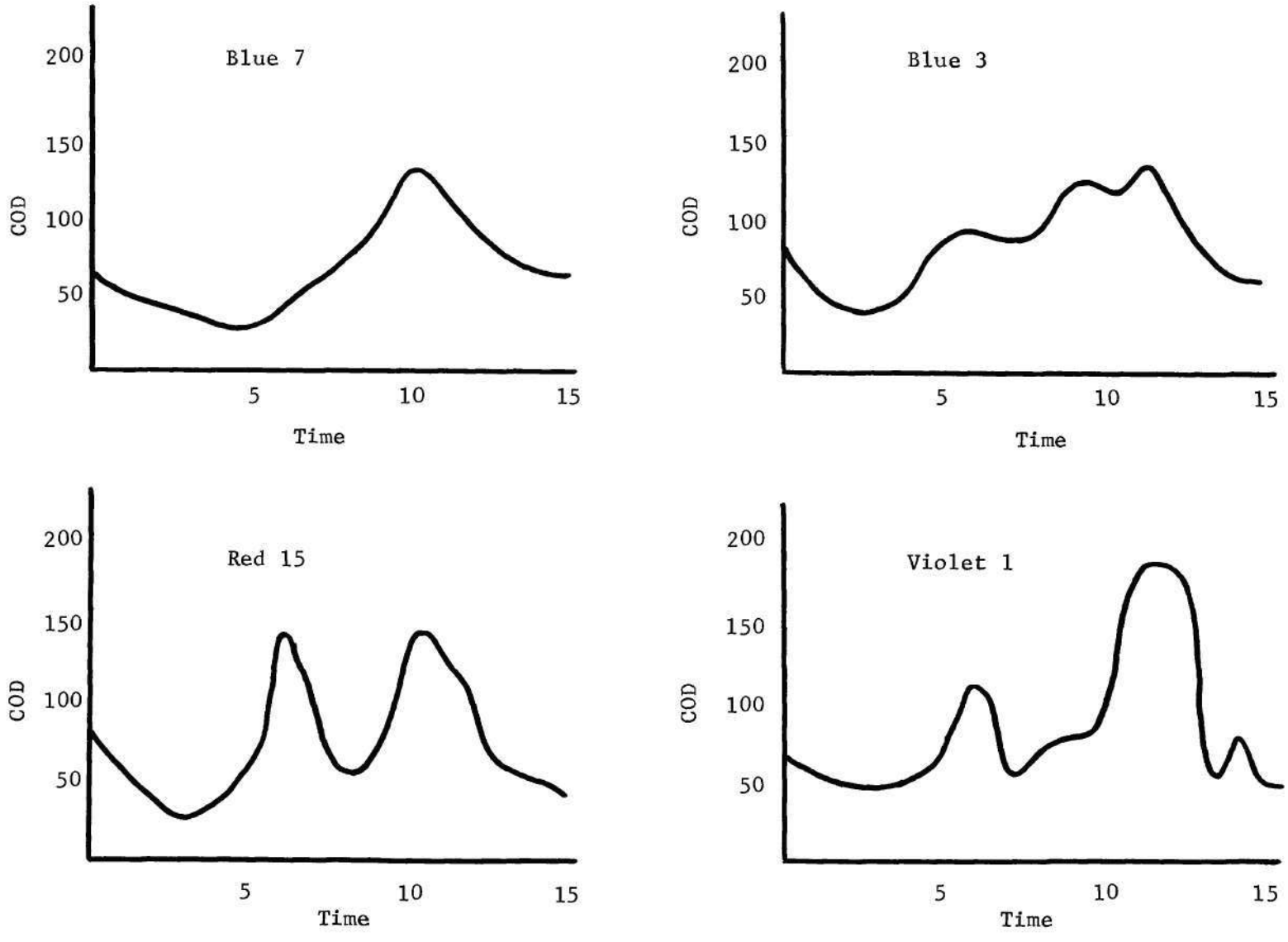


Figure 7. COD (mg/l) vs. Time (days)

