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STUDIES OF REAGENTS FOR COLORIMETRIC DETERMINATION OF VITAMIN A IN FOODS AND FEEDS

by

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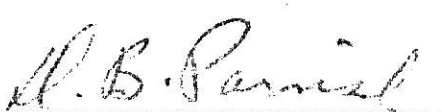

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Introduction

An intense search for simple, sensitive and accurate methods for the determination of vitamin A in biological materials has been underway for the last two decades. Many methods, such as u.v. spectrophotometry, colorimetry, fluorimetry, and polarography have been used and even efforts to apply infrared and nuclear magnetic resonance have been made (1,2). However, for routine analysis of vitamin A in foods and feeds, colorimetry is most feasible at this time. The colorimetric method is simple both in instrumentation and operation and is the method adopted by the Association of Official Analytical Chemists (AOAC). The colorimetric method is relatively accurate, sensitive, and instrumentation is less expensive than other common methods. It is suitable for routine analytical and control procedures in food and feed manufacturing.

The AOAC method uses saturated antimony trichloride solution in chloroform as the colorimetric reagent. The main drawbacks in this method are that the blue color developed starts fading in 3 to 5 seconds, and also, any traces of moisture seriously affect the reagent. Other suitable colorimetric reagents that could be used for vitamin A analysis in foods and feeds are trifluoroacetic acid and trichloroacetic acid (3,4). The color developed in those cases is also unstable but the reagents are not affected by moisture.

The purpose of this work was: (a) to make a systematic study of the blue-color reaction for analysis of vitamin A using selected color-developing reagents and reagent solvents, (b) to observe the effects

of carotenoids (carotenes, monohydroxycarotenoids and dihydroxycarotenoids), sterols (alpha-tocopherol, cholesterol, cholesterol acetate, and diethylstilbesterol), moisture and radiation in the visible region under normal assay conditions, and (c) to determine the most suitable colorimetric reagent for the determination of vitamin A in foods and feeds. The reagents used in this study were antimony trichloride, trichloroacetic acid and trifluoroacetic acid. The solvents were chloroform, 1,2-dichloroethane and dichloromethane.

Review of Literature

Development of colorimetric methods for vitamin A

The earliest mention of a chemical test for the identity of cod-liver oil, based upon the reaction of the oil with sulfuric acid, was in 1880 (5). It appears that this was really a test for vitamin A, used before the vitamin was discovered. Many observations were made that cod-liver oil would give color reactions with various types of reagents such as arsenic trichloride (6), sulfuric acid (7), and antimony trichloride (8). Unknowingly, early analysts were determining identity of cod-liver and similar oils having vitamin A activity by the chemical reaction of the vitamin A present.

The first attempt at developing a true colorimetric test for vitamin A was by Drummond and Watson (9), who observed a parallelism between the intensity of the color given by cod-liver oil reacting with sulfuric acid and the vitamin A potency of the oil in feeding experiments. Until at least 1925 it was not known that the colorimetric test depended on the presence of vitamin A (10). Carr and Price (11) in 1926 studied colorimetric reactions more thoroughly and proposed use of antimony trichloride in chloroform as a quantitative reagent for vitamin A. This test has been more thoroughly investigated than any other, and many conditions relative to use of it have been published. Earlier workers found that the reaction and intensity of blue color were affected by traces of moisture and alcohol, by temperature, by the concentration of antimony trichloride, and by the presence of impurities such as chlorine and phosgene

in the chloroform (8,12-14,16-18). It was found that carotenoids also gave a blue color with antimony trichloride (26). A new color test was devised using catechol to distinguish vitamin A from carotenoid substances (27). However, the Carr-Price method eventually became the colorimetric test for determination of retinol (19), retinal (20), retinoic acid (21), 3-dehydroretinol (22), and other related compounds (19). Tsukida et al. (28), in their studies on degradation products encountered in the vitamin A analysis, tabulated the changes in colors and absorbance maxima for vitamin A compounds when reacted with antimony trichloride.

Earlier attempts to stabilize the blue color that is produced by the Carr-Price reaction were unsuccessful (11). Caldwell and Parrish (24) suggested that the photometers for determining vitamin A by the Carr-Price reaction should employ low intensity of incident light to reduce fading of the blue color to a minimum and make possible more precise photometric readings. Caldwell et al. (25) showed the necessity for calibration of each photometer for the determination of vitamin A, and use of a correction factor for the presence of carotenoids. Several attempts were made to modify the Carr-Price method including the use of catechol (27) and guaiacol (29). Modifications of the antimony trichloride reagent have been reported (30). Dann and Evelyn (31) applied the Evelyn Photoelectric Colorimeter to measure absorbance of the antimony trichloride blue-color reaction. The Evelyn Photoelectric Colorimeter is still a reliable instrument for routine analysis of vitamin A.

Several other color tests for vitamin A were tried by earlier workers (11,32,33), but they have found little use for quantitative procedures

and routine application. Dugan and Frigerio (34) investigated the reactions of various Lewis acids with vitamin A and its derivatives. They have shown that trifluoroacetic acid has sensitivity and specificity similar to that of the antimony trichloride reaction. Using trifluoroacetic acid as the chromogenic agent, Neeld and Pearson (3) devised macro- and micro- methods for the determination of serum vitamin A. It was claimed that trifluoroacetic acid is less toxic than antimony trichloride (3,35) and that it entirely eliminates the moisture problem encountered with antimony trichloride (3). The main drawbacks of the trifluoroacetic acid method are the evanescent nature of the blue color and the extreme volatility of the solvents used (3). Tiews and Zentz (36) reported that continual use of trifluoroacetic acid was rather toxic, causing severe headaches. The trifluoroacetic acid method needs consideration because it was 10% more sensitive than antimony trichloride (5:1 ratio of saturation), and the colored species formed with extracts of saponified oils is slightly more stable than that formed by the latter reagent. Correspondence of the results obtained indicated that the former method substitutes satisfactorily for the latter (35). Recently, Bradley and Hornback (39) reported an improved trifluoroacetic acid method for routine analysis of micro-levels of vitamin A in plasma or serum, claiming reproducibility exceeding that of published procedures.

Among the Lewis acids producing color with vitamin A that were mentioned in earlier literature, trichloroacetic acid is particularly interesting (6). Nogrády (38) indicated that antimony trichloride was replaceable by trichloroacetic acid to give a similar color development.

Karrer and Jucker (37) also have shown that polyene substances give blue or violet colors with trichloroacetic acid. Bayfield (4) described a colorimetric method for the determination of vitamin A in serum and liver using a trichloroacetic acid reagent. The advantages of the method are that the reagent is readily available, is comparatively easy to handle in preparation, and the apparatus may be cleaned easily. Trichloroacetic acid has possible advantages over trifluoroacetic acid and antimony trichloride from a toxicity point of view, and the moisture problem that is encountered with the Carr-Price method is eliminated (4).

Other colorimetric reagents reported in the literature for the estimation of vitamin A are few in number and most of them have not been tried for application to the routine analysis of vitamin A in foods and feeds. Some of the proposed reagents are specific for the isomers of vitamin A. Sobel and Werbin (42) described a method based on the color reaction between vitamin A and activated glycerol dichlorohydrin. Tiews and Zentz (36) used 0.125% ferric chloride in acetyl chloride to determine vitamin A content in blood serum and liver. Craig et al. (15) used a saturated solution of ferrous sulfate in glacial acetic acid as the chromogen. Reagents suggested for the colorimetric determination of vitamin A aldehyde (retinylaldehyde) are thiobarbituric acid (49), o-aminophenol (50), 4-aminobenzoic or 4-aminosalicylic acid in the presence of 2N-hydrochloric acid (51), and 2,4-dinitrophenyl hydrazine (23).

The chemistry of the color-producing reaction

The majority of methods for the estimation of vitamin A are based more on unsaturation than on the single slightly reactive group. The

subject also is complicated by isomers and by precursors such as carotene (40). Kürösy (43) suggested that an interaction between strong acids and carotenoids can be explained by the conjugated chain acting as an electron donor towards a proton or Lewis acid, and as an acceptor towards anions. Wasserman (41) measured light absorption and electrical conductance of vitamin A acetate in benzene with and without trichloroacetic acid and has shown that the polyene is protonated in the presence of acid. Wassermann (41) attempted to find whether the blue-colored derivatives that are obtained in the Carr-Price and trichloroacetic acid reactions are similar species to carbonium-ion pairs. Dugan et al. (35), in studies of the reaction of vitamin A compounds with a number of Lewis acids, found that the trifluoroacetic acid reagent produced a spectrally identical colored species to that formed by antimony trichloride in the Carr-Price reaction. They proposed that the reaction could be a coupling of the empty orbitals of the Lewis acids with the π electrons of the conjugated polyene, followed by dismutation into two charged species. The absorption maxima of the species produced were characteristic of the carotenoid but independent of the Lewis acid. Dugan et al. (35) suggested that the decay of the blue species is promoted by oxygen, perhaps with formation of aliphatic peroxides. Blatz et al. (44,45) studied the carbonium ions generated by vitamin A related polyenes, and characterized the polyenylic cations spectroscopically. Blatz and Pippert (46) suggested that a carbonium-ion intermediate was the new long-wavelength-absorbing species. In a later study Blatz et al. (47) examined spectroscopically the electrochromic species generated by reacting trichloroacetic

acid and by reacting iodine dissolved in 1,2-dichloroethane with retinol and related polymers. They showed that carbonium-ion intermediates were formed during dehydration reactions between vitamin A and the acid. The resulting carbonium ions of a given polyene are identical regardless of whether formed by iodine or trichloroacetic acid. Recently, Blatz and Estrada (48) studied the species formed during the Carr-Price reaction by following the reaction spectroscopically at various temperatures and concentrations. The resulting colored species were found to be retinylic and anhydroretinylic cations identical to those reported in the literature for the reaction of polyenes with acids. The reaction pathway is given in Figure 4, and discussed in a later section.

Materials and Methods

Instruments and apparatus

Evelyn Photoelectric Colorimeter. Honeywell, Model 4600. Filters used – 620 nm and 440 nm.

Matched absorption tubes. 18 x 150 mm, glass tubes.

Chromatography tubes. 15 x 170 mm and 5 x 100 mm, glass tubes.

Refluxing apparatus. All glass T-joint Liebeg condensers and 300 ml boiling flasks. Heat supplied by steam bath.

Separatory funnels. 250 and 500 ml, Pyrex, ground glass stopper and stopcock.

Evaporation assembly. Water aspirator connected to an apparatus holding 2 absorption tubes. Solvent evaporated using 65° water bath and gentle agitation.

Other glassware. Pyrex.

Reagents

Antimony trichloride[1]. Reagent grade. Prepare 20% (w/v) solution in the respective solvent. Add 3.0% acetic anhydride (reagent grade)[2].

Trifluoroacetic acid[2]. Reagent grade. Prepare 1:2 (v/v) solution in the respective solvent.

Trichloroacetic acid[1,3]. Reagent grade; 50 g in 25 ml (w/v) of the respective solvent.

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[1] Mallinckrodt Chemical Works, St. Louis, Mo. 63160, used for most studies.

[2] Eastman Kodak Co., Rochester, N.Y. 14650

[3] Fisher Scientific Co., Chemical Manufacturing Division, Fair Lawn, N.J. 07410

Solvents used for preparing the reagents. Chloroform[4], 1,2-dichloroethane[1] (ethylene dichloride) and dichloromethane[4] (methylene dichloride).

