PRELIMINARY INVESTIGATION
OF THE
CHROMATOGRAPHY OF BEEF-LIVER CATALASE

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Chemistry 80 Thesis

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Foreword

This entry in the Don Baxter Prize competition is a report on research carried out over two summers, 1962 and 1963. Therefore, it is partially on research that was reported in last year's competition.

Acknowledgement

I would like to express my sincere appreciation for the patience and guidance given me by Dr. W. A. Schroeder during my introductory venture into research chemistry. Working with him and his associates has been very interesting and beneficial, and I would also like to express my gratitude to Dr. Anil Saha for his very helpful assistance.

Summary

A description is presented of the methods and results of liquid-column chromatography of beef-liver catalase using DEAE-cellulose, DEAE-Sephadex, and Dowex-2x10 as adsorbents; varying concentrations and combinations of tris-hydroxymethyl aminomethane and HCl, NaCl, sodium phosphates, and KCN buffers as eluents; and spectrophotometric and Kat. F. data on individual chromatographic fractions as bases for analysis and interpretation of the chromatograms. Peaks were identified, were analyzed for iron and amino acid composition, and were separately rechromatographed.

Introduction

The general problem, to which this research presents a small part of the solution, is to determine the structure and properties of catalase, an enzyme known for its ability to catalyze the decomposition of hydrogen peroxide to water and gaseous oxygen. It is now generally accepted that the catalase molecule has a molecular weight of about 250,000 and contains four iron atoms. [1] Methods used to analyze catalase are electrophoresis, breakdown by enzymes, and chromatography.

More specifically, this research is concerned with a study of methods of separation, purification, and investigation of catalase with column chromatography, by varying adsorbents, eluents and their concentrations, sequences, etc., sample sizes, and various other chromatographic techniques. One of the present methods used to purify catalase is repeated recrystallization from $(NH_4)_2SO_4$ solutions of varying pH. Separation of horse-liver catalase from beef-liver catalase has already been developed by chromatographic means. [2] Purification by the chromatographic method, when successfully developed, could easily be scaled up to produce purified catalase in quantity for further work on its structure.

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[1] R. Lemberg and J. W. Legge, Hematin Compounds and Bile Pigments, Interscience Publishers, Inc., New York, 1949, p. 414.

[2] W. A. Schroeder, Anil Saha, William D. Fenninger, and Johnson T. Cua, Biochem. Biophys. Acta, 58 (1962) 611-613.

Experimental

The chromatographic column used was about 50 cm. in overall length with an internal diameter of 1 cm. An ion exchange adsorbent was used to pack the column, such that the adsorbent column itself was about 1 cm. by 42 cm. The column was kept at a constant equilibrium temperature of 6° C by cold water circulating in a jacket around the column.

The sample, in many cases, was 1 ml. of beef-liver catalase solution (20 mg./ml.) from the California Corporation for Biochemical Research. The sample, after having been dialyzed overnight in 250 ml. of the first eluent, was put on top of the adsorbent column. A continuous flow of eluent was provided by a reservoir connected to the top of the column with Tygon (later, polyethylene) tubing, the reservoir being at a height appropriate to the desired flow rate.

Chromatographic fractions were collected in 15 cm. test tubes placed in an automatic, weight-driven, time-controlled fraction collector. The flow rate and fraction collector were regulated so that each fraction contained about two milliliters of effluent, collected over a period of twenty minutes. Often the buffer was changed during the run to an increased concentration after 50 to 150 ml. of a lower concentration buffer had been passed through.

Spectrophotometric absorptions at 280 millimicrons and 405 millimicrons (or 277 and 422 millimicrons for cyanide solutions, as will be explained later) were then determined for individual fractions with a Beckman Model DU spectrophotometer using hydrogen lamp radiation.

The results were graphed, absorption vs. total volume of effluent, for interpretation. It is profitable to determine the 280/405 ratio (or the 277/422 ratio in the case of cyanide solutions). This ratio is the ratio of the absorption at 280 millimicrons, which is indicative of the general protein content of the sample, to the absorption at 405 millimicrons, which is indicative of the heme content of the sample and, therefore, possibly, of the "pure" catalase content.

With this experimental set-up two problems were encountered. When about six feet of Tygon tubing were used to bring buffer from the reservoir to the top of the column, absorption at 280 millimicrons was present in a substantial quantity, even when blank chromatograms using water as a "sample" were run. This source of absorption was eliminated by the use of polyethylene tubing in place of the Tygon tubing. It was also found that the tris-hydroxymethyl aminomethane and hydrochloric acid buffers, which will be called tris buffers, after standing for more than a couple days after dilution from 1.0 M solution to 0.1 M or 0.2 M solution, spontaneously began to build up considerable absorption. This source of spurious absorption was largely eliminated by the use of relatively new dilute buffer solutions.

