ULTRAVIOLET ABSORPTION CURVES FOR VITAMIN A USING TUNGSTEN AND HYDROGEN DISCHARGE LIGHT SOURCES

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ABSTRACT

The spectrophotometric method for determining vitamin A potency has been generally adopted by the vitamin liver oil industry. The practice of using a tungsten light source for making measurements at certain wave lengths in the ultraviolet region, however, has not been considered reliable by the instrument manufacturer. To check on this, a series of experiments were conducted with two types of light sources. From the data obtained, it was concluded that reliable results may be obtained by use of the tungsten lamp.

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INTRODUCTION

The vitamin liver oil industry has generally adopted the spectrophotometric method of assay for determining vitamin A potency. Rapid adoption of this method was due mainly to the ease and rapidity with which vitamin A could be estimated in fish liver oils. Reproducibility of the results obtained with the spectrophotometric method was found to be much better than with methods previously used.

Although determination of vitamin A ordinarily depends upon the measurement of maximum absorption at 325 to 328 Mu., it is sometimes necessary to determine the absorption at 300 and 350 Mu. in order to properly evaluate the value obtained at 328 Mu. Ratio of the absorption at 300 Mu. to that at 328 Mu. along with the ratio of the absorption at 350 Mu. to that at 328 Mu. aids materially in deciding whether or not the reading at 328 Mu. is due entirely to vitamin A or due to the presence of appreciable quantities of non-specific absorbing substances.

Investigation has shown that vitamin A exhibits the following ratios when the whole oil is dissolved in ethanol or isopropanol: $\frac{E\ (300\ Mu.)}{E\ (328\ Mu.)}$ less than 0.73 and $\frac{E\ (350\ Mu.)}{E\ (328\ Mu.)}$ less than 0.65. These ratios were established as the minimum standards acceptable to the War Food Administration for the purchase of vitamin A oils and vitamin A concentrates by the Government. The now defunct WFA handled war period purchases of vitamin A oils and concentrates for Lend-Lease shipments, etc. War Food Administration standard requirements were specifically promulgated to obtain products of satisfactory quality and stability.

The Beckman Photoelectric Quartz Spectrophotometer, equipped with proper phototubes and accessories, is designed to operate with accuracy from 220 Mu. in the ultraviolet up to 1,100 Mu. in the infrared. A 6-volt, 25-watt tungsten lamp light source is satisfactory for measurements above 320 Mu. However, for measurements in the ultraviolet region below 320 Mu., it is stated by the manufacturer that a hydrogen discharge light source must be used. In addition, when the hydrogen discharge tube is employed, directions by the instrument manufacturer call for use of absorption cells of fused silica and a blue ultraviolet sensitive phototube with an ultraviolet transmitting envelope.

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Even though many of the vitamin laboratories equipped with the Beckman Spectrophotometer possess a hydrogen discharge lamp and its power supply unit, little use has been made of these accessories. Some of the reasons for lack of use of the hydrogen discharge light source for photometric measurements at 300 Mu. were: the comparatively short life of the early lamp models, the high cost of lamp replacement, difficulties with the power supply unit, and hesitancy on the part of the analysts to make the necessary instrument changes. As a result, most of the vitamin laboratories have apparently relied on the 300 Mu. measurements made with the tungsten light source even though this practice is not considered reliable by the instrument manufacturer.

In order to evaluate, critically, the practice of determining, with a tungsten light source, the absorption of vitamin A oils at 300 Mu. ultraviolet, a series of experiments were carried out using two different types of oil containing vitamin A. In one series, the light source used was the hydrogen discharge lamp. In the other series, the light source was the tungsten lamp previously described.

EXPERIMENTAL METHODS AND DATA

All measurements were made with a Beckman Quartz Spectrophotometer, Model DU, calibrated from 200 to 2,000 Mu. This instrument is equipped with a blue ultraviolet sensitive phototube with an ultraviolet transmitting envelope. This tube

Table 1 - Extinction Ratios Determined with Tungsten and Hydrogen Discharge Light Sources on Grayfish Liver Oil[1]