Solvents used for extraction procedures. Hexane[5], methanol (95%), cyclohexane[1] and cyclohexene[6].

Eluant for chromatography. 4, 10, and 20% (v/v) acetone in hexane.

Sodium sulfate[7]. Anhydrous powder, reagent grade.

Potassium hydroxide[1]. Reagent grade. Prepare 40% (w/v) in water just before using.

U.S.P. vitamin A reference standard[8]. 34.4 mg trans-vitamin A acetate (equivalent to 30.00 mg trans-vitamin A alcohol) per gram (100,000 U.S.P. units per gram).

Adsorbent. Silica gel G[3] and Hyflo Super-Cel[9] mixed in 1:1 (w/w) proportion.

Rochelle salt[1]. N.F. powder. Prepare 10% (w/v) solution and use for cleaning glassware used for antimony trichloride reagent.

Sterols. DL-alpha-tocopherol[10], cholesterol[11], cholesteryl acetate[11], and diethylstilbesterol[11]. Prepare 1.0% solution of cholesterol acetate in hexane and 0.1% solution of other sterols in hexane.

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[4] 'Baker Analyzed' Reagent, J. T. Baker Chemical Co., Phillipsburg, N.J. 08865

[5] Skellysolve B commercial hexane. Skelly Oil Co.

[6] Matheson Coleman and Bell, Manufacturing Chemists, Norwood, Ohio 45212

[7] Allied Chemical, General Chemical Division, Morristown, N.J. 07960

[8] U.S. Pharmacopeial Convention, 4630 Montgomery Ave. Bethesda, Md. 20014

[9] Johns-Manville Products Corporation, 22 E. 40th St., New York, N.Y. 10016

[10] General Biochemicals, Laboratory Park, Chagrin Falls, Ohio 44022

[11] Nutritional Biochemical Corporation, Cleveland, Ohio 44128

Methods

Extraction procedures and carotenoid separations. The AOAC method [(52) sections 39.018–39.021] was followed for the extraction and chromatographic separation of carotenoid pigments (carotenes, monohydroxycarotenoids and dihydroxycarotenoids from dried alfalfa leaf. Minor modifications in the meathods were (a) use of proportionately larger sample size and quantities of reagents, and (b) hydrolysis of sample by boiling $\frac{1}{2}$ hr. The carotenoid pigments separated were concentrated separately by evaporation under vacuum and stored as hexane solutions in brown bottles under refrigeration.

Standard curves for vitamin A. Snip off the end of the vitamin A capsule, expel and weigh the solution. Dissolve it in the solvent used and dilute to 250 ml in a volumetric flask. Make 5 or more appropriate dilutions for blue-color reading by Carr-Price or similar type reaction in the photoelectric colorimeter. Measure absorbance for a standard solution in each solvent selected and construct standard curves.

Comparison of stability of color using vitamin A standard solutions. The comparison of stability of blue color produced when vitamin A was reacted with various colorimetric reagents was studied by measuring absorbance at different time intervals and constructing fading curves[1]. The general plan for various reagents and solvents studied is given below.

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[1] Following the conventional use with the Evelyn Photoelectric Colorimeter, the letter L is used for absorbance in the figures.

Reagent used[1]		
I. Antimony trichloride ($SbCl_3$)	in chloroform ($CHCl_3$)	with 3% Ac_2O[2]
"	in 1,2-dichloroethane ($C_2H_4Cl_2$)	" "
"	in dichloromethane (CH_2Cl_2)	without "
II. Trifluoroacetic acid (CF_3COOH)	in chloroform ($CHCl_3$)	without Ac_2O with "
"	in 1,2-dichloroethane ($C_2H_4Cl_2$)	without " with
"	in dichloromethane (CH_2Cl_2)	without "
III. Trichloroacetic acid (CCl_3COOH)	in chloroform ($CHCl_3$)	without Ac_2O with "
"	in 1,2-dichloroethane ($C_2H_4Cl_2$)	without " with "
"	in dichloromethane (CH_2Cl_2)	without "

Comparison of stability of color produced by carotenoids with various reagents. The comparison of initial increase in color development and the stability of the color developed for individual carotenoids (carotenes, monohydroxycarotenoids and dihydroxycarotenoids) when reacted with various blue-color-producing reagents was studied by measuring the absorbance at different time intervals and constructing fading curves for

---

[1] For each determination 9.0 ml of the reagent was added to 1.0 ml of vitamin A in the solvents listed.

[2] Acetic anhydride.

each of the respective carotenoids. One milliliter of the carotenoid solution in hexane was evaporated under reduced pressure and redissolved in the selected solvent (chloroform, 1,2-dichloroethane, or dichloromethane). Nine milliliters of reagent was added to produce the blue color. The general plan followed, reagents used, and carotenoids studied are given below.

I.	$SbCl_3$ in $CHCl_3$	with 3% Ac_2O[1]	with C[2],	MHC[3]	and DHC[4,5]	
	" in $C_2H_4Cl_2$	" "	" "	"	"	
	" in CH_2Cl_2	without "	" "	"	"	
II.	CF_3COOH in $CHCl_3$	without Ac_2O	" "	"	"	
		with "	" "	"	"	
	" in $C_2H_4Cl_2$	without "	" "	"	"	
		with "	" "	"	"	
	" in CH_2Cl_2	without "	" "	"	"	
III.	CCl_3COOH in $CHCl_3$	without Ac_2O	" "	"	"	
		with "	" "	"	"	
	" in $C_2H_4Cl_2$	without "	" "	"	"	
		with "	" "	"	"	
	" in CH_2Cl_2	without "	" "	"	"	

---

[1] Acetic anhydride.

[2] Carotenes.

[3] Monohydroxycarotenoids.

[4] Dihydroxycarotenoids.

[5] Each carotenoid was studied in a separate trial.

Studies on the effects of sterols on the blue color developed by various reagents with vitamin A. The sterols were initially dissolved in hexane. The concentrations of sterol solutions used were:

DL-alpha tocopherol	0.1%
Cholesterol	0.1%
Cholesteryl acetate	1.0%
Diethylstilbesterol	0.1%

The solution of diethylstilbesterol in hexane was made as a saturated solution and was approximately 0.1% concentration.

One milliliter of a sterol solution in hexane was evaporated under vacuum and redissolved in 1.0 ml of the different solvents containing vitamin A. Nine milliliters of the reagent in the same solvent was added rapidly and the absorbance was noted at different time intervals. The general plan followed was to obtain data for the blue-color reaction of vitamin A in the various solvents — one set of data when no sterol was added, and one when each sterol above was added separately as outlined in the following.

	No sterol	With Toc.[1]	With Ch.[2],	With Ch.Ac.[3],	With DES[4]
I. $SbCl_3$ in $CHCl_3$					
" in $C_2H_4Cl_2$	"	"	"	"	"
" in CH_2Cl_2	"	"	"	"	"
II. CF_3COOH in $CHCl_3$	"	"	"	"	"
" in $C_2H_4Cl_2$	"	"	"	"	"
" in CH_2Cl_2	"	"	"	"	"
III. CCl_3COOH in $CHCl_3$	"	"	"	"	"
" in $C_2H_4Cl_2$	"	"	"	"	"
" in CH_2Cl_2	"	"	"	"	"

Comparison of stability of color produced by various reagents with vitamin A in the presence of carotenoids. The carotenoids were present in the following concentrations[5] in hexane.

	µg/ml
Carotenes	10.10
Monohydroxycarotenoids	4.80
Dihydroxycarotenoids	8.30

One milliliter of each carotenoid in hexane was evaporated under reduced pressure in matched tubes. The carotenoid was redissolved in

---

[1] DL-alpha tocopherol.

[2] Cholesterol.

[3] Cholesteryl acetate.

[4] Diethylstilbesterol.

[5] All concentrations measured as carotene at 440 nm.

1.0 ml of selected solvent containing vitamin A. Nine milliliters of the reagent was rapidly added to 1.0 ml of this mixture and absorbance of the color produced was measured at different time intervals to obtain data for fading curves. The selected reagents and the general plan were as follows.

$SbCl_3$ in $CHCl_3$	with C[1],	MHC[2],	and DHC[3,4]
Vitamin A in $CHCl_3$[5]			
$SbCl_3$ in CH_2Cl_2	"	"	"
Vitamin A in CH_2Cl_2[6]			
CF_3COOH in $CHCl_3$	"	"	"
Vitamin A in $CHCl_3$[5]			
CF_3COOH in CH_2Cl_2	"	"	"
Vitamin A in CH_2Cl_2[6]			
CCl_3COOH in $CHCl_3$	"	"	"
Vitamin A in $CHCl_3$[5]			
CCl_3COOH in CH_2Cl_2	"	"	"
Vitamin A in CH_2Cl_2[6]			

---

[1] Carotenes.

[2] Monohydroxycarotenoids.

[3] Dihydroxycarotenoids.

[4] Each vitamin A - carotenoid mixture was studied in a separate trial.

[5] Concentration 2.90 µg/ml.

[6] Concentration 2.78 µg/ml.

Comparison of hydrocarbon sample solvents which might be used both for extraction procedures and analysis of vitamin A. The suitability of hexane, cyclohexane and cyclohexene as solvents for vitamin A without transfer to usual solvents (chloroform, 1,2-dichloroethane and dichloromethane) for colorimetric analysis was studied. One milliliter of hexane containing 2.84 µg vitamin A/ml was evaporated under reduced pressure, redissolved in a test solvent and used for analysis. The general plan was as follows.

	Vitamin A solution[1]	Reagent used[2]
I.	Vitamin A in hexane	$SbCl_3$ in $CHCl_3$ CF_3COOH in $CHCl_3$
II.	Vitamin A redissolved in $CHCl_3$	$SbCl_3$ in $CHCl_3$ CF_3COOH in $CHCl_3$
III.	Vitamin A redissolved in cyclohexane	$SbCl_3$ in $CHCl_3$ CF_3COOH in $CHCl_3$
IV.	Vitamin A redissolved in cyclohexene	$SbCl_3$ in $CHCl_3$ CF_3COOH in $CHCl_3$

[1] The results of I, III and IV were compared with that of II to determine their stability.

[2] 9.0 ml of the reagent was used.

Results and Discussion

Standard curves for vitamin A with various reagents. Standard curves for the determination of vitamin A using various color-producing reagents are given on Plates 1 and 2 of the Appendix. The slopes for different standard curves are presented in Table 1. Trichloroacetic acid dissolved in dichloromethane had the lowest slope, followed by antimony trichloride in dichloromethane. In general, lower slope values signify greater sensitivity, i.e., more color development per unit vitamin A. The slopes tabulated do not deviate markedly from each other. The standard curves for vitamin A using antimony trichloride and trifluoroacetic acid dissolved in chloroform are presented in Figure 1. The similarity of these two standard curves is consistent with the results obtained by Neeld and Pearson (3). Trifluoroacetic acid reagent has proved to be slightly more sensitive than antimony trichloride reagent in this and later work. That is compatible with the observation of Dugan and Fregerio (35) that trifluoroacetic acid reagent is 10% more sensitive than the antimony trichloride reagent.