Results and Discussion

Chromatograms using tris buffer at pH 7.5 as eluent and DEAE-cellulose (Schleicher and Schuell, selected reagent ion exchange cellulose, #72 DEAE type 40, .97 meq./ml.) as adsorbent will be used as the basis for discussion because this system was the most successful and extensively examined of those tried. In a typical chromatogram

tris buffer of 0.11 M to 0.15 M concentration eluted a first peak. 0.2 M tris buffer was then used to bring through a second peak, which was usually on the same order of magnitude as the first peak with respect to the portion of the sample contained in the whole of the peak; see Figure 1.

The effect of varying the concentration of tris buffer was observed as follows. 0.1 M would not elute the first peak if less than a few hundred milliliters were used. 0.11 M would elute the first peak (most of the time), although slowly by spreading the first peak over a large volume of effluent and many fractions. 0.12 M gave a sharper first peak. 0.15 M gave a sharp peak that was almost entirely contained in 10 to 15 ml. of effluent. 0.2 M always brought out the second peak sharply in about 10 ml. of effluent.

The 280/405 ratio for the dialyzed sample was observed to be consistently around 1.14 for the beef-liver catalase of the one lot number used during the first summer of the two summers in which this investigation took place. By spreading or smearing out the first peak with 0.11 M tris, the 280/405 ratio approached 1.00 in the densest portion of the peak and slowly and gradually rose as the peak tailed out. The second peak ratio was 1.14 to 1.16 or greater at the minimum ratio point.

A shift in the concentration-elution relation was observed in the second summer. 0.09 M would slowly elute the first peak in the fashion that 0.11 M had the preceding summer. 0.1 M and 0.12 M had an elution effect previously given by 0.12 M and 0.15 M, respectively. The origin of this shift could not be traced, although different

samples and buffer stock solutions were used. Concentrations used during the first summer will be denoted by "M," while those used during the second summer will be denoted by "M."

The amounts of catalase in the peaks were determined by summing the products of the volume of the fraction and the optical density at 405 millimicrons. Peak I was 2 times as large as peak II when a 0.09 M and 0.2 M buffer sequence was used, 9 times as large with a 0.1 M and 0.2 M sequence, and 38 times as large with a 0.12 M and 0.2 M sequence. When about 400 ml. of 0.09 M was used instead of the 150 ml. ordinarily used, peak I was 5 times as large as peak II. When a 0.09 M and 0.11 M and 0.2 M buffer sequence was used, three distinct peaks were eluted. These results made it apparent that the number, size, and shape of the peaks could be varied widely by an appropriate sequence of buffer concentrations. This observation was further supported by the large, broad, single peak that was eluted with a linear-gradient buffer system running from 0.05 M to 0.2 M.

Chromatographic behavior varied when catalase samples of different lot numbers were chromatographed in the same manner. Peak I ranged from 1.5 to 2.8 times as large as peak II with a 0.09 M and 0.2 M buffer sequence. The 280/405 ratio of the dialyzed sample before chromatography varied from 1.12 to 1.20 depending on the lot number, but it was constant within 0.02 for any given lot number. Similarly, the minimum 280/405 ratio in each peak depended on the lot number, although these minima could be controlled to a slight extent by the buffer sequence. In peak I the minimum ranged from 0.99 to 1.09; in peak II it ranged from 1.15 to 1.30. In the three-peak chromatogram,

which had a dialyzed sample 280/405 ratio of 1.19, the 280/405 ratio minima of the peaks were 1.07, 1.14, and 1.31, in that order.

Several variations in the chromatographic method were tried. A chromatogram which was run at room temperature instead of the usual 6° C showed no change in basic chromatographic behavior.

When the sample size was increased from 1.0 ml. to 2.5 ml., nothing substantially different was observed except, of course, proportionately larger absorption readings. Consequently, 2.5 ml. samples were used during the second summer to provide sizeable optical densities even for peaks spread thinly over a large volume.

As a variation in eluent, pH 8.0 tris buffer was used. From previous work done by Dr. Anil Saha, it was known that higher concentrations were needed for the same elution effect given by pH 7.5 tris buffers. 0.17 M pH 8.0 tris buffer was found to have about the same elution power as 0.11 M pH 7.5 tris buffer; 0.22 M pH 8.0 tris seemed less effective than 0.2 M pH 7.5 tris, but it still brought out the second peak. Other than this difference in concentration-elution effect, the chromatogram was similar to previous runs.

When NaCl-tris buffers at pH 7.5 were tried, it was found that a 0.1 M tris+ 0.01 F NaCl buffer had much the same elution effect as 0.11 M pH 7.5 tris; and 0.1 M tris+ 0.1 F NaCl, that of 0.2 M pH 7.5 tris. If any difference could be detected, it was that the salt, when it was used to replace an equal concentration of tris in a buffer, was only slightly less effective than the tris it replaced.

