Effects of Beta-Carotene Isomerization on Its Absorption at 326 Millimicrons

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THE importance of the influence of the carotenoid pigments upon the absorption at 326 mμ is well known to workers who are attempting to estimate vitamin A concentrations by spectrophotometric methods. The usual procedure is to measure the absorption due to carotene at 450 mμ and use a certain percentage of this value as the correction in the ultraviolet for vitamin A determination. Considerable discrepancy exists in these correction values as reported in the literature. Gillam (*4*) reports a factor of 6.5%, Steenbock (*1*) 10%, McNicholas (*5*) 5%, and Peterson (*8*) values ranging from 5 to 8.3%.

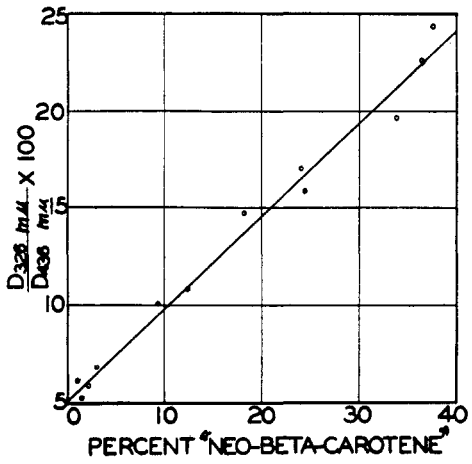

Figure 1. Relation between Optical Density Ratios and Calculated Per Cent Isomerization of β-Carotene

Recent studies on the carotenoids (*2, 10*) have shown that heat is one of the factors responsible for their isomerization. All the methods in general use for vitamin A determination require heating at some place in the procedure. It therefore appeared probable that isomerization phenomena were partially responsible for the range of correction values listed. Accordingly, a series of β-carotene extracts was analyzed spectrophotometrically at wave lengths of 436, 478, and 326 mμ, and the per cent isomerization to "neo-β-carotene" was calculated by the method of Beadle and Zscheile (*2*). The ratios of optical densities at 326 mμ to those at 436 mμ were also calculated. (The optical density is defined by the expression $\frac{\log_{10} \frac{I_0}{I}}{l}$ where I_0 is the intensity of radiation through the solvent, I is the intensity of radiation through the solution, and l is the cell thickness.) The results are shown in Table I. The value of 326 mμ in the ultraviolet has been chosen because the recent work of Zscheile and Henry (*11*) and of Morgareidge (*7*) has shown this to be closer to the absorption maximum of vitamin A than the previously used 328 mμ.

A series of pure β-carotene samples (obtained from Dr. Salmon, Alabama Agricultural Experiment Station) dissolved in redistilled Skellysolve B, had been set aside in tightly stoppered test tubes, in the dark, at different temperatures, in order to study the kinetics of isomerization to the neo-β-carotene reported by Beadle and Zscheile (*2*). When the analyses of these samples were made for the per cent of isomerization to neo-β-carotene the optical density at 326 mμ was also determined.

The carotene was extracted from the alfalfa in a Waring Blendor with an alcohol–Skellysolve B mixture. The extract was filtered, washed with water, extracted three times with Skellysolve B, concentrated to approximately 60 ml., dried with sodium sulfate, and adsorbed on a column of 2 parts of Hyflo Super-Cel and 1 part of magnesia (Micron brand No. 2641). The carotene was separated from the xanthophylls and chlorophylls by elution with a 4% acetone–Skellysolve B solution. This is, essentially, the method of Moore and Ely (*6*), as modified by Wall and Kelley (*9*). This fraction, no doubt, contained a small percentage of α-carotene. Since acetone exhibits considerable absorption at 326 mμ, it was removed from the eluted carotene solution by washing with water. The purified extract was then dried over anhydrous sodium sulfate before making absorption measurements.

Figure 1 shows that a straight-line relationship exists between the extent of β-carotene isomerization and the calculated optical density ratios; and, therefore, explains the apparently anomalous correction values which have been reported. The values obtained in this study include the range of corrections previously reported. It would appear that the density ratio for pure β-carotene is about 5.0% in Skellysolve B. Estimating from the data given by Zechmeister and Polgár (*10*), the value is about 6% in hexane. The equation of the line in Figure 1 is:

$$\frac{D_{326\ m\mu}}{D_{436\ m\mu}} \times 100 = 5.0 + 0.480 \times \%\ \text{"neo-}\beta\text{-carotene"}$$

Care in the use of reported correction values is necessary. The shift in absorption maxima in various solvents is well known. The type of instrument on which the calibration is made is also of importance. Such factors as slit width and scattered radiation will also undoubtedly influence correction values. The data presented here were taken on the Beckman (*3*) spectrophotometer, using slit widths of 0.02 mm. at 478 mμ, 0.04 mm. at 436 mμ, and 0.34 mm. at 326 mμ.

SUMMARY

The isomerization of β-carotene is at least partially responsible for the wide range of correction values reported for vitamin A analysis in the ultraviolet. The correction required at 326 mμ for β-carotene in Skellysolve B has been calculated on the basis of data taken on the Beckman spectrophotometer and shown to be a linear function of the per cent isomerization.