Stability of the blue color produced by various reagents with vitamin A standard solutions. A comparison of stabilities of the blue color produced by various reagents with vitamin A standard solutions was made by noting the absorbance at different time intervals after adding the color-developing reagent and constructing fading curves. The results are given in the Appendix, Tables 1-23, and corresponding fading curves are shown on Plates 3-5. The initial absorbance reading was at 5 sec

Table 1. The slopes for different standard curves for the determination of vitamin A using various color-producing reagents[a]

Reagent	Solvent			Vit. A in $C_2H_4Cl_2$; reagents in $CHCl_3$
	$CHCl_3$	CH_2Cl_2	$C_2H_4Cl_2$	
$SbCl_3$	14.40	12.86	15.23	13.57
CF_3COOH	13.90	14.17	16.43	14.44
CCl_3COOH	- -	12.58	13.79	14.70

[a] Calculated from curves on Plates 1 and 2, Appendix

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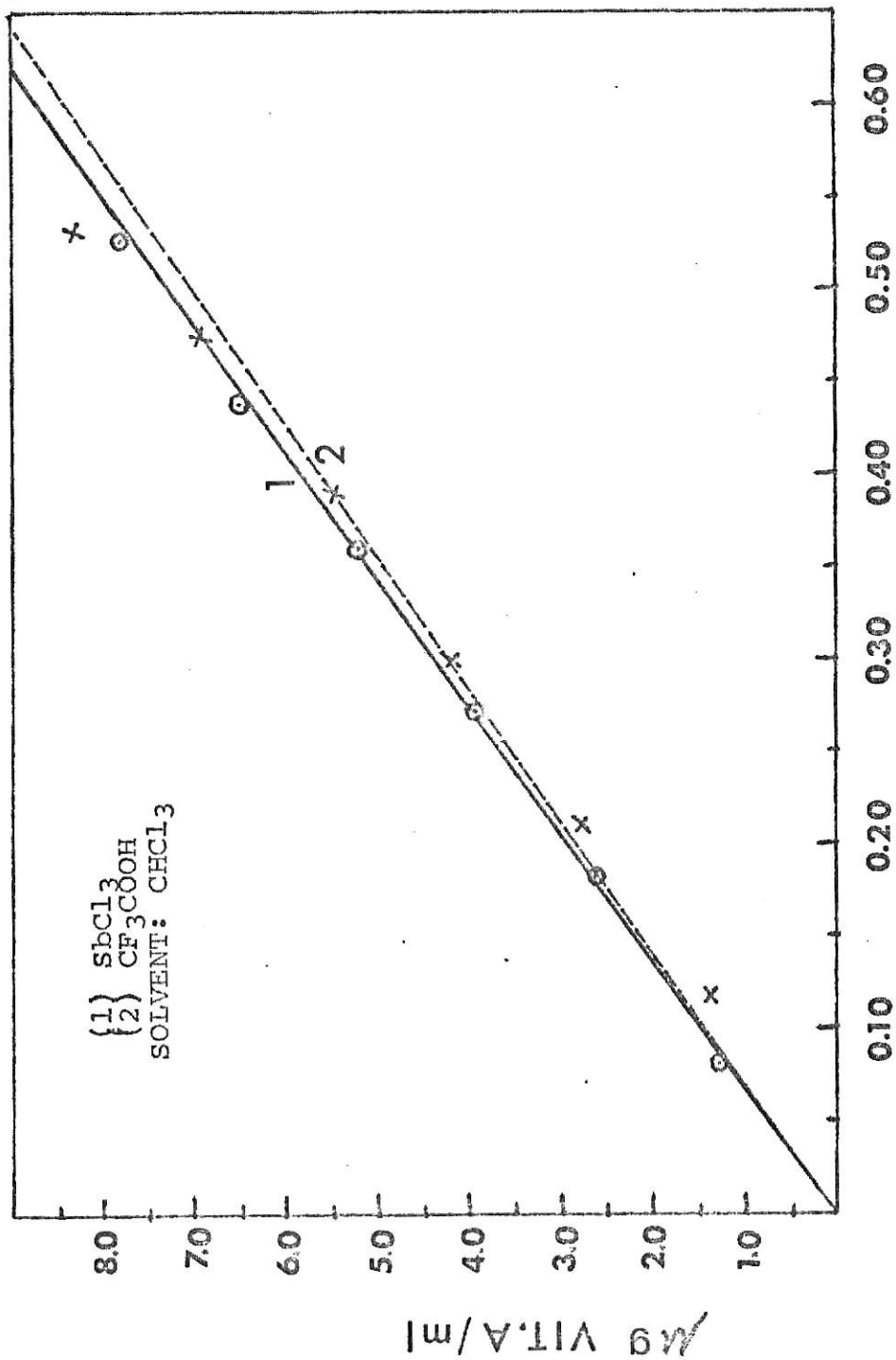

FIG.1. STANDARD CURVES FOR VITAMIN A USING DIFFERENT COLOR DEVELOPING REAGENTS

and the second at 15 sec, after addition of the reagent (9.0 ml) to vitamin A solution (1.0 ml) in the matched tube placed in the photoelectric colorimeter. The third reading was taken at 30 sec and subsequent measurements at intervals of 30 sec to 4 min total time.

Trifluoroacetic acid dissolved in dichloromethane produced the most stable blue color with vitamin A (Table 14 and Plate 5, Appendix). There was a change of about 0.02 in absorbance three minutes after the addition of this reagent. The next most stable color was produced by trichloroacetic acid in dichloromethane. The intensity of initial blue color produced was greater for trichloroacetic acid reagent than that for trifluoroacetic acid reagent when dichloromethane was the solvent (Figure 2). In view of the intensity of initial blue color and greater stability of the color, trichloroacetic acid in dichloromethane might be a better reagent for the determination of vitamin A than antimony trichloride in chloroform. Trifluoroacetic acid in each of the solvents (chloroform, dichloromethane and 1,2-dichloroethane) produced a more stable color compared to antimony trichloride in the same solvents.

Trichloroacetic acid in chloroform produced a highly unstable color which faded rapidly. Bayfield (4) used trichloroacetic acid in chloroform for determining vitamin A in serum and liver. The quantity of trichloroacetic acid reagent used by Bayfield (4) was one-third that used in the present study. The quantity of reagent added appears to be an important consideration. It also is important that the colorimetric reagent added mix instantaneously with the vitamin A solution for maximum intensity of the initial blue color. The absorbance of the initial blue

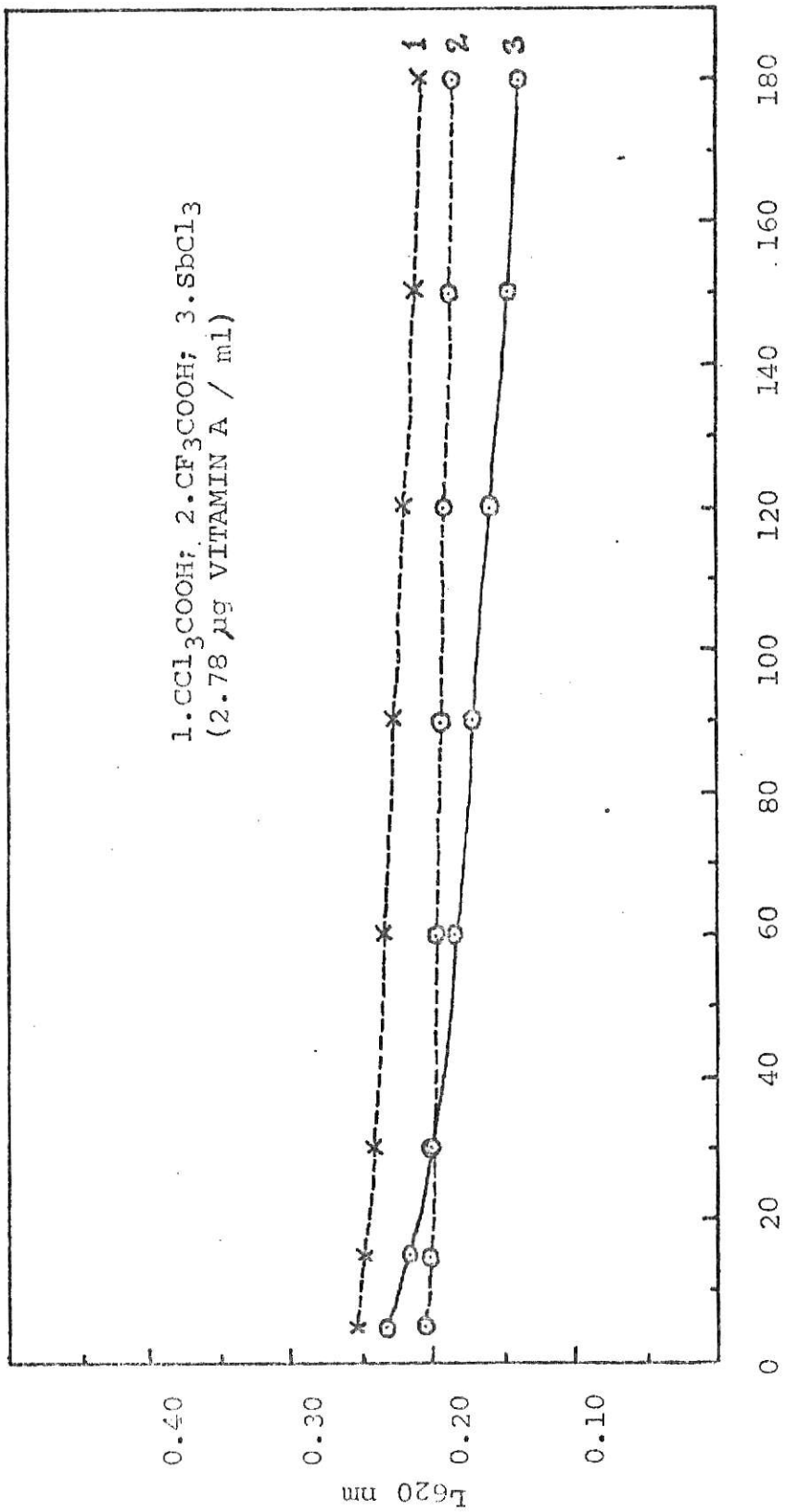

FIG. 2. FADING CURVES FOR THE BLUE-COLOR REACTION OF VITAMIN A IN CH_2Cl_2 WITH THREE REAGENTS

color is measured before rapid fading commences to determine the vitamin A present in the solution.

The addition of small quantities of acetic anhydride to the reagents prior to use. The present of 3% acetic anhydride in antimony trichloride reagent in chloroform (52) is advantageous in protecting the reagent from traces of moisture, especially troublesome when humidity is high[1]. The moisture problem does not occur with either trifluoroacetic acid- or trichloroacetic acid-in-chloroform reagents (3,4). In the present determinations, one drop of acetic anhydride was added to the sample solution containing vitamin A just before addition of the reagent. The presence of acetic anhydride appeared unnecessary and did not affect the results of some of the reagents (Plates 3-5, Appendix). When 3% acetic anhydride was added to reagents prepared with antimony trichloride or trichloroacetic acid in dichloromethane and stored for more than one week, they became unusable and developed a reddish-brown color. To overcome the difficulties in the case of those two reagents 3% acetic anhydride was not included in the reagent but a drop of acetic anhydride was added to the sample solution just prior to determination. This was advantageous in the case of antimony trichloride reagent most of the time and for trichloroacetic acid reagent when carotenoids were present. Antimony trichloride reagent with 3% acetic anhydride was stable for several days when the solvent was chloroform or 1,2-dichloroethane. Turbidity problems due to moisture did not arise at any time with trifluoroacetic

---

[1] Previous experience in this laboratory.

acid reagent prepared in any of the solvents used, and addition of acetic anhydride did not seem to be of any advantage.