Investigation of the effect of cyanide on the spectrum of catalase

revealed that catalase dialyzed in tris buffer containing cyanide had a maximum absorption at 422 millimicrons rather than at 405 millimicrons like the catalase dialyzed in tris buffer. The other maximum, at 277 millimicrons, was the same in both samples. It was noted that the cyanide not only shifted the 405 millimicron absorption maximum, but also reduced the magnitude of that maximum somewhat, thereby giving a larger 277/422 ratio than an analogous non-cyanide buffer 280/405 ratio. For comparison of the spectra see Figures 2 and 3. In hopes of stabilizing the catalase during chromatography by complexing heme with cyanide, 0.09 M pH 7.5 tris+ 0.001 F KCN and 0.2 M pH 7.5 tris+ 0.001 F KCN were used in a chromatogram. Peak II was nearly as large as peak I; the 277/422 ratios were high, 1.62, 1.42, and 1.54 for the dialyzed sample, peak I, and peak II, respectively. At pH 7.5 part of the cyanide was found to leave the fractions after a couple of days so that the fractions had to be slightly diluted with more concentrated KCN solution to bring out the 422 millimicron maximum for spectrophotometric reading.

A phosphate-cyanide buffer (Na_2HPO_4--NaH_2PO_4--KCN) was tried; the chromatogram was about the same as that of pH 7.5 tris except for concentration-elution effect differences. A 0.1 M Na^++ 0.001 F KCN pH 7.5 buffer appeared to have the eluting power of 0.12 M pH 7.5 tris buffer, and 0.15 M Na^++ 0.001 F KCN was comparable with 0.2 M pH 7.5 tris.

All chromatograms discussed to this point were run on DEAE-cellulose adsorbent. Dowex-2x10 resin (200-400 mesh, anion exchange resin, medium porosity, quaternary ammonium, total capacity 3.0±0.3 meq./

dry gram, Dow Chemical Co.) was tried as an adsorbent with phosphate (sodium) buffers with concentrations ranging from 0.1 M in phosphate+ 0.001 F KCN to 0.0005 M in phosphate. The breakthrough volumes, which are the volumes of effluent between the application of the sample and the appearance of the sample in the effluent, were those calculated for a sample which was not adsorbed on the resin at all.

One chromatogram was run using DEAE-Sephadex A-25 adsorbent (medium particle size, anion exchanger, capacity 3.2 meq./gram, Pharmacia) and a 0.09 M pH 7.5 tris and 0.2 M pH 7.5 tris buffer sequence. Peak I was 3.6 times larger than peak II, and peak I had a small secondary peak superimposed on it about 35 ml. after the crest of the main peak.

With the chromatographic systems described, it was observed that the main part of the peak was always eluted with the concentration front. The conditions of chromatography were limited by the insolubility of catalase in a range around its isoelectric point of 5.6.

Fractions containing the greater portions of the two peaks were centrifuged to concentrate them for samples of peak I and peak II catalase for rechromatography. During the centrifugation and treatment of the samples, the 280/405 ratio of the peak I sample rose from 1.0 to 1.2, while the 280/405 ratio of the peak II sample increased from 1.3 to 1.5. Upon rechromatography of the peak I sample, only a very small first peak was eluted with 0.12 M pH 7.5 tris, but a large second peak with a ratio minimum of 1.04 was eluted with 0.2 M pH 7.5 tris. The peak II sample rechromatography gave no first peak with 0.12 M pH 7.5 tris, but 0.2 M pH 7.5 tris gave a large

second peak with a ratio minimum of 1.16.

Because the ratio changes indicated that the centrifugation process might be changing the samples, peak I and peak II were rechromatographed without centrifugation. Peak I, which had a 280/405 ratio of 1.13 after dialysis, was split into two peaks with ratios of 1.08 and 1.18 upon rechromatography with a 0.09 M pH 7.5 tris and 0.2 M pH 7.5 tris buffer sequence. Under the same treatment peak II, which had a ratio of 1.29 after dialysis, was eluted with 0.2 M pH 7.5 tris in a single peak having a ratio of 1.32.

Kat. F. values, which indicate the catalytic activity by measuring the rate of decomposition of hydrogen peroxide in the presence of catalase, were determined by a method based on that described by Bonnichsen, Chance, and Theorell.[3] It was found that the Kat. F. values of the dialyzed samples and of peak I samples were in the range of 50,000 to 60,000, while the peak II values were usually between 40,000 and 50,000. A general decrease in Kat. F. value with increasing 280/405 ratio was observed. The Kat. F. values of catalase in cyanide solutions appeared to be 10% or 20% high, but this was probably due to the fact that the same concentration of catalase has a lower 422 millimicron reading in cyanide solution than it would have at 405 millimicrons in a non-cyanide solution, thus leading to an underestimation of the amount of catalase present.