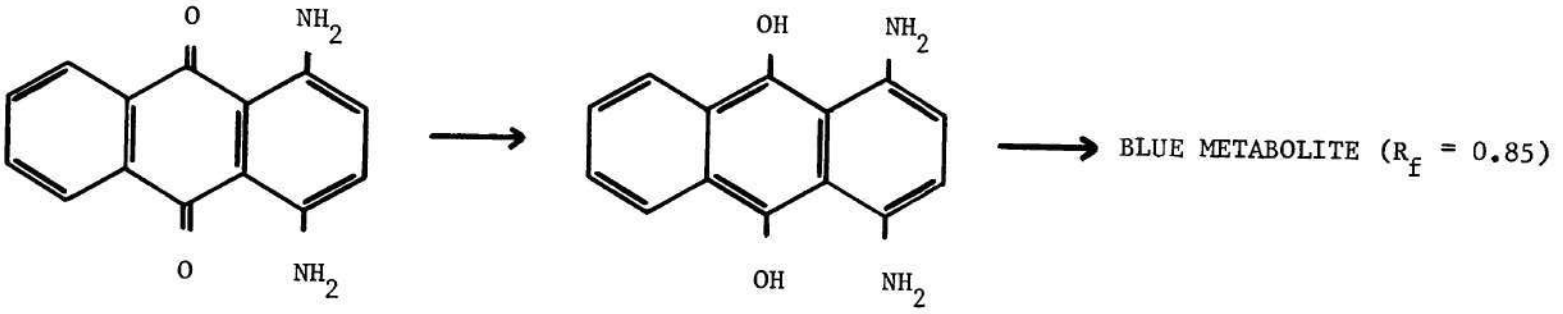


Figure 8. Suggested Degradation Pathway of Disperse Violet 1

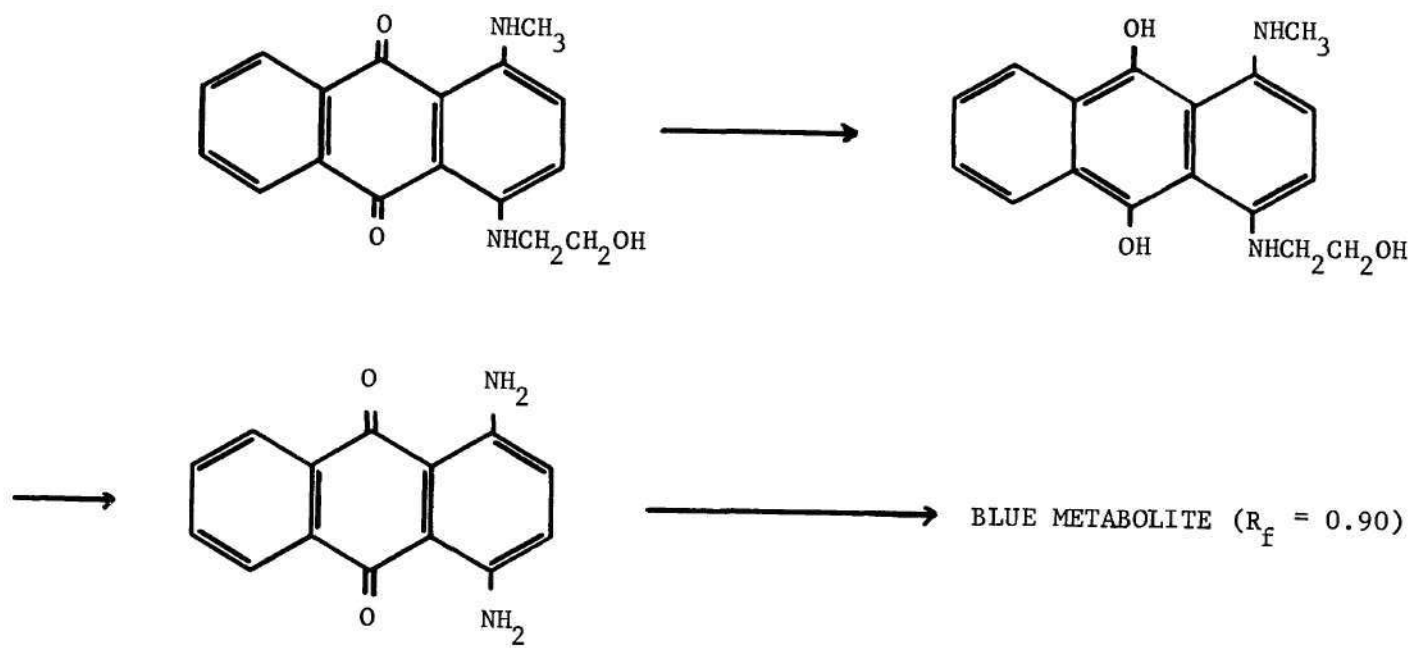


Figure 9. Suggested Degradation Pathway of Disperse Blue 3