The influence of carotenes, monohydroxycarotenoids and dihydroxycarotenoids on color production and stability. Results from studies on the color produced by carotenoids with various reagents used for determination of vitamin A are presented in Tables 24-62 and Plates 6 and 7 of the Appendix. All the carotenoids reacted with the various colorimetric reagents used to produce bluish colors measurable at 620 nm. However, the behavior of individual carotenoids in producing the maximum color intensity and fading is different from that produced by vitamin A. After the addition of the reagent to carotenoid solutions an initial rapid increase of color was observed, followed by a slow fading. This initial increase of color could not be seen within the first 5 sec when the reagent was reacted with vitamin A alone. (Plates 3-7, Appendix). The blue color produced by vitamin A appeared to have maximum intensity of color initially at approximately 5 sec after the addition of the reagents and then started fading slowly. Among the three different carotenoid groups studied (carotenes, monohydroxycarotenoids and dihydroxycarotenoids), in general, dihydroxycarotenoids produced an increase of initial color which in some cases continued to 60 sec (Plate 6, Appendix). The initial increase in color intensity produced by carotenoids when reacted with the trichloroacetic acid and trifluoroacetic acid reagents in dichloromethane was relatively low and would be advantageous in vitamin A determinations when carotenoids are present. Hence, these reagents were included for further studies involving mixtures of carotenoids and vitamin A.

Studies with vitamin A and carotenoid mixtures. The observations made on the color produced by vitamin A and carotenoid mixtures reacted with colorimetric reagents and changes in the color with time are given in Tables 108-125 and Plate 8 in the Appendix. Vitamin A solutions, containing individual carotenoids in either chloroform or dichloromethane, were reacted with the colorimetric reagents, antimony trichloride, trifluoroacetic acid or trichloroacetic acid. The initial absorbances obtained for the carotenoid and vitamin A mixtures (taken from Tables 108-125, Appendix) are summarized in Table 2. The intensity of initial blue color produced when trichloroacetic acid in dichloromethane was reacted with carotenoid-vitamin A mixtures most closely resembled intensity of the initial color produced by antimony trichloride in dichloromethane. Those two reagents produced more intense initial color than the other reagents studied (Table 2). When chloroform was the solvent, the initial color produced by trifluoroacetic acid and trichloroacetic acid was lower than the initial color produced by the antimony trichloride reagent.

The use of dichloromethane as a solvent for trichloroacetic acid remarkably improved the chromogenicity of that reagent. Trichloroacetic acid in dichloromethane also produced a more stable color when this reagent was reacted with vitamin A alone (Plate 5, Appendix). A further advantage of the latter reagent is that when carotenoids were reacted with the reagent, there was a lesser build up of color initially than was the case with all the other reagents (Plate 7, Appendix). However, when this reagent was reacted with a mixture of carotenoids and vitamin A, the stability of color produced was not greater than that produced by antimony

Table 2. The initial absorbances at 620 nm obtained for carotenoid[a] and vitamin A[b] mixtures reacted with selected blue color producing reagents in solvents indicated

Carotenoid and vitamin A mixture	$SbCl_3$		CF_3COOH		CCl_3COOH	
	in $CHCl_3$	in CH_2Cl_2	in $CHCl_3$	in CH_2Cl_2	in $CHCl_3$	in CH_2Cl_2
Carotenes and vitamin A	0.349	0.387	0.337	0.357	0.260	0.385
Monohydroxy-carotenoids and vitamin A	0.359	0.398	0.328	0.347	0.252	0.382
Dihydroxy-carotenoids and vitamin A	0.417	0.409	0.357	0.385	0.286	0.409

[a] Carotenes, 10.10 μg/ml; monohydroxycarotenoids, 4.80 μg/ml; and dihydroxycarotenoids, 8.30 μg/ml

[b] Concentration of vitamin A in $CHCl_3$, 2.90 μg/ml; in CH_2Cl_2, 2.78 μg/ml

trichloride in dichloromethane (Tables 111-113 and 123-125, Appendix). To eliminate the turbidity of solutions, both reagents required the addition of acetic anhydride (one drop) prior to the colorimetric analysis of vitamin A present in the carotenoid mixture. The determination of vitamin A when present in admixture with dihydroxycarotenoids required a little more acetic anhydride (one more drop) to eliminate turbidity when antimony trichloride or trichloroacetic acid were used in dichloromethane. Both of these reagents appeared to be suitable for the determination of vitamin A present in carotenoid mixtures only when acetic anhydride was added just before the determination. The trifluoroacetic acid reagent did not create any turbidity problems when reacted with vitamin A mixtures.

Influence of sterols, radiation in the visible region, and moisture on the stability of color produced by various blue-color-producing reagents when reacted with vitamin A. The influence of sterols (DL-alpha-tocopherol, cholesterol, cholesteryl acetate and diethylstilbesterol) on the blue color produced by various reagents with vitamin A is shown in Table 3, Figure 3, and Tables 63-107 in the Appendix. The stability of color produced by vitamin A with trichloroacetic acid dichloromethane was influenced less by the presence of sterols, especially in the case of DL-alpha-tocopherol, than was color produced by the other reagents. When the solvent was 1,2-dichloroethane, sterols, in general, caused slightly more rapid fading of the blue color. The presence of cholesterol, cholesteryl acetate and diethylstilbesterol did not markedly influence the stability of the blue color produced by antimony trichloride in chloroform.

The effect of radiation in the visible region on selected reagents

Table 3. Influence of sterols on stability of the blue color produced by various reagents reacted with vitamin A[a]

Sterols	$SbCl_3$ in			CF_3COOH in			CCl_3COOH in		
	$CHCl_3$[b]	$C_2H_4Cl_2$[c]	CH_2Cl_2[d]	$CHCl_3$[b]	$C_2H_4Cl_2$[c]	CH_2Cl_2[d]	$CHCl_3$[b]	$C_2H_4Cl_2$[c]	CH_2Cl_2[d]
No sterols added	0.135	0.08	0.095	0.02	0.05	0.02	0.025	0.04	0.05
Cholesterol	0.133	0.096	0.092	0.02	0.06	0.04	0.03	0.06	0.06
Cholesteryl acetate	0.139	0.09	0.105	0.02	0.07	0.04	0.02	0.06	0.06
Diethyl-stilbesterol	0.148	0.11	0.107	0.05	0.07	0.05	0.02	0.07	0.08
DL-alpha-tocopherol	0.197	0.10	0.092	0.07	0.06	0.025	0.04	0.06	0.08

[a] Values are change (decrease) in absorbance three minutes after the addition of reagent

[b] 2.90 µg vitamin A/ml chloroform

[c] 2.86 µg vitamin A/ml 1,2-dichloroethane

[d] 2.78 µg vitamin A/ml dichloromethane

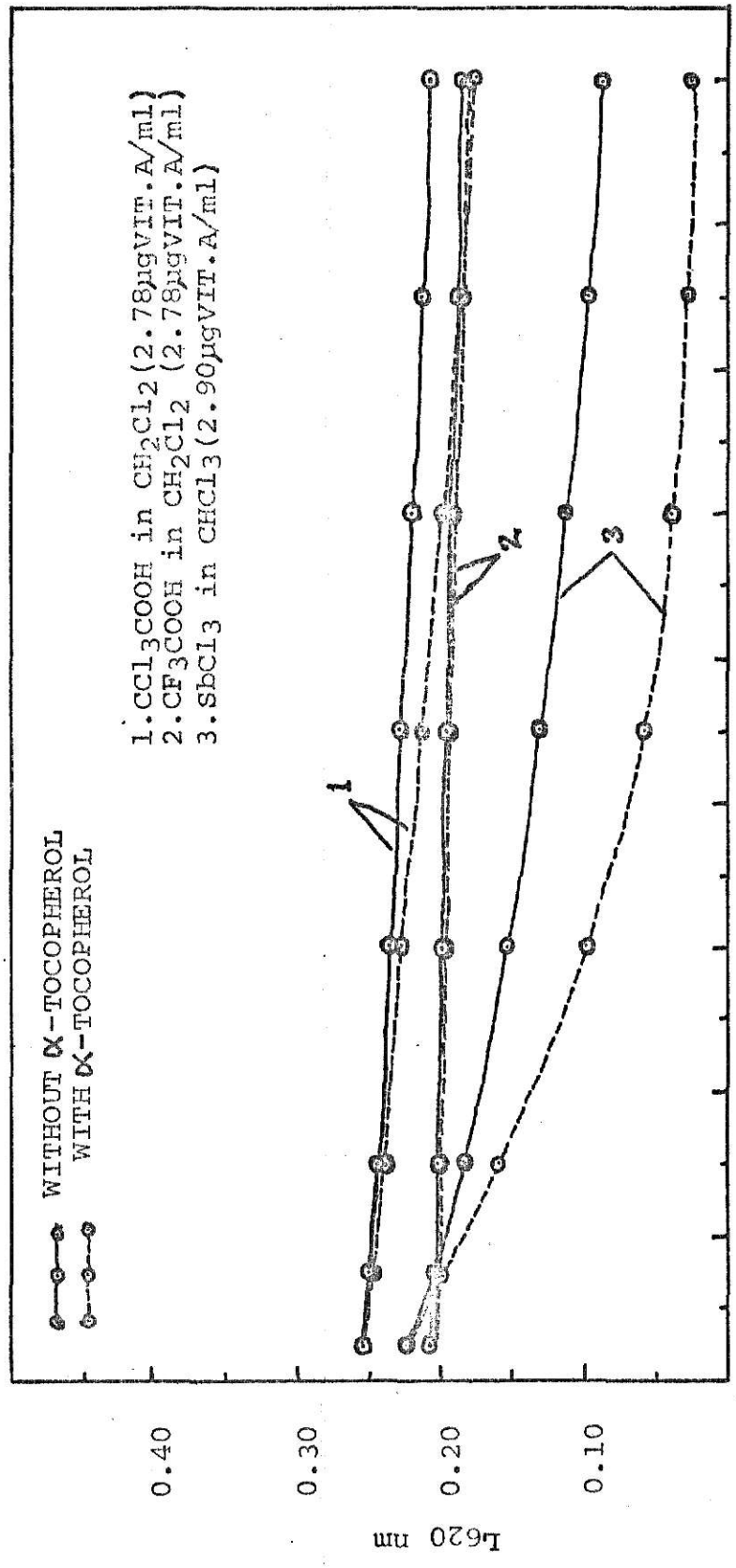

FIG.3. FADING CURVES FOR VITAMIN A IN SOLVENTS AND REAGENTS INDICATED.
EFFECT OF 0.10% DL-ALPHA TOCOPHEROL ON FADING

was studied. The stabilities of blue color produced by the reagents when reacted with vitamin A were compared by measuring absorbance at different times. The results are presented in Tables 134-143 in the Appendix. The trichloroacetic acid reagent in chloroform exposed to fluorescent light (daylight bulb) for 26 hr under ordinary laboratory conditions lost the capacity to produce blue color with vitamin A. This observation was in agreement with the finding of Bayfield (4) with the same reagent. When trichloroacetic acid was dissolved in dichloromethane and exposed to fluorescent light (daylight bulb) for about 24 hr, no decrease in absorbance of the blue color produced with vitamin A was observed. This reagent also retained its chromogenicity when kept under subdued fluorescent light (daylight bulb) and in a colorless glass bottle for about four weeks.