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[3] Roger K. Bonnichsen, Britton Chance, and Hugo Theorell, Acta Chem. Scand., 1 (1947) 685-709.

The amino acid composition of peak I and peak II samples, determined by Dr. Anil Saha with a Beckman Spinco Automatic Amino Acid Analyzer, Model 120, revealed only slight differences between the two. (Starred values (*) have been corrected for hydrolytic destruction.)

Amino Acid Composition

Amino Acid	Residues/250,000 grams	
	Peak I	Peak II
Lysine	110.1	111.3
Histidine	83.6	87.2
Arginine	120.7	130.5
Aspartic Acid	301.3	287.8
Threonine	92.2*	90.3*
Serine	95.6*	94.5*
Glutamic Acid	207.1	203.9
Proline	168.8	174.6
Glycine	149.7	151.9
Alanine	157.6	156.8
Cystine/2	16.3*	9.8*
Valine	146.4	144.8
Methionine	45.1	45.5
Isoleucine	77.1	76.9
Leucine	154.1	150.9
Tyrosine	76.4*	81.9*
Phenylalanine	134.9	133.9

The weight percentages of iron in peak I and in peak II samples were determined by Mrs. Barbara Olson using spectrophotometric measurements of the ferrous--4,7-diphenyl-1,10-phenanthroline complex as described by Diehl and Smith.[4] Peak I contained 0.101% iron, while peak II was very slightly higher with 0.103% iron.

Conclusions

Of the methods of chromatography tried, pH 7.5 tris buffer with DEAE-cellulose gave the best apparent separation and purification. pH 8.0 tris, pH 7.5 tris + NaCl, and phosphate buffers with DEAE-cellulose and pH 7.5 tris with DEAE-Sephadex were also satisfactory, but these methods had no advantage over pH 7.5 tris with DEAE-cellulose except, possibly, in the cost of NaCl and phosphate buffers. The tris-KCN buffer on DEAE-cellulose and phosphate buffer on Dowex-2x10 were unsatisfactory.

It is apparent that the number, size, and shape of the peaks can be varied by an appropriate choice of buffer sequence. The particular sample of catalase, distinguished by lot number, had a considerable effect on the distribution of substance between the peaks and in the minimum 280/405 ratio of the peaks. With an increasing 280/405 ratio a decrease in the Kat. F. value was observed.

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[4] Harvey Diehl and G. Frederick Smith, <u>The Iron Reagents: Bathophenanthroline, 2,4,6-Tripyridyl-s-triazine, and Phenyl-2-pyridyl Ketoxime</u>, The G. Frederick Smith Chemical Company, Columbus, Ohio, 1960, especially pp. 19-21.

Prospectus

More research on the composition and chromatography of catalase will be needed, especially with regard to the rechromatography of peak I and peak II samples. The significance of the secondary peak obtained with DEAE-Sephadex should be investigated. Left open is the question of whether the samples are a continuously varying substance or a mixture of distinct compounds which were not resolved well by these methods of chromatography. The adsorbents and eluents tried have not exhausted all combinations or possibilities. Extension of these methods to other forms of catalase such as horse-liver, human-erythrocyte, and rabbit-erythrocyte catalases and to other similar biochemicals is another definite possibility for further research.

TABULAR DATA SUMMARY OF CHROMATOGRAMS NO. 01319 THROUGH 01342

Chromatogram no.	Adsorbent	Size of column (cm)	Sample Volume (ml)	Sample Material	pH of buffer	Eluent Molarity (tris unless otherwise stated) (M)	Eluent Break-through volume (ml)	Eluent Minimum 280/405 277/422 ratio	Eluent Total volume (ml)
01319	DEAE	1x46	1	BLC	7.5	.1			43.1
						.2	29.0	1.1524	
01320	DEAE	1x40	1	BLC	7.5	.15	23.5	1.3590	
01321	DEAE	1x45.5	1	BLC	7.5	.1			57.6
						.2	28.2	1.2969	
01322	DEAE	1x41.3	1	BLC	7.5	.12	29.4	1.2510	151.1
						.2	26.1	1.7462	
01323	DEAE	1x42	1	BLC	7.5	.11	29.1	1.3396	131.6
						.2	28.4	1.2500	
01324	DEAE	1x42	1	BLC	7.5	.1			49.4
						.1+ .01 NaCl	24.3	1.2583	100.2
						.1+ .02 NaCl			175.8
						.1+ .1 NaCl	24.6	1.4781	
01325	DEAE	1x43.1	.7	BLC	7.5	.1+ .01 NaCl	30.2	1.6354	115.6
						.1+ .1 NaCl	28.0	1.3621	
01326	DEAE	1x41	.55	BLC	7.5	.1+ .001 KCN			66.0
						.2+ .001 KCN	26.2	1.6923	
01327	DEAE	1x42.7	1	BLC	8.0	.15			147.5
						.17	29.4	1.1938	176.0
						.22	29.6	2.1826	
01328	DEAE	1x42.0	1	BLC	7.5	.1 Na^+(phosphate)+ .001 KCN	28.7	1.3600	151.1
						.15 Na^+(phosphate)+ .001 KCN	27.2	2.0227	