Wave Length	Tungsten Lamp		Hydrogen Discharge Lamp		Wave Length	Tungsten Lamp		Hydrogen Discharge Lamp	
λ	E	$\frac{E\,\lambda}{E\,328}$	E	$\frac{E\,\lambda}{E\,328}$	λ	E	$\frac{E\,\lambda}{E\,328}$	E	$\frac{E\,\lambda}{E\,328}$
220	-	-	.780	1.452	300	.370	.685	.365	.679
230	-	-	.680	1.266	310	.470	.870	.467	.869
240	-	-	.570	1.061	320	.525	.972	.522	.972
250	-	-	.290	.540	325	.543	1.005	.538	1.001
260	-	-	.173	.322	328	.540	1.000	.537	1.000
270	-	-	.187	.348	330	.534	.988	.527	.981
280	-	-	.233	.433	340	.438	.811	.435	.810
290	-	-	.292	.543	350	.314	.581	.308	.573

1/ Oil was dissolved in redistilled isopropanol.

has high sensitivity over the range 200-625 Mu. A second caesium oxide phototube with high sensitivity from 600-1,000 Mu. was part of the instrument's equipment.

Table 2 - Extinction Ratios Determined with Tungsten and Hydrogen Discharge Light Sources on Distilled Vitamin A Concentrate[1]

Wave Length	Tungsten Lamp		Hydrogen Discharge Lamp		Wave Length	Tungsten Lamp		Hydrogen Discharge Lamp	
λ	E	$\frac{E\,\lambda}{E\,328}$	E	$\frac{E\,\lambda}{E\,328}$	λ	E	$\frac{E\,\lambda}{E\,328}$	E	$\frac{E\,\lambda}{E\,328}$
220	-	-	.186	.321	300	.362	.626	.361	.624
230	-	-	.165	.285	310	.482	.833	.475	.821
240	-	-	.140	.242	320	.552	.955	.550	.951
250	-	-	.123	.212	325	.577	.998	.575	.994
260	-	-	.120	.207	328	.578	1.000	.578	1.000
270	-	-	.134	.231	330	.570	.986	.568	.982
280	-	-	.182	.314	340	.474	.820	.470	.813
290	-	-	.259	.448	350	.333	.576	.333	.576

1/ Concentrate was dissolved in redistilled isopropanol.

The tungsten light source consisted of a 6-volt, 25-watt tungsten filament lamp operated from a storage battery. The hydrogen discharge lamp was that fur-

nished by the instrument manufacturer and was operated from the hydrogen lamp power supply unit connected to the 110-volt, 60-cycle, alternating current house line. Samples of the oils were dissolved in redistilled isopropanol and fused silica absorption cells were used in making all photometric density readings.