Antimony trichloride reacts with even traces of water and yields insoluble antimony oxychloride, causing turbidity during analysis which makes measurement of the blue color impossible and forms opaque films which are difficult to remove (35). Trifluoroacetic acid is water soluble and does not form insoluble films or turbid solutions on contact with water. The stability of color produced by trichloroacetic acid reagent with vitamin A in the presence of moisture was studied and the results are given in Tables 144 and 145 in the Appendix. The intensity of initial blue color produced was not affected by the presence of moisture, which is consistent with the observation of Bayfield (4). However, the rate of fading when moisture was present (one drop of water added prior to analysis) appeared to be slightly greater.

Other solvents suitable for both extraction and analysis of vitamin A. In the routine analysis of vitamin A, the hydrocarbon solvent hexane, containing vitamin A, was evaporated under reduced pressure and the vitamin was redissolved in one of the solvents (chloroform, dichloromethane or 1,2-dichloroethane) for the purpose of colorimetric determination. The suitability of hexane and other solvents such as cyclohexane and cyclohexene for use in the analysis of vitamin A, without transfer of the vitamin to chloroform, dichloromethane or 1,2-dichloroethane, was tested using antimony trichloride and trifluoroacetic acid reagents. The intensity of blue color produced in the determination was compared with the result obtained when vitamin A was transferred to chloroform (Table 4). The stability of such a color as a function of time can be compared using the data in Tables 126-133 in the Appendix. In general, hexane and cyclohexane appeared to be suitable solvents for the analysis of vitamin A without transferring to chloroform, at least with the concentrations and other conditions studied. Sato (53) determined vitamin A using cyclohexane for the extraction procedure. Recently, Grimm and Tiews (54) reported what they called an improved method of vitamin A determination in foods using a 1,2-dichloroethane extraction.

Cyclohexene produced not only a lower intensity of initial blue color but the color produced was also more unstable. Due to those reasons and others, such as production of secondary color on standing and unpleasant odor, cyclohexene is regarded as unsuitable for the colorimetric analysis of vitamin A.

Table 4. Maximum absorbance[a] for the blue color reaction of vitamin A[b] with the reagents[c] and sample solvents indicated

Sample solvent	Absorbance	
	$SbCl_3$ in $CHCl_3$	CF_3COOH in $CHCl_3$
Chloroform	0.179	0.226
Hexane	0.174	0.216
Cyclohexane	0.174	0.226
Cyclohexene	0.152	0.197

[a] 620 nm

[b] 2.84 µg/ml

[c] 9.0 ml reagent directly added to 1.0 ml of the solvent containing vitamin A

The blue-colored species in the vitamin A reaction — absorption maxima, stability, chemical nature and reaction pathways. The reaction pathway proposed by Blatz and Estrada (48) for the production of colored species from retinol or retinyl acetate reacted with antimony trichloride is given in Figure 4. Retinol (I) absorbs at about 325 nm. It is dehydroxylated by antimony trichloride to produce the retinylic cation (IIa and IIb) which absorbs at about 587 nm, and a complex antimony anion. The retinylic cation loses a proton to form anhydroretinol (III) which absorbs at about 368 nm. Anhydroretinol (III) can act as a base and add the acid antimony trichloride to either end of the polyene chain. If antimony trichloride adds to C-4, a new retinylic cation species (Va,Vb) is formed. Apparently, this absorbs at about 586 nm. On the other hand, antimony trichloride could add to C-15 to produce the anhydroretinylic cation (IVa,IVb) absorbing at about 619 nm. Since the products formed are mainly anhydroretinylic cations (absorption 619 nm) with a smaller amount of retinylic cation (absorption about 586 nm)(48), the intensity of blue color was measured at 620 nm in the present study.

The rapid fading of the blue color observed in the present study and similar vitamin A studies might be explained by the proposal of Blatz and Pippert (46) that the cation formed could cyclize to produce a tetraenylic species which undergoes a slow polymerization spreading the absorbance over ultraviolet, visible and near-infrared regions of the spectrum. The trifluoroacetic acid reagent produces spectrally identical colored species to those formed by antimony trichloride (35). In the present study, in general, the blue species formed with trifluoroacetic

Figure 4. Reaction pathway in the Carr-Price reaction (48). Retinol (I) is dehydroxylated to give the retinylic cation (IIa,IIb, λ_{max} 586 nm); II deprotonates to give anhydroretinol (III, λ_{max} 368 nm); III adds antimony trichloride at C-4 to give the new retinylic cation (Va,Vb λ_{max} 619 nm), or at C-15 to give the anhydroretinylic cation (IVa,IVb, λ_{max} 586 nm).

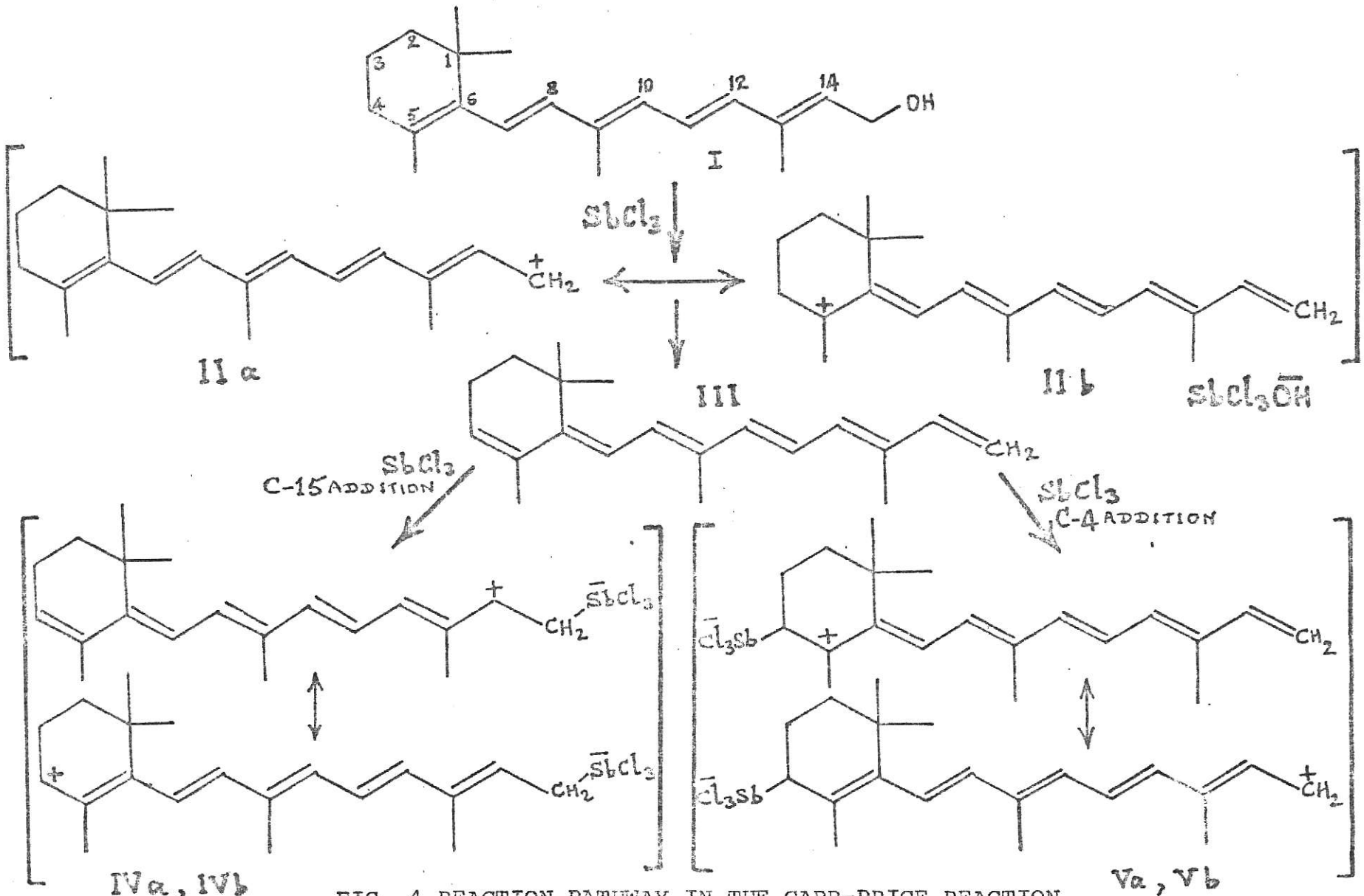

FIG. 4. REACTION PATHWAY IN THE CARR-PRICE REACTION

acid was slightly more stable than species formed with antimony trichloride. That observation is consistent with those of other workers (35). Measurement of the absorbance of the species at 620 nm also is justified on the basis of earlier studies (3,20,35).

Application of findings to routine vitamin A analysis. Trichloroacetic acid in dichloromethane produced somewhat higher absorbance and stable color, is less affected by moisture than antimony trichloride in chloroform, and at present is less costly. Thus it possibly is a better reagent for the routine determination of vitamin A in foods and feeds than antimony trichloride in chloroform. To compare the suitability of the better colorimetric reagents investigated in the present study for routine vitamin A determinations, a technician in the laboratory determined vitamin A in samples of foods and feeds, including liquid feed supplement, mixed animal feed, breakfast cereal, instant breakfast drink mix, and a pet food. The analytical results on all products were similar regardless of the reagent used — antimony trichloride in chloroform or dichloromethane, or trichloroacetic acid and trifluoroacetic acid in dichloromethane.

Summary

A systematic study of the blue-color reaction for analysis of vitamin A was made using three color-developing reagents (antimony trichloride, trifluoroacetic acid, and trichloroacetic acid) and three solvents (chloroform, dichloromethane, and 1,2-dichloroethane). Observations were made on the effects of carotenoids (carotenes, monohydroxycarotenoids, and dihydroxycarotenoids), sterols (alpha-tocopherol, cholesterol, cholesteryl acetate, and diethylstilbesterol), moisture, and radiation in the visible region on the color reaction under normal assay conditions to find the most suitable colorimetric reagent for the determination of vitamin A in foods and feeds. In general, trifluoroacetic acid, trichloroacetic acid and antimony trichloride in dichloromethane produced the most stable colors. Trichloroacetic acid in dichloromethane produced the highest intensity of blue color. Trifluoroacetic acid in dichloromethane was a relatively good reagent, but it produced a slightly lower intensity of blue color than trichloroacetic acid in dichloromethane. The analytical results on foods and feeds, including liquid feed supplement, mixed animal feed, breakfast cereal, instant breakfast drink mix, and a pet food were similar regardless of the reagent used - antimony trichloride in chloroform or dichloromethane, or trichloroacetic acid and trifluoroacetic acid in dichloromethane.

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APPENDIX

Appendix Tables 1-23. Comparison of stabilities of the blue color produced by various reagents with vitamin A standard solutions.