Chromatogram no.	Adsorbent	Size of column (cm)	Sample Volume (ml)	Sample Material	pH of buffer	Eluent Molarity (tris unless otherwise stated) (M)	Eluent Breakthrough volume (ml)	Eluent Minimum 280/405 277/422 ratio	Eluent Total volume (ml)
01329	Dowex-2x10	1x41.8	1	BLC	7.5	.1 phosphate+ .001 KCN	10.3	1.368	
01330	DEAE	1x42.0	.7	BLC	7.5	.1+ .01 NaCl	35.0	1.370	138.1
						.1+ .1 NaCl	28.3	1.264	
01331	Dowex-2x10	1x40.2	1	BLC	7.75	.02 phosphate+ .001 KCN	9.8	1.312	
01332	DEAE	1x41.0	2.5	BLC	7.5	.11	33.9	.992	175.3
						.2	26.3	.922	
01333	Dowex-2x10	1x39.6	1	BLC	7.9	.001 Na_2HPO_4	11.4	1.221	
01334	DEAE	1x41.9	.7	H_2O	7.5	.1+ .01 NaCl			172.2
						.1+ .1 NaCl			
01335	Dowex-2x10	1x39.2	1	BLC	7.6	.0005 Na_2HPO_4	9.7	1.122	
01336	DEAE	1x42.2	.7	H_2O	7.5	.1+ .01 NaCl			152.4
						.1+ .1 NaCl			
01337	DEAE	1.2x37.4	1	BLC	7.5	.1			146.8
						.15	38.1	1.001	151.1
						.2	37.5	1.232	
01338	DEAE	1x40.7	2.4	BLC	7.5	.11	37.7	.993	191.2
						.2	26.1	1.156	

Chromatogram no.	Adsorbent	Size of column (cm)	Sample Volume (ml)	Sample Material	pH of buffer	Eluent Molarity (tris unless otherwise stated) (M)	Eluent Breakthrough volume (ml)	Eluent Minimum 280/405 277/422 ratio	Eluent Total volume (ml)
01339	DEAE	1x40.8	2.4	BLC	7.5	.11	74.0	.857	173.8
						.12	27.3	.978	297.2
						.2	24.6	1.139	
01340	DEAE	1x40.5	2.4	BLC	7.5	.05			116.6
						.12	38.0	1.010	199.9
						.2	24.7	1.163	
01341	DEAE	1x41.5	.2	BLC	7.5	.11			143.3
						.12	26.9	1.000	194.0
						.2	26.4	1.044	
01342	DEAE	1x41.5	.3	BLC	7.5	.11			134.4
						.12			201.0
						.2	26.5	1.160	

TABULAR DATA SUMMARY OF CHROMATOGRAMS NO. 01343 THROUGH 01359

All of the chromatograms no. 01343 through 01359 were done during the second summer with about 2.5 ml. of beef-liver catalase on DEAE-cellulose columns about 42 cm. long except chromatogram no. 01359 which was done using 1.0 ml. of beef-liver catalase on a 42 cm. DEAE-Sephadex column.

	Sample				Eluent		
Chromatogram no.	Control no. (a lot no.)	280/405 277/422 ratio	Kat. F.	Peak I / Peak II	Molarity (M) (pH 7.5 tris unless otherwise stated)	Minimum 280/405 277/422 ratio	Kat. F. of minimum-ratio fraction
01343	6133437	1.185		37.82	.12 .2	1.101 1.300	
01344	6133437	1.188	49,082	9.92	.1 .2	1.088 1.303	62,449 43,103
01345	6352235	1.125	48,839	4.82	.1 .2	1.013 1.162	54,629 38,498
01346	6352235	1.118	56,818	2.09	.09 .2	1.017 1.154	59,737 49,431
01347	6133437	1.204	53,346	2.77	.09 .2	1.078 1.279	50,461 38,889
01348	6133437			2.28	.09 .2	1.064 1.298	
01349	6133437			9.00	.1 .2	1.080 1.335	
01350	Peak I ch. 01349	1.133	54,308		.05 .09 .2	 1.078 1.182	 53,723 49,727