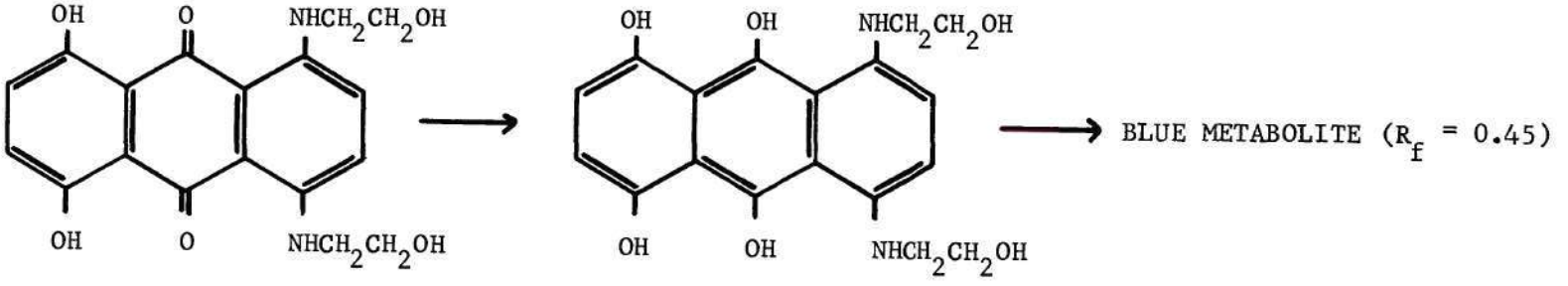


Figure 10. Suggested Degradation Pathway of Disperse Blue 7

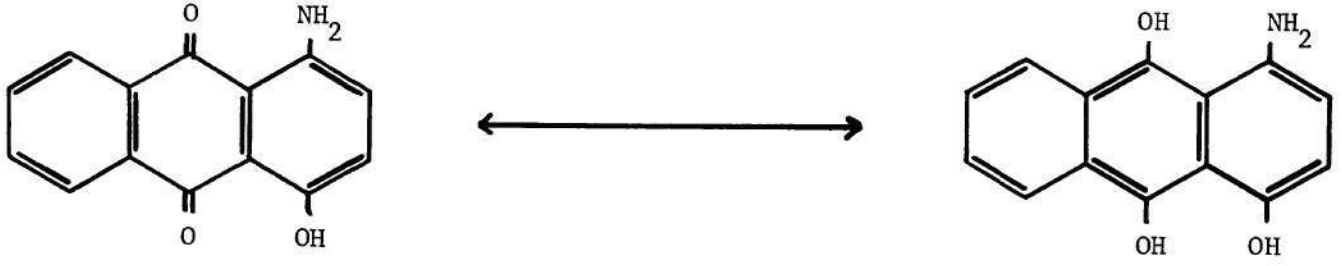


Figure 11. Red 15 Equilibrium in Activated Sludge