Table 1. $SbCl_3$ in $CHCl_3$-with 3% Ac_2O

Time (sec.)	% T	L_{620}
Start	67.00	0.174
15	68.50	0.164
30	71.00	0.149
60	75.00	0.125
90	78.50	0.105
120	82.00	0.086
150	84.75	0.072
180	87.00	0.060
210	89.00	0.050
240	90.75	0.042

Concn: 2.88 µg vit. A/ml

Table 2. $SbCl_3$ in $CHCl_3$-with 3% Ac_2O

Time (sec.)	% T	L_{620}
Start	47.50	0.323
15	50.50	0.297
30	54.00	0.268
60	60.00	0.222
90	65.50	0.184
120	70.00	0.155
150	74.00	0.131
180	77.25	0.112
210	80.00	0.097
240	82.25	0.085

Concn: 5.76 µg vit. A/ml

Table 3. $SbCl_3$ in $C_2H_4Cl_2$-with 3% Ac_2O

Time (sec.)	% T	L_{620}
Start	67.25	0.172
15	69.00	0.161
30	72.00	0.143
60	76.50	0.116
90	80.25	0.096
120	83.50	0.078
150	86.00	0.066
180	88.00	0.056
210	89.50	0.048
240	91.00	0.041

Concn: 2.76 µg vit. A/ml

Table 4. $SbCl_3$ in $C_2H_4Cl_2$-with 3% Ac_2O

Time (sec.)	% T	L_{620}
Start	46.25	0.335
15	49.00	0.310
30	53.00	0.276
60	60.50	0.218
90	67.00	0.174
120	73.00	0.137
150	77.75	0.109
180	82.00	0.086
210	85.00	0.071
240	87.75	0.057

Concn: 5.53 µg vit. A/ml

Table 5. $SbCl_3$ in CH_2Cl_2-without Ac_2O

Time (sec.)	% T	L_{620}
Start	58.50	0.233
15	60.75	0.216
30	62.75	0.202
60	65.50	0.184
90	67.50	0.171
120	69.50	0.158
150	71.25	0.147
180	72.75	0.138

Concn: 2.78 µg vit. A/ml

Table 6. CF_3COOH in $CHCl_3$-without Ac_2O

Time (sec.)	% T	L_{620}
Start	69.00	0.161
15	70.00	0.155
30	71.50	0.146
60	74.00	0.131
90	76.25	0.118
120	78.50	0.105
150	80.50	0.094
180	82.25	0.085
210	84.00	0.076
240	85.50	0.068

Concn: 2.88 µg vit. A/ml

Appendix Tables 1-23 (contd)

Table 7. CF_3COOH in $CHCl_3$-without Ac_2O

Time (sec.)	% T	L_{620}
Start	45.25	0.344
15	46.50	0.332
30	48.50	0.314
60	51.75	0.286
90	55.25	0.258
120	58.00	0.237
150	60.75	0.216
180	62.75	0.202
210	64.75	0.189
240	66.50	0.177

Concn: 5.41 µg vit. A/ml

Table 8. CF_3COOH in $CHCl_3$-with Ac_2O

Time (sec.)	% T	L_{620}
Start	69.00	0.161
15	70.50	0.152
30	71.00	0.149
60	73.75	0.132
90	76.50	0.116
120	79.00	0.102
150	81.50	0.089
180	83.25	0.080
210	85.00	0.071
240	86.50	0.063

Concn: 2.88 µg vit. A/ml

Table 9. CF_3COOH in $CHCl_3$-with Ac_2O

Time (sec.)	% T	L_{620}
Start	45.00	0.347
15	46.50	0.332
30	48.25	0.317
60	51.75	0.286
90	55.50	0.256
120	58.75	0.231
150	61.50	0.211
180	64.25	0.192
210	66.25	0.179
240	68.25	0.166

Concn: 5.41 µg vit. A/ml

Table 10. CF_3COOH in $C_2H_4Cl_2$-without Ac_2O

Time (sec.)	% T	L_{620}
Start	71.00	0.149
15	71.25	0.147
30	72.50	0.140
60	75.00	0.125
90	77.50	0.111
120	80.00	0.097
150	82.50	0.084
180	84.25	0.074
210	85.50	0.068
240	87.00	0.061

Concn: 2.76 µg vit. A/ml

Table 11. CF_3COOH in $C_2H_4Cl_2$-without Ac_2O

Time (sec.)	% T	L_{620}
Start	49.00	0.310
15	50.50	0.297
30	53.00	0.276
60	57.00	0.244
90	60.00	0.222
120	63.50	0.197
150	67.50	0.171
180	70.00	0.155
210	73.00	0.137
240	75.50	0.122

Concn: 5.53 µg vit. A/ml

Table 12. CF_3COOH in $C_2H_4Cl_2$-with Ac_2O

Time (sec.)	% T	L_{620}
Start	70.50	0.152
15	71.00	0.149
30	72.75	0.138
60	75.25	0.124
90	78.00	0.108
120	80.25	0.096
150	82.25	0.085
180	84.25	0.074
210	86.00	0.066
240	87.25	0.059

Concn: 2.76 µg vit. A/ml

Appendix Tables 1-23 (contd)

Table 13. CF_3COOH in $C_2H_4Cl_2$-with Ac_2O

Time (sec.)	% T	L_{620}
Start	48.75	0.312
15	50.75	0.295
30	53.00	0.276
60	57.25	0.242
90	61.50	0.211
120	65.25	0.186
150	68.75	0.163
180	72.00	0.143
210	74.75	0.126
240	77.50	0.111

Concn: 5.53 µg vit. A/ml

Table 14. CF_3COOH in CH_2Cl_2-without Ac_2O

Time (sec.)	% T	L_{620}
Start	62.25	0.206
15	62.75	0.202
30	63.00	0.201
60	63.50	0.197
90	64.00	0.194
120	64.50	0.191
150	65.00	0.187
180	65.50	0.184

Conc: 2.78 µg vit. A/ml

Table 15. CCl_3COOH in $CHCl_3$-without Ac_2O

Time (sec.)	% T	L_{620}
Start	76.00	0.119
15	88.50	0.053
30	90.75	0.042
60	93.25	0.030
90	94.75	0.024
120	95.75	0.019
150	96.50	0.016

Concn: 2.70 µg vit. A/ml

Table 16. CCl_3COOH in $CHCl_3$-without Ac_2O

Time (sec.)	% T	L_{620}
Start	59.00	0.229
15	79.00	0.102
30	84.50	0.073
60	88.50	0.053
90	91.50	0.039
120	93.75	0.028
150	95.50	0.020
180	96.50	0.016

Concn: 5.41 µg vit. A/ml

Table 17. CCl_3COOH in $CHCl_3$-with Ac_2O

Time (sec.)	% T	L_{620}
Start	76.75	0.115
15	90.00	0.046
30	92.50	0.034
60	95.00	0.022
90	96.50	0.016

Concn: 2.70 µg vit. A/ml

Table 18. CCl_3COOH in $CHCl_3$-with Ac_2O

Time (sec.)	% T	L_{620}
Start	59.00	0.229
15	79.00	0.102
30	83.00	0.081
60	88.25	0.054
90	91.75	0.037
120	94.00	0.027
150	95.50	0.020
180	96.25	0.017

Concn: 5.41 µg vit. A/ml

Appendix Tables 1-23 (contd)

Table 19. CCl_3COOH in $C_2H_4Cl_2$-without Ac_2O

Time (sec.)	% T	L_{620}
Start	70.00	0.155
15	70.25	0.153
30	72.00	0.143
60	74.75	0.126
90	77.75	0.109
120	80.25	0.096
150	82.25	0.085
180	84.25	0.074
210	86.00	0.064
240	88.00	0.0543

Concn: 2.76 µg vit. A/ml

Table 20. CCl_3COOH in $C_2H_4Cl_2$-without Ac_2O

Time (sec.)	% T	L_{620}
Start	45.00	0.347
15	46.75	0.330
30	48.75	0.312
60	52.50	0.280
90	56.25	0.250
120	59.75	0.224
150	62.75	0.202
180	65.25	0.186
210	68.00	0.168
240	70.00	0.155

Concn: 5.41 µg vit. A/ml

Table 21. CCl_3COOH in $C_2H_4Cl_2$-with Ac_2O

Time (sec.)	% T	L_{620}
Start	69.00	0.161
15	69.75	0.157
30	71.25	0.147
60	74.00	0.131
90	76.75	0.115
120	79.00	0.102
150	81.00	0.092
180	83.00	0.081
210	84.25	0.074
240	85.75	0.067

Concn: 2.76 µg vit. A/ml

Table 22. CCl_3COOH in $C_2H_4Cl_2$-with Ac_2O

Time (sec.)	% T	L_{620}
Start	45.00	0.347
15	47.75	0.321
30	50.00	0.301
60	54.25	0.266
90	58.00	0.237
120	61.75	0.209
150	64.75	0.189
180	67.50	0.171
210	70.00	0.155
240	72.25	0.141

Concn: 5.41 µg vit. A/ml

Table 23. CCl_3COOH in CH_2Cl_2-without Ac_2O

Time (sec.)	% T	L_{620}
Start	55.75	0.254
15	56.25	0.250
30	57.00	0.244
60	58.00	0.234
90	59.25	0.227
120	60.25	0.220
150	61.25	0.213
180	62.25	0.206

Concn: 2.78 µg vit. A/ml

Appendix Tables 24-62. Studies with carotenes (C), monohydroxycarotenoids (MHC) and dihydroxycarotenoids (DHC)[a].

Table 24. C - $SbCl_3$ in $CHCl_3$[b]

Time (sec.)	% T	L_{620}
Start	48.00	0.319
15	44.50	0.352
30	45.00	0.342
60	43.25	0.364
90	40.50	0.392
120	40.00	0.398
150	39.75	0.401
180	39.75	0.401
210	40.00	0.398
240	40.00	0.398

Table 25. MHC - $SbCl_3$ in $CHCl_3$[b]

Time (sec.)	% T	L_{620}
Start	61.00	0.215
15	54.50	0.264
30	52.75	0.278
60	52.75	0.278
90	52.75	0.278
120	52.75	0.278
150	52.75	0.278
180	52.75	0.278
210	52.75	0.278
240	52.75	0.278

Table 26. DHC - $SbCl_3$ in $CHCl_3$[b]

Time (sec.)	% T	L_{620}
Start	47.25	0.325
15	39.00	0.409
30	38.25	0.417
60	38.50	0.414
90	39.00	0.409
120	39.25	0.406
150	39.50	0.403
180	39.75	0.401
210	40.00	0.398
240	40.25	0.395

Table 27. C - $SbCl_3$ in $C_2H_4Cl_2$[b]

Time (sec.)	% T	L_{620}
Start	61.00	0.215
15	54.75	0.262
30	54.25	0.266
60	52.00	0.284
90	51.50	0.288
120	51.25	0.290
150	51.25	0.290
180	51.25	0.290

Table 28. MHC - $SbCl_3$ in $C_2H_4Cl_2$[b]

Time (sec.)	% T	L_{620}
Start	68.00	0.168
15	57.50	0.240
30	56.00	0.252
60	55.25	0.258
90	55.25	0.258
120	55.50	0.256
150	56.00	0.252
180	56.25	0.250
210	56.50	0.248
240	57.00	0.244

Table 29. DHC - $SbCl_3$ in $C_2H_4Cl_2$[b]

Time (sec.)	% T	L_{620}
Start	55.00	0.260
15	42.00	0.377
30	40.00	0.398
60	39.25	0.406
90	39.50	0.403
120	40.00	0.398
150	40.50	0.392
180	41.00	0.387
210	41.25	0.385
240	41.75	0.380

[a] Concentrations, read as carotene: C, 10.10 µg/ml; MHC, 4.8 µg/ml; DHC, 8.30 µg/ml.

[b] Reagent contained 3% Ac_2O.