Chromatogram no.	Sample: Control no. (a lot no.)	Sample: 280/405 277/422 ratio	Sample: Kat. F.	Peak I / Peak II	Eluent: Molarity ($\underline{M}$) (pH 7.5 tris unless otherwise stated)	Eluent: Minimum 280/405 277/422 ratio	Eluent: Kat. F. of minimum-ratio fraction
01351	Peak II ch. 01348	1.294	49,848		.09		
					.2	1.322	40,046
01352	6023435	1.115	50,822	1.62	.09	0.993	61,571
					.2	1.171	44,046
01353	6133437	1.182	53,091	5.16	.09	1.065	54,897
					.2	1.378	36,522
01354	6133437	1.192	53,396		.09	1.073	43,367
					.11	1.144	50,176
					.2	1.312	32,985
01355	6133437	1.620	64,931	1.06	.09+ .001 $\underline{F}$ KCN	1.423	
					.2+ .001 $\underline{F}$ KCN	1.536	
01356	6133437	1.200			linear gradient .05 to .2	1.061	
01357 (room temperature)	6133437	1.189			.05		
					.07	1.074	
					.08	1.122	
					.09	1.125	
					.1	1.140	
					.2	1.253	
01358	6323239	1.193	47,694	1.48	.09	1.086	50,000
					.2	1.246	42,544
01359	6133437	1.196		3.59	.09	1.071	
					.2	1.278	