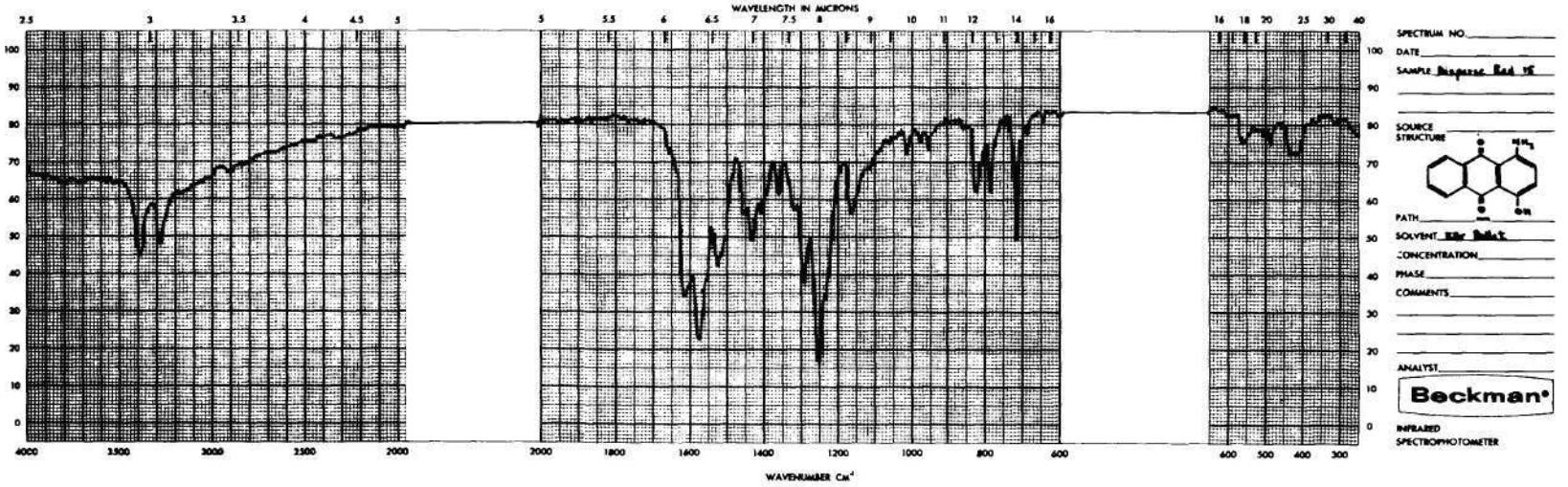


Figure 12. The Infrared Spectrum of Disperse Red 15

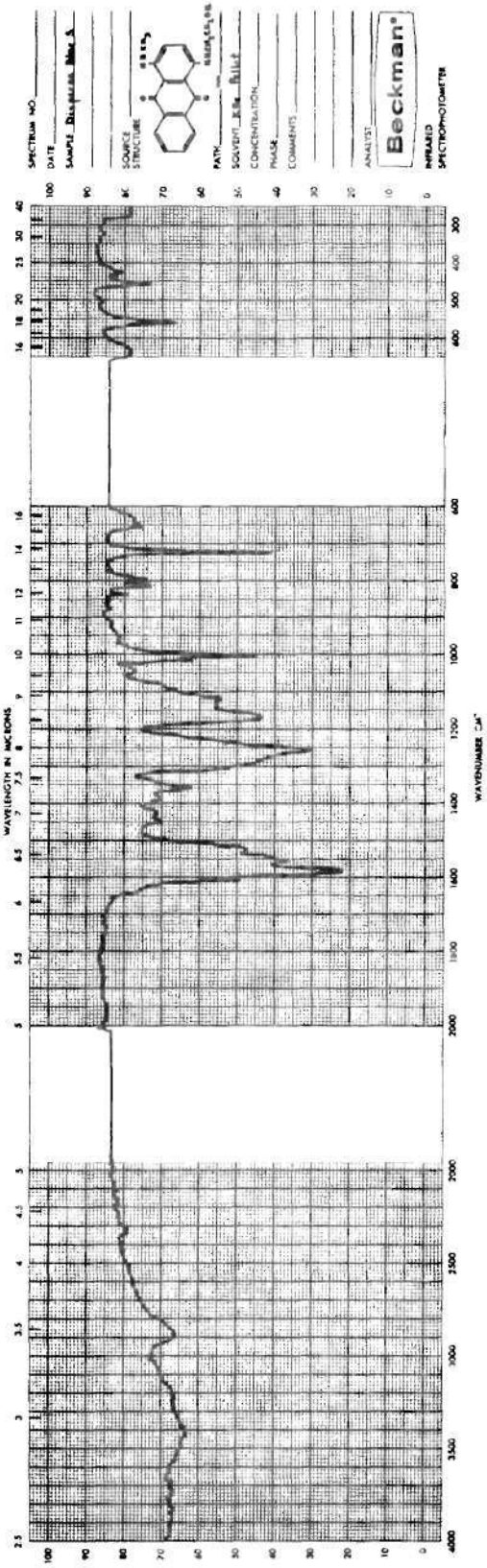


Figure 13. The Infrared Spectrum of Disperse Blue 3

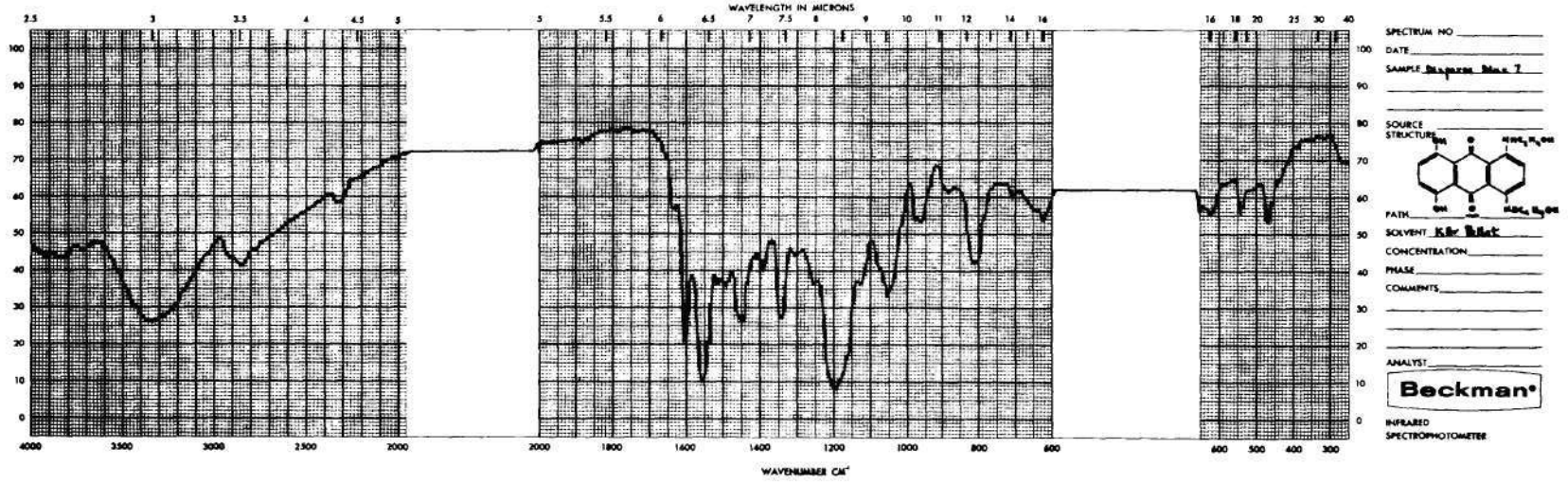