Appendix Tables 24-62 (contd)

Table 30. C - $SbCl_3$ in CH_2Cl_2 [c]

Time (sec.)	% T	L_{620}
Start	69.25	0.160
15	65.50	0.184
30	64.50	0.191
60	63.25	0.199
90	62.75	0.202
120	62.50	0.204
150	62.25	0.206
180	62.25	0.206

Table 31. MHC - $SbCl_3$ in CH_2Cl_2 [c]

Time (sec.)	% T	L_{620}
Start	70.00	0.155
15	67.00	0.174
30	64.00	0.194
60	61.25	0.213
90	60.50	0.218
120	60.00	0.222
150	59.75	0.224
180	59.50	0.226

Table 32. DHC - $SbCl_3$ in CH_2Cl_2 [c]

Time (sec.)	% T	L_{620}
Start	65.00	0.187
15	61.00	0.215
30	60.00	0.222
60	59.50	0.226
90	59.50	0.226
120	59.50	0.226
150	59.25	0.227
180	59.00	0.229

Table 33. C - CF_3COOH in $CHCl_3$ [d]

Time (sec.)	% T	L_{620}
Start	56.00	0.252
15	46.00	0.337
30	40.75	0.390
60	40.00	0.398
90	39.75	0.401
120	39.75	0.401
150	40.00	0.398
180	40.25	0.395
210	40.50	0.392
240	40.75	0.390

Table 34. C - CF_3COOH in $CHCl_3$ [b]

Time (sec.)	% T	L_{620}
Start	55.00	0.260
15	46.00	0.337
30	40.50	0.392
60	39.00	0.409
90	39.00	0.409
120	39.00	0.409
150	39.50	0.403
180	40.00	0.398
210	40.25	0.395
240	40.75	0.390

Table 35. MHC - CF_3COOH in $CHCl_3$ [d]

Time (sec.)	% T	L_{620}
Start	62.00	0.208
15	53.00	0.276
30	50.50	0.297
60	50.50	0.297
90	51.25	0.290
120	52.00	0.284
150	53.25	0.274
180	54.25	0.266
210	55.25	0.258
240	56.25	0.250

[c] Two drops of acetic anhydride are added just before estimation.
[d] No Ac_2O used in reagent.

Table 36. MHC - CF_3COOH in $CHCl_3$[b]

Time (sec.)	% T	L_{620}
Start	62.50	0.204
15	54.50	0.264
30	51.00	0.292
60	50.75	0.295
90	51.50	0.289
120	52.50	0.280
150	53.50	0.272
180	54.25	0.266
210	55.50	0.256
240	56.25	0.250

Table 37. DHC - CF_3COOH in $CHCl_3$[d]

Time (sec.)	% T	L_{620}
Start	50.00	0.301
15	40.25	0.395
30	37.75	0.423
60	38.25	0.417
90	39.25	0.406
120	40.50	0.392
150	41.75	0.380
180	43.25	0.364
210	44.25	0.354
240	45.50	0.342

Table 38. DHC - CF_3COOH in $CHCl_3$[b]

Time (sec.)	% T	L_{620}
Start	50.00	0.301
15	40.00	0.398
30	37.75	0.423
60	38.00	0.420
90	39.00	0.409
120	40.50	0.392
150	42.00	0.377
180	43.25	0.364
210	44.50	0.352
240	45.50	0.342

Table 39. C - CF_3COOH in $C_2H_4Cl_2$[d]

Time (sec.)	% T	L_{620}
Start	51.25	0.290
15	49.25	0.308
30	45.50	0.342
60	39.00	0.409
90	36.25	0.441
120	35.00	0.456
150	35.00	0.456
180	35.00	0.456
210	35.50	0.450
240	36.00	0.444

Table 40. C - CF_3COOH in $C_2H_4Cl_2$[b]

Time (sec.)	% T	L_{620}
Start	50.00	0.301
15	46.50	0.332
30	42.00	0.377
60	36.75	0.435
90	35.00	0.456
120	34.50	0.462
150	34.50	0.462
180	35.00	0.456
210	35.25	0.453
240	36.00	0.444

Table 41. MHC - CF_3COOH in $C_2H_4Cl_2$[d]

Time (sec.)	% T	L_{620}
Start	61.00	0.215
15	55.50	0.256
30	50.50	0.297
60	47.00	0.328
90	46.00	0.337
120	46.25	0.335
150	46.50	0.332
180	47.25	0.325
210	47.75	0.321
240	48.25	0.317

Appendix Tables 24-62 (contd)

Table 42. MHC - CF_3COOH in $C_2H_4Cl_2$ [b]

Time (sec.)	% T	L_{620}
Start	60.00	0.222
15	52.00	0.284
30	47.00	0.328
60	43.25	0.364
90	42.75	0.369
120	43.25	0.364
150	43.75	0.359
180	44.50	0.352
210	45.25	0.344
240	46.00	0.337

Table 43. DHC - CF_3COOH in $C_2H_4Cl_2$ [d]

Time (sec.)	% T	L_{620}
Start	50.25	0.299
15	41.50	0.382
30	37.00	0.432
60	33.50	0.475
90	33.25	0.478
120	34.00	0.469
150	35.25	0.453
180	36.25	0.441
210	37.00	0.432
240	37.75	0.423

Table 44. DHC - CF_3COOH in $C_2H_4Cl_2$ [b]

Time (sec.)	% T	L_{620}
Start	50.00	0.301
15	39.75	0.401
30	34.25	0.465
60	31.00	0.509
90	31.25	0.505
120	32.00	0.495
150	33.25	0.478
180	33.75	0.472
210	35.00	0.456
240	36.25	0.441

Table 45. C - CF_3COOH in CH_2Cl_2 [d]

Time (sec.)	% T	L_{620}
Start	67.00	0.174
15	63.00	0.201
30	58.50	0.233
60	51.25	0.290
90	50.75	0.295
120	51.00	0.292
150	56.00	0.252
180	56.25	0.250

Table 46. MHC - CF_3COOH in CH_2Cl_2 [d]

Time (sec.)	% T	L_{620}
Start	68.75	0.163
15	67.00	0.174
30	64.50	0.190
60	64.00	0.194
90	64.25	0.192
120	64.75	0.189
150	65.25	0.186
180	65.75	0.182

Table 47. DHC - CF_3COOH in CH_2Cl_2 [d]

Time (sec.)	% T	L_{620}
Start	66.00	0.181
15	61.00	0.215
30	58.50	0.233
60	57.50	0.240
90	58.00	0.237
120	58.50	0.233
150	59.25	0.227
180	60.00	0.222

Table 48. C - CCl_3COOH in $CHCl_3$ [a]

Time (sec.)	% T	L_{620}
Start	62.00	0.208
15	53.00	0.276
30	51.50	0.288
60	51.00	0.292
90	50.00	0.301
120	49.50	0.305
150	49.00	0.310
180	48.50	0.314
210	48.25	0.317
240	47.75	0.321

Table 49. C - CCl_3COOH in $CHCl_3$ [b]

Time (sec.)	% T	L_{620}
Start	61.50	0.211
15	53.50	0.272
30	51.75	0.286
60	50.25	0.299
90	48.75	0.312
120	48.00	0.319
150	47.25	0.325
180	47.25	0.325
210	47.00	0.328
240	46.75	0.330

Table 50. MHC - CCl_3COOH in $CHCl_3$ [a]

Time (sec.)	% T	L_{620}
Start	69.00	0.161
15	60.50	0.218
30	57.00	0.244
60	54.50	0.264
90	54.50	0.264
120	55.25	0.258
150	56.00	0.252
180	56.50	0.248
210	57.00	0.244
240	57.50	0.240

Table 51. MHC-CCl_3COOH in $CHCl_3$ [b]

Time (sec.)	% T	L_{620}
Start	69.50	0.158
15	60.50	0.218
30	56.75	0.246
60	54.75	0.262
90	54.75	0.262
120	55.25	0.258
150	56.00	0.252
180	56.50	0.248
210	57.00	0.244
240	57.50	0.240

Table 52. DHC - CCl_3COOH in $CHCl_3$ [a]

Time (sec.)	% T	L_{620}
Start	59.00	0.229
15	47.50	0.323
30	43.75	0.359
60	41.75	0.380
90	42.25	0.374
120	43.75	0.359
150	44.75	0.349
180	45.75	0.340
210	46.50	0.332
240	47.50	0.323

Table 53. DHC - CCl_3COOH in $CHCl_3$ [b]

Time (sec.)	% T	L_{620}
Start	58.50	0.233
15	49.50	0.305
30	44.25	0.354
60	41.75	0.380
90	42.25	0.374
120	43.50	0.362
150	44.50	0.352
180	45.50	0.342
210	46.25	0.335
240	47.00	0.328

Table 54. C - CCl_3COOH in $C_2H_4Cl_2$ [d]

Time (sec.)	% T	L_{620}
Start	61.00	0.215
15	51.50	0.288
30	48.50	0.314
60	47.75	0.321
90	47.75	0.321
120	47.50	0.323
150	47.50	0.323
180	47.50	0.323
210	47.50	0.323
240	47.50	0.323

Table 55. C - CCl_3COOH in $C_2H_4Cl_2$ [b]

Time (sec.)	% T	L_{620}
Start	54.75	0.262
15	47.50	0.323
30	47.00	0.328
60	46.75	0.330
90	46.50	0.332
120	46.50	0.332
150	46.50	0.332
180	46.50	0.332
210	46.75	0.330
240	47.00	0.328

Table 56. MHC - CCl_3COOH in $C_2H_4Cl_2$ [d]

Time (sec.)	% T	L_{620}
Start	61.75	0.209
15	55.50	0.256
30	50.75	0.295
60	49.00	0.310
90	49.75	0.303
120	50.50	0.297
150	51.25	0.290
180	51.75	0.286
210	52.25	0.282
240	52.75	0.278

Table 57. MHC - CCl_3COOH in $C_2H_4Cl_2$ [b]

Time (sec.)	% T	L_{620}
Start	62.00	0.208
15	51.50	0.288
30	47.00	0.321
60	48.00	0.319
90	49.00	0.310
120	50.00	0.301
150	50.50	0.297
180	51.25	0.290
210	51.75	0.286
240	52.25	0.282

Table 58. DHC - CCl_3COOH in $C_2H_4Cl_2$ [d]

Time (sec.)	% T	L_{620}
Start	55.75	0.254
15	42.25	0.374
30	37.00	0.432
60	36.00	0.444
90	37.25	0.429
120	38.50	0.414
150	39.50	0.403
180	40.50	0.392
210	41.50	0.382
240	42.25	0.374

Table 59. DHC - CCl_3COOH in $C_2H_4Cl_2$ [b]

Time (sec.)	% T	L_{620}
Start	53.00	0.276
15	39.50	0.403
30	36.50	0.438
60	37.00	0.432
90	39.25	0.406
120	40.00	0.398
150	41.25	0.385
180	42.00	0.377
210	43.00	0.367
240	43.75	0.359

Appendix Tables 24-62 (contd)

Table 60. C - CCl_3COOH in CH_2Cl_2[d]

Time (sec.)	% T	L_{620}
Start	70.75	0.150
15	65.75	0.182
30	63.50	0.197
60	- -	- -
90	62.00	0.208
120	62.00	0.208
150	62.00	0.208
180	62.00	0.208

Table 61. MHC - CCl_3COOH in CH_2Cl_2[d]

Time (sec.)	% T	L_{620}
Start	70.50	0.152
15	70.00	0.155
30	70.00	0.155
60	70.50	0.152
90	71.25	0.147
120	71.50	0.146
150	72.00	0.143
180	72.25	0.141

Table 62. DHC - CCl_3COOH in CH_2Cl_2[d]

Time (sec.)	% T	L_{620}
Start	65.00	0.187
15	64.00	0.194
30	63.25	0.199
60	63.50	0.197
90	64.25	0.192
120	65.00	0.187
150	65.50	0.184
180	66.00	0.181

Appendix Tables 63-107.[e] Effect of sterols on the blue color produced by various reagents with vitamin A. 0.1% DL-α tocopherol (Toc.), 0.1% cholesterol (Ch.), 1.0% cholesterol acetate (Ch.Ac.) and ∿ 0.1% diethylstilbestrol (DES)[f].