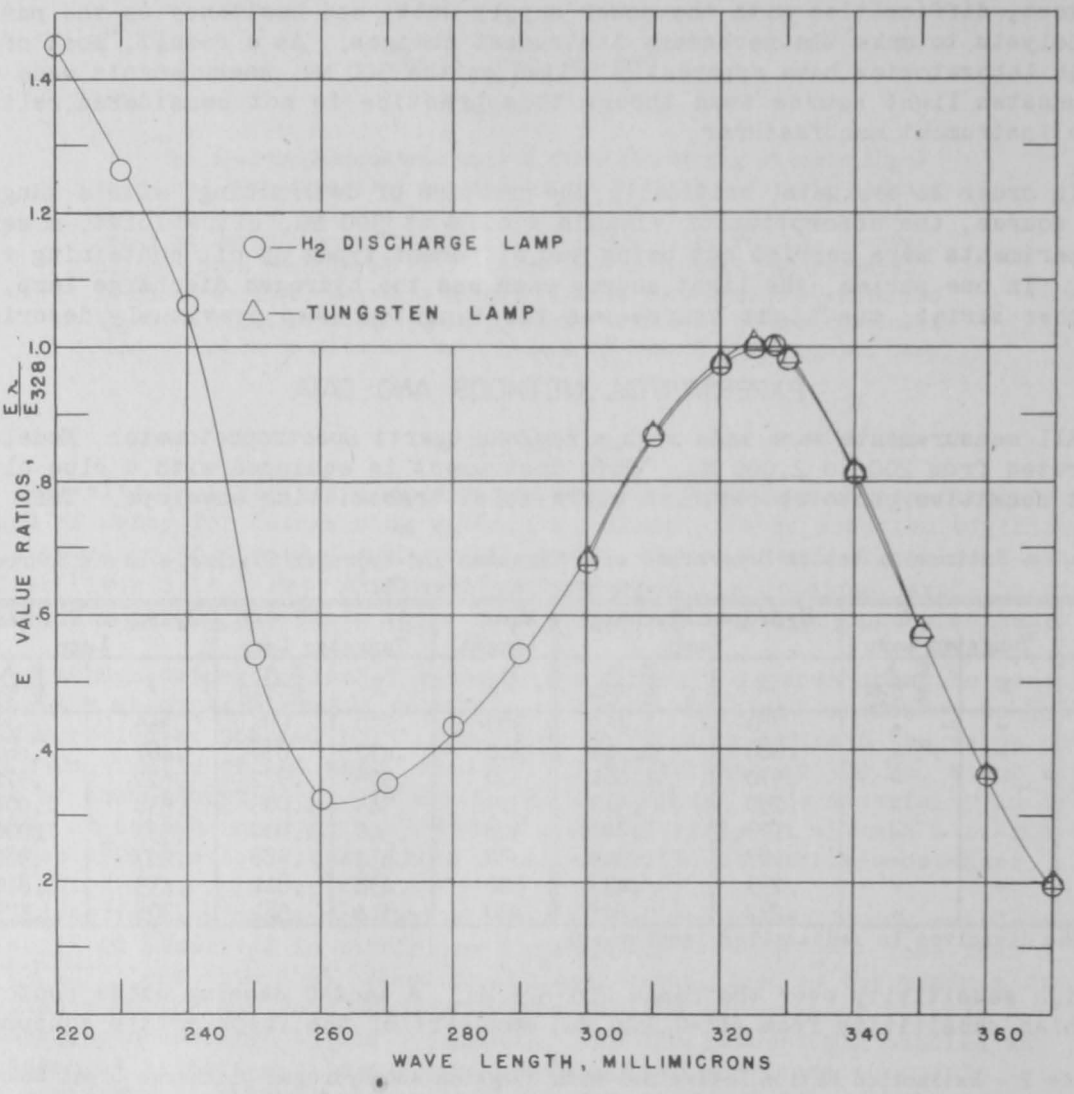

FIGURE 1 - ULTRAVIOLET ABSORPTION CURVES FOR GRAYFISH LIVER OIL

One of the samples used in the experiments was a commercially prepared grayfish liver oil (Squalus suckleyi) which had been held for approximately one year in refrigerated storage. The vitamin A content of this oil was in the medium potency range, being 18,400 units per gram. The other sample used was a distilled fish liver oil concentrate with a declared potency of 198,300 units per gram.

Photometric density readings were made at 325 Mu. and at 328 Mu., as well as at 10 Mu. intervals from 220 to 350 Mu. with the hydrogen discharge lamp and from 300 to 350 Mu. with the tungsten light source. Table 1 (see p. 23) presents ratios of extinction coefficients at a given wave length ($E\lambda$) to those at 328 Mu. (E 328) calculated from absorption readings using both light sources with grayfish liver oil. The ratios determined for the distilled vitamin A concentrate are given in

Table 2 (see p. 23). It will be noted that the ratios from 300 to 350 Mu. are practically the same with both light sources. This is more readily apparent from a graphic presentation of the data in the form of curves given in Figure 1 (see p. 24) and Figure 2. Some of the points on the curves for the different light sources used actually coincide.