Table I. Effect of β-Carotene Isomerization on Absorption at 326 Mμ

Sample	Optical Density 436 mμ	478 mμ	326 mμ	"Neo-β-Carotene", %	$\frac{D_{326}}{D_{436}} \times 100$
β-Carotene					
Sample 1	0.730	0.781	0.109	18.3	14.9
Sample 2	0.692	0.762	0.075	12.5	10.8
Sample 3	0.611	0.704	0.036	2.2	5.9
Sample 4	0.597	0.690	0.031	1.5	5.2
Sample 5	0.988	1.030	0.156	24.5	15.8
Sample 6	0.900	0.898	0.176	33.8	19.6
Sample 7	0.678	0.779	0.048	3.0	7.1
Sample 8	0.641	0.744	0.040	1.0	6.2
Alfalfa leaf extract	0.805	0.842	0.138	24.0	17.1
Alfalfa leaf extract	0.256	0.286	0.026	9.4	10.1
Alfalfa leaf extract (refluxed 30 hours)	1.293	1.267	0.316	37.5	24.4
Alfalfa leaf extract (refluxed 30 hours)	0.825	0.811	0.187	36.5	22.7

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Apparatus for Rapid Polarographic Analysis

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A VARIETY of different types of cells, each of which has its merits, has been proposed for polarographic analysis (*3*). Fundamentally, these all fall into two categories; (1) those in which a quiet pool of mercury in direct contact with the solution being analyzed constitutes the second electrode, which were recommended originally by Heyrovský and much used in the earlier work with the dropping electrode, and (2) those in which a saturated calomel, or other nonpolarizable reference electrode, is used as the second electrode. For reasons already discussed (*3*) cells of the latter type, such as that described by Lingane and Laitinen (*5*), are preferable for general use, particularly in research studies when the polarographic behavior of a substance is being investigated for the first time. However, for more or less routine analyses of substances of well-known polarographic characteristics cells of the first type often are more convenient. The cell shown in Figure 1 has proved to be very useful for a variety of analyses.

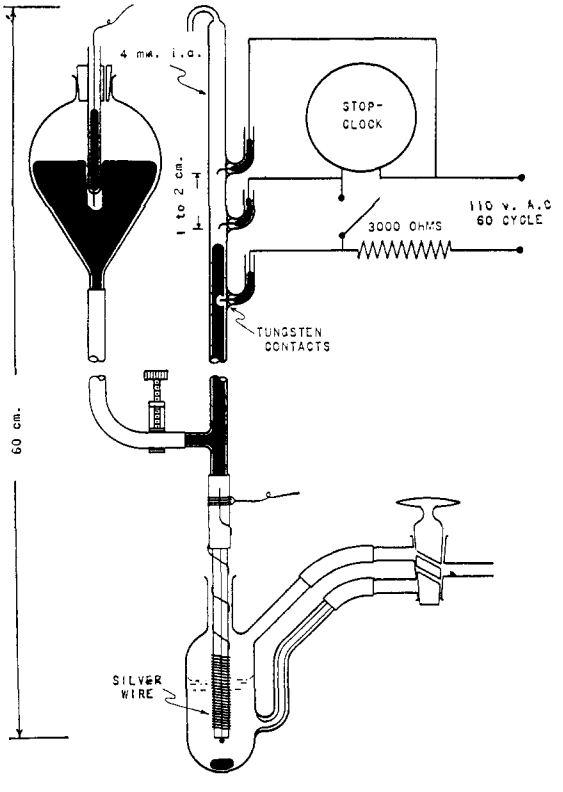

Figure 1. Polarographic Cell

SIMPLIFIED POLAROGRAPHIC CELL

In this simple cell the inconvenient classical mercury pool anode has been replaced by a silver–silver chloride anode, which consists of No. 22 silver wire wound as a tight cylinder directly on the dropping electrode capillary, as shown, with its free end spiraled up to the rubber connecting tube where it is held in place by a wrapping of copper wire. The silver wire cylinder is about 2 cm. long, and its lower end extends to within about 3 or 4 mm. from the tip of the dropping electrode. To ensure a reproducible potential, it is advisable to deposit electrolytically a thin coating of silver chloride on the silver electrode before use. The apparent area of the electrode is about 5 sq. cm., which is amply large to prevent appreciable polarization. As a matter of fact, the area of the electrode immersed in the solution may be as small as 1 sq. cm. without significant polarization occurring with currents of the usual magnitude.

This silver–silver chloride electrode may be employed whenever the solution investigated contains chloride ion, and when it does not contain substances which will dissolve silver chloride (metathesis of the silver chloride to a more insoluble salt is permissible). For example, it may be used with any of the common supporting electrolytes containing alkali or alkaline earth halides, hydrochloric acid, acidic, neutral, or basic tartrate solutions containing chloride ion, sodium hydroxide, in solutions of the tetraalkylammonium halides or hydroxides, etc. The electrode may not be used in ammoniacal solutions, in cyanide solutions, or in general whenever the solution contains substances that form very stable complex ions with silver, because in such cases the silver chloride coating will be dissolved and the polarogram will show a diffusion current of the silver complex. A safe criterion that may be applied in doubtful cases consists of adding a drop or two of 0.1 *N* silver nitrate to about 10 cc. of the solution to be investigated, and if a precipitate forms (it need not be silver chloride) the silver chloride electrode may be used safely.

The potential of the silver–silver chloride electrode in any particular medium may be determined either by direct measurement against the saturated calomel electrode (for which purpose an H-cell with saturated calomel anode, *5*, is convenient), or by comparing the apparent half-wave potential of some substance as determined with the silver–silver chloride anode with the known value referred to the saturated calomel electrode. The potential of the silver–silver chloride electrode is subtracted algebraically from observed half-wave potentials to refer the latter to the standard saturated calomel electrode. In any given chloride-containing medium the potential of the silver–silver chloride electrode is 0.046 volt more negative than that of a calomel electrode in the same solution.