Figure 14. The Infrared Spectrum of Disperse Blue 7

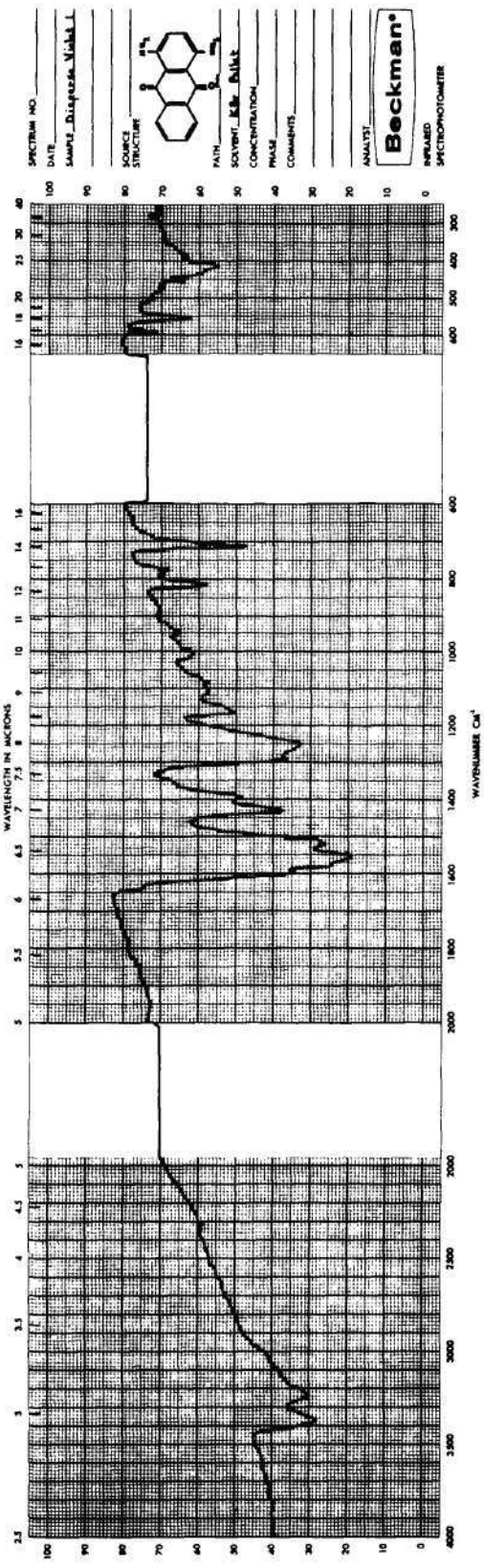


Figure 15. The Infrared Spectrum of Disperse Violet 1

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