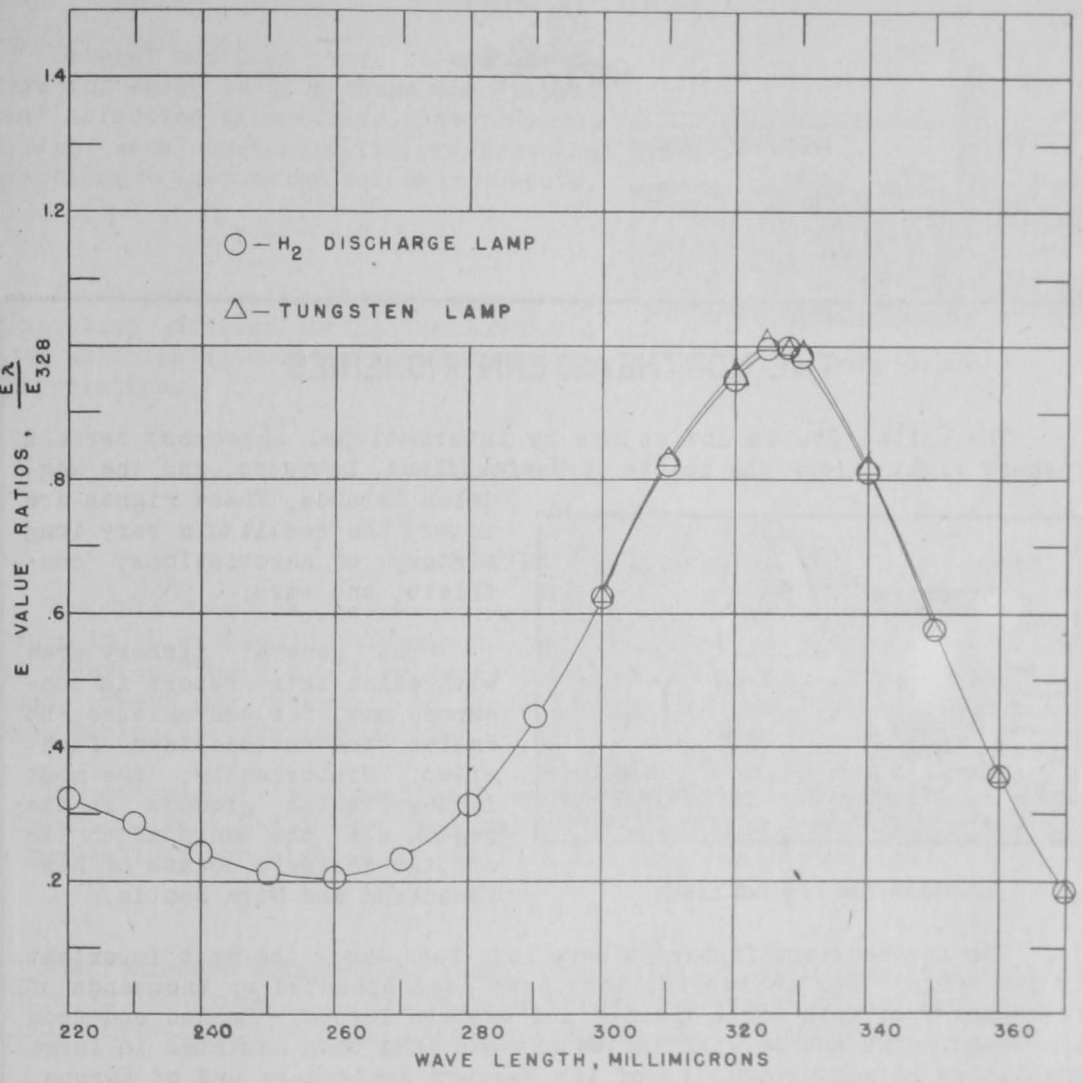

FIGURE 2 - ULTRAVIOLET ABSORPTION CURVES FOR DISTILLED VITAMIN A CONCENTRATE

It was concluded from the data obtained with the two vitamin oils studied that the ratios $\frac{E\ 300}{E\ 328}$ and $\frac{E\ 350}{E\ 328}$ are as reliable when determined with the tungsten lamp as they are with the hydrogen discharge light source.

A comparison of the absorption curves below 300 Mu. for the two oils, Figures 1 and 2, indicates that the lower potency grayfish liver oil possesses considerable absorption below 260 Mu. in the ultraviolet while the curve with distilled vitamin A concentrate showed practically no absorption in this region. This may be due in part to the amount of oil in solution since concentration of the grayfish liver oil in the solution used for making the measurements was approximately 10 times that of the distilled concentrate because of the large difference in vitamin A

potency. This absorption in the far ultraviolet might also be due, in part at least, to substances other than vitamin A found in natural fish oils but not ordinarily present in a distilled vitamin A concentrate. Additional experiments upon other fish liver oils and composites would undoubtedly yield more information on which to base an explanation of these observations.

THE NORTHEASTERN FISHERIES

The United States now enjoys by international agreement certain fishery rights along the coasts of Newfoundland, Labrador, and the Magdalen Islands. These rights are in part the result of a very long history of negotiations, conflicts, and wars.

NORTHEASTERN FISHING BANKS

The general fishery area with which this report is concerned may, for convenience, be called the northeastern fisheries. Historically, the most famous fishing grounds in the region are the banks which lie off the southern coasts of Newfoundland and Nova Scotia.

The northeastern fisheries have long been among the most important in the world. For centuries, they have been operated by thousands of fishermen from both North America and western Europe. Salted cod from the Grand Banks and neighboring waters has long been marketed in large quantities in many countries of the Western Hemisphere and of Europe, and it is a particularly important food staple in the West Indies.

Compared with other economic enterprises, the northeastern fisheries were far more prominent in earlier times than they are now. Although fishing in this area has increased materially in recent decades, there has been a far greater expansion of agriculture, mining, and manufacturing on the North American continent.

--U. S. Tariff Commission, Report No. 152