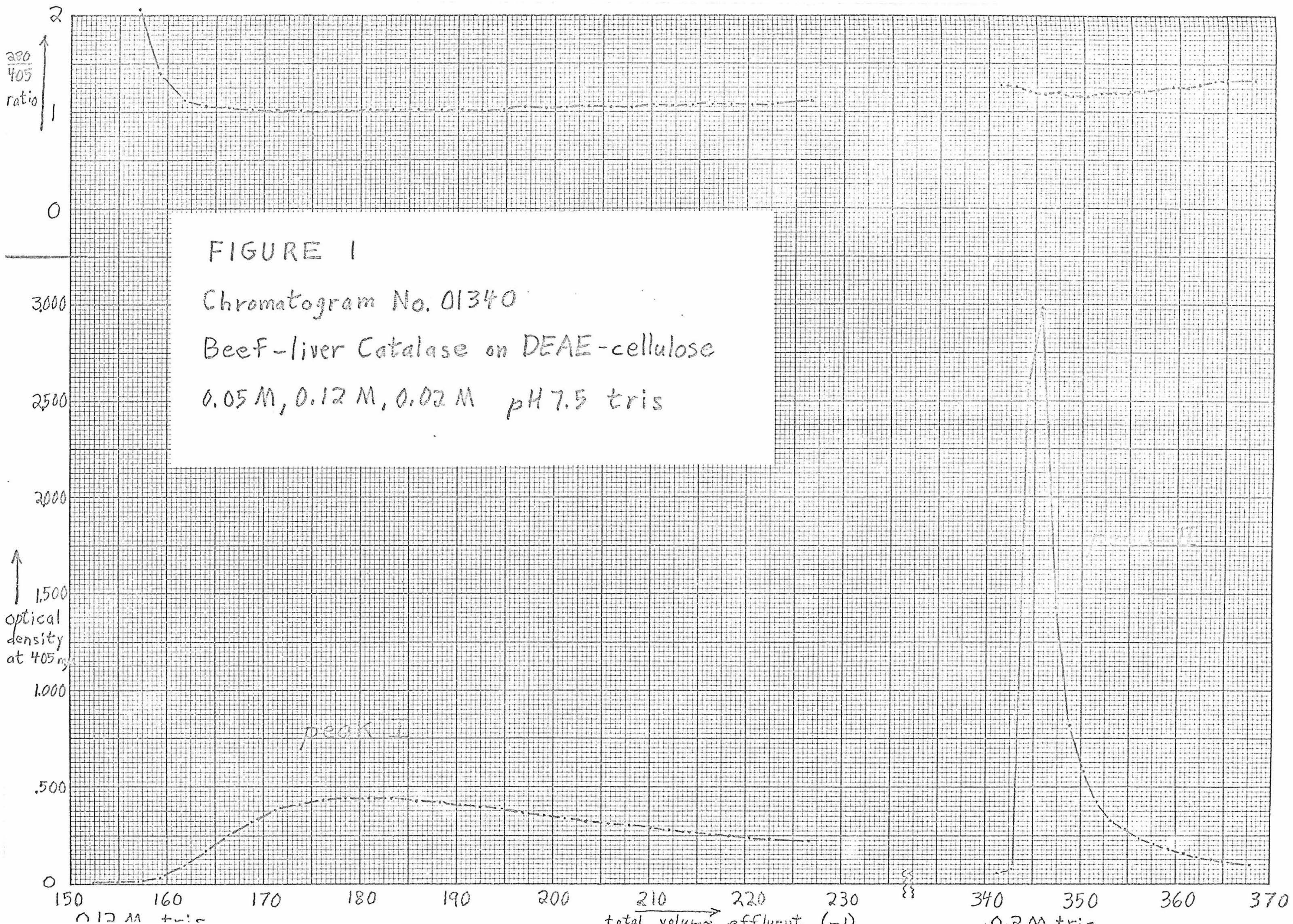
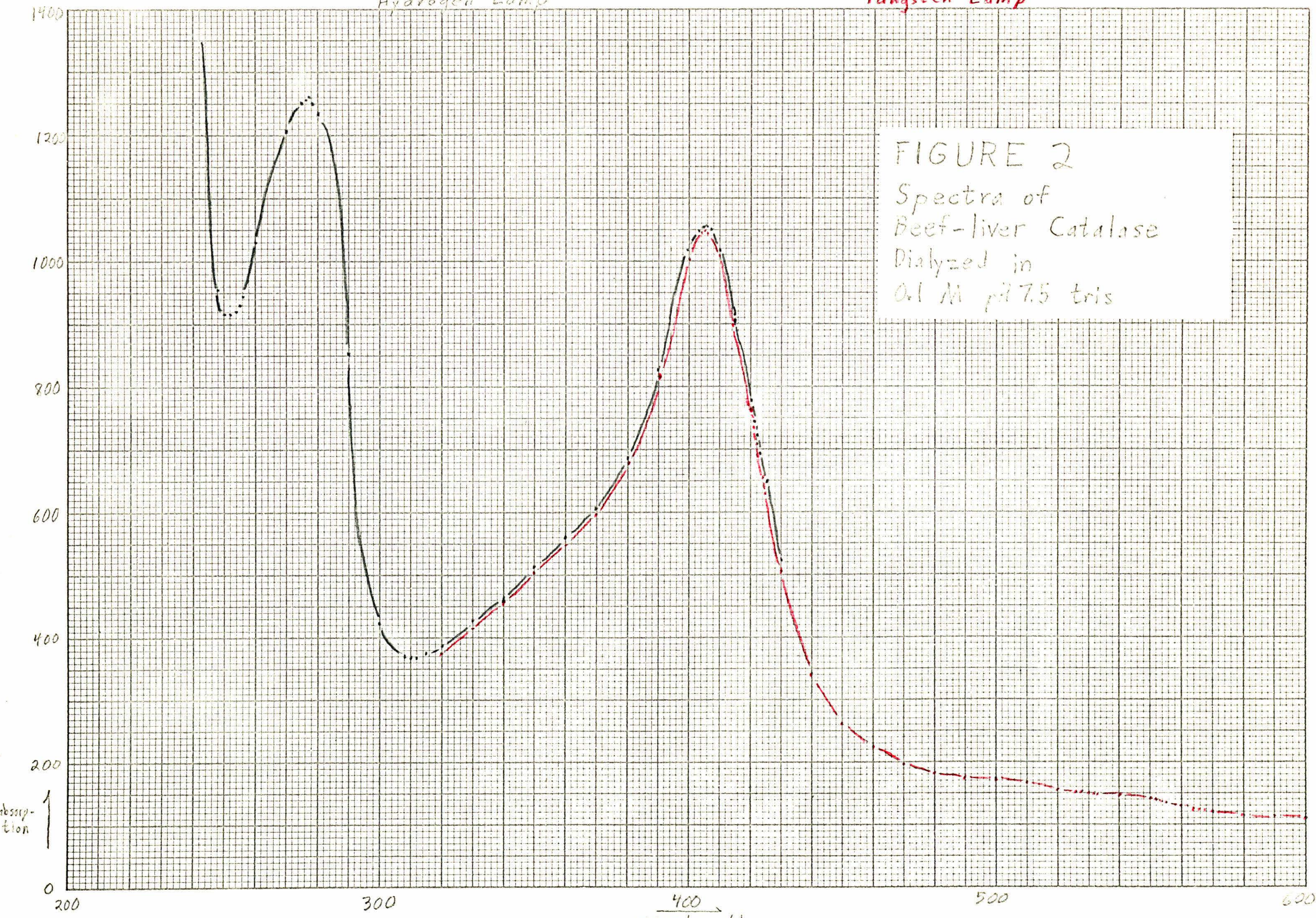


FIGURE 1
Chromatogram No. 01340
Beef-liver Catalase on DEAE-cellulose
0.05 M, 0.12 M, 0.02 M pH 7.5 tris
280/405 ratio
2
1
0
3.000
2.500
2.000
1.500
optical density at 405 mμ
1.000
.500
0
peak I
peak II
150
160
170
180
190
200
210
220
230
340
350
360
370
total volume effluent (ml)


Hydrogen Lamp
Tungsten Lamp
FIGURE 2
Spectra of
Beef-liver Catalase
Dialyzed in
0.1 M pH 7.5 tris
1400
1200
1000
800
600
400
200
0
absorp-
tion
200
300
400
500
600
wavelength
(mμ)

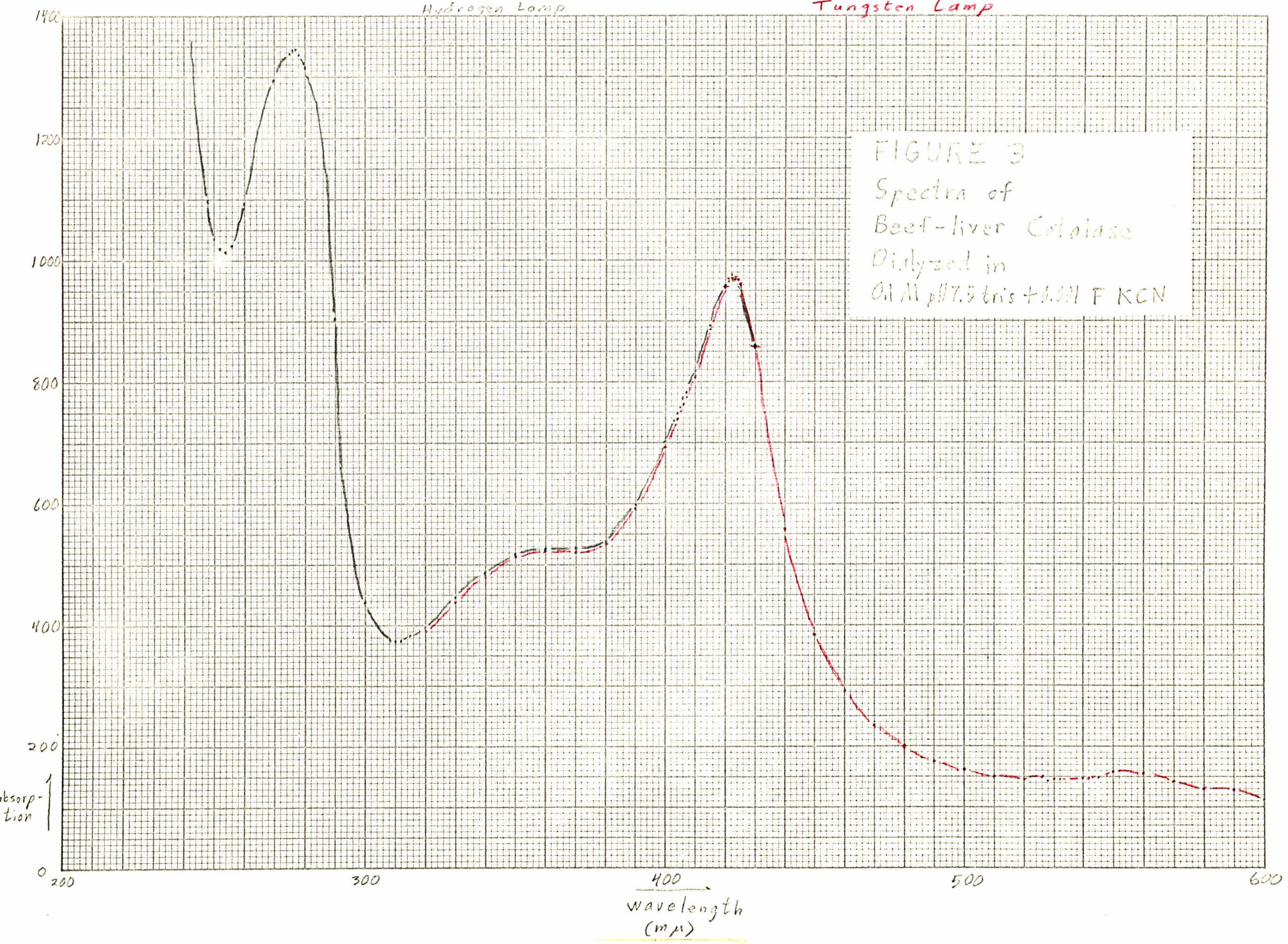


FIGURE 3
Spectra of
Beef-liver Catalase
Dialyzed in
0.1 M pH7.5 tris + 0.001 F KCN
Hydrogen Lamp
Tungsten Lamp
1400
1200
1000
800
600
400
200
0
absorp-
tion
200
300
400
500
600
wavelength
(mμ)