Table 63. $SbCl_3$ in $CHCl_3$[b] (No sterol)

Time (sec.)	% T	L_{620}
Start	60.00	0.222
15	62.50	0.204
30	65.50	0.184
60	70.25	0.153
90	74.00	0.131
120	77.25	0.112
150	79.75	0.098
180	81.75	0.0875

Table 64. $SbCl_3$ in $CHCl_3$[b] (Toc.)

Time (sec.)	% T	L_{620}
Start	60.00	0.222
15	63.50	0.197
30	69.50	0.158
60	80.00	0.097
90	87.50	0.058
120	91.50	0.039
150	93.50	0.029
180	94.50	0.025

Table 65. $SbCl_3$ in $CHCl_3$[b] (Ch.)

Time (sec.)	% T	L_{620}
Start	58.00	0.237
15	60.00	0.222
30	62.75	0.202
60	68.25	0.166
90	72.75	0.138
120	75.50	0.122
150	77.75	0.109
180	78.75	0.104

Table 66. $SbCl_3$ in $CHCl_3$[b] (Ch.Ac.)

Time (sec.)	% T	L_{620}
Start	58.25	0.235
15	60.25	0.220
30	63.50	0.197
60	69.50	0.158
90	74.00	0.131
120	77.25	0.112
150	79.25	0.101
180	80.25	0.096

Table 67. $SbCl_3$ in $CHCl_3$[b] (DES)

Time (sec.)	% T	L_{620}
Start	58.00	0.237
15	60.25	0.220
30	63.75	0.196
60	70.00	0.155
90	75.00	0.125
120	78.25	0.107
150	80.25	0.096
180	81.50	0.089

Table 68. $SbCl_3$ in $C_2H_4Cl_2$[b] (No sterol)

Time (sec.)	% T	L_{620}
Start	58.00	0.237
15	58.50	0.233
30	59.75	0.224
60	61.75	0.209
90	63.75	0.196
120	65.75	0.182
150	67.50	0.171
180	69.00	0.161

[e] Vitamin A concn: in $CHCl_3$, 2.90 µg/ml; in $C_2H_4Cl_2$, 2.86 µg/ml; in CH_2Cl_2, 2.78 µg/ml.

[f] Symbols in parentheses indicate sterol present.

Appendix Tables 63-107 (contd)

Table 69. $SbCl_3$ in $C_2H_4Cl_2$[b] (Toc.)

Time (sec.)	% T	L_{620}
Start	58.75	0.230
15	60.25	0.220
30	61.75	0.209
60	64.75	0.189
90	67.50	0.171
120	70.00	0.155
150	72.25	0.141
180	74.00	0.131

Table 70. $SbCl_3$ in $C_2H_4Cl_2$[b] (Ch.)

Time (sec.)	% T	L_{620}
Start	58.50	0.233
15	60.00	0.222
30	61.50	0.211
60	64.25	0.1922
90	67.00	0.174
120	69.25	0.160
150	71.25	0.147
180	73.00	0.137

Table 71. $SbCl_3$ in $C_2H_4Cl_2$[b] (Ch.Ac.)

Time (sec.)	% T	L_{620}
Start	59.25	0.227
15	60.50	0.218
30	61.75	0.209
60	64.50	0.191
90	67.25	0.172
120	69.50	0.158
150	71.50	0.146
180	73.50	0.134

Table 72. $SbCl_3$ in $C_2H_4Cl_2$[b] (DES)

Time (sec.)	% T	L_{620}
Start	60.00	0.222
15	61.75	0.209
30	63.25	0.199
60	66.25	0.179
90	69.25	0.160
120	72.00	0.143
150	74.50	0.128
180	77.00	0.113

Table 73. $SbCl_3$ in CH_2Cl_2[d] (No. sterol)

Time (sec.)	% T	L_{620}
Start	58.50	0.233
15	60.75	0.216
30	62.75	0.202
60	65.50	0.184
90	67.50	0.171
120	69.50	0.158
150	71.25	0.147
180	72.75	0.138

Table 74. $SbCl_3$ in CH_2Cl_2[d] (Toc.)

Time (sec.)	% T	L_{620}
Start	58.50	0.233
15	60.25	0.220
30	62.00	0.208
60	64.75	0.189
90	67.25	0.172
120	69.00	0.161
150	71.00	0.149
180	72.25	0.141

Appendix Tables 63-107 (contd)

Table 75. $SbCl_3$ in CH_2Cl_2[d] (Ch.)

Time (sec.)	% T	L_{620}
Start	58.50	0.233
15	60.50	0.218
30	62.25	0.206
60	65.00	0.187
90	67.25	0.172
120	69.25	0.160
150	70.75	0.150
180	72.25	0.141

Table 76. $SbCl_3$ in CH_2Cl_2[d] (Ch.Ac.)

Time (sec.)	% T	L_{620}
Start	58.75	0.231
15	60.75	0.216
30	62.25	0.206
60	65.50	0.184
90	68.50	0.164
120	70.75	0.150
150	72.75	0.138
180	74.75	0.126

Table 77. $SbCl_3$ in CH_2Cl_2[d] (DES)

Time (sec.)	% T	L_{620}
Start	58.50	0.233
15	60.75	0.216
30	62.50	0.204
60	65.50	0.184
90	68.75	0.163
120	71.25	0.147
150	72.75	0.138
180	74.75	0.126

Table 78. CF_3COOH in $CHCl_3$[d] (No sterol)

Time (sec.)	% T	L_{620}
Start	57.25	0.242
15	57.50	0.240
30	58.00	0.237
60	58.25	0.235
90	59.00	0.229
120	59.25	0.227
150	59.75	0.224
180	60.25	0.220

Table 79. CF_3COOH in $CHCl_3$[d] (Toc.)

Time (sec.)	% T	L_{620}
Start	57.25	0.242
15	57.50	0.240
30	58.50	0.233
60	60.25	0.220
90	62.25	0.206
120	64.00	0.194
150	65.50	0.184
180	66.75	0.176

Table 80. CF_3COOH in $CHCl_3$[d] (Ch.)

Time (sec.)	% T	L_{620}
Start	58.00	0.237
15	58.00	0.237
30	58.25	0.235
60	58.50	0.233
90	59.00	0.229
120	59.50	0.226
150	60.00	0.222
180	60.25	0.220

Appendix Tables 63-107 (contd)

Table 81. CF_3COOH in $CHCl_3$[d] (Ch.Ac.)

Time (sec.)	% T	L_{620}
Start	57.75	0.238
15	58.00	0.237
30	58.25	0.235
60	58.50	0.233
90	58.75	0.231
120	59.50	0.226
150	60.00	0.222
180	60.50	0.218

Table 82. CF_3COOH in $CHCl_3$[d] (DES)

Time (sec.)	% T	L_{620}
Start	58.75	0.231
15	59.25	0.227
30	60.25	0.220
60	62.00	0.208
90	63.00	0.201
120	64.25	0.192
150	65.50	0.184
180	66.50	0.177

Table 83. CF_3COOH in $C_2H_4Cl_2$[d] (No sterol)

Time (sec.)	% T	L_{620}
Start	54.25	0.266
15	54.50	0.264
30	55.50	0.256
60	56.50	0.248
90	58.00	0.237
120	59.00	0.229
150	60.00	0.222
180	61.00	0.215

Table 84. CF_3COOH in $C_2H_4Cl_2$[d] (Toc.)

Time (sec.)	% T	L_{620}
Start	54.00	0.268
15	54.50	0.264
30	55.50	0.256
60	57.00	0.244
90	58.50	0.233
120	60.00	0.222
150	61.25	0.213
180	62.50	0.204

Table 85. CF_3COOH in $C_2H_4Cl_2$[d] (Ch.)

Time (sec.)	% T	L_{620}
Start	54.50	0.264
15	55.50	0.256
30	56.25	0.250
60	57.75	0.238
90	59.00	0.229
120	60.50	0.218
150	61.75	0.209
180	62.75	0.202

Table 86. CF_3COOH in $C_2H_4Cl_2$[d] (Ch.Ac.)

Time (sec.)	% T	L_{620}
Start	55.00	0.260
15	56.25	0.250
30	57.00	0.244
60	58.50	0.233
90	60.00	0.222
120	61.50	0.211
150	62.75	0.202
180	64.00	0.194

Appendix Tables 63-107 (contd)

Table 87. CF_3COOH in $C_2H_4Cl_2$[d] (DES)

Time (sec.)	% T	L_{620}
Start	54.00	0.268
15	55.50	0.256
30	56.25	0.250
60	58.25	0.235
90	59.50	0.226
120	61.00	0.215
150	62.50	0.204
180	63.50	0.197

Table 88. CF_3COOH in CH_2Cl_2[d] (No sterol)

Time (sec.)	% T	L_{620}
Start	62.25	0.206
15	62.75	0.202
30	63.00	0.201
60	63.50	0.197
90	64.00	0.194
120	64.50	0.191
150	65.00	0.187
180	65.50	0.184

Table 89. CF_3COOH in CH_2Cl_2[d] (Toc.)

Time (sec.)	% T	L_{620}
Start	62.25	0.206
15	62.50	0.204
30	62.75	0.202
60	63.50	0.197
90	64.00	0.194
120	64.75	0.189
150	65.50	0.184
180	66.00	0.181

Table 90. CF_3COOH in CH_2Cl_2[d] (Ch.)

Time (sec.)	% T	L_{620}
Start	62.75	0.202
15	63.50	0.197
30	64.25	0.192
60	65.25	0.186
90	66.25	0.179
120	67.25	0.172
150	68.00	0.168
180	69.00	0.161

Table 91. CF_3COOH in CH_2Cl_2[d] (Ch.Ac.)

Time (sec.)	% T	L_{620}
Start	62.75	0.202
15	63.75	0.196
30	64.25	0.192
60	65.25	0.186
90	66.50	0.177
120	67.50	0.171
150	68.75	0.163
180	69.50	0.158

Table 92. CF_3COOH in CH_2Cl_2[d] (DES)

Time (sec.)	% T	L_{620}
Start	63.25	0.199
15	63.75	0.196
30	65.00	0.187
60	66.25	0.179
90	67.50	0.171
120	69.00	0.161
150	70.00	0.155
180	71.00	0.149

Appendix Tables 63-107 (contd)

Table 93. CCl_3COOH in $CHCl_3$[d] (No sterol)

Time (sec.)	% T	L_{620}
Start	60.00	0.222
15	60.25	0.220
30	60.75	0.216
60	61.25	0.213
90	62.00	0.208
120	62.50	0.204
150	63.00	0.201
180	63.50	0.197

Table 94. CCl_3COOH in $CHCl_3$[d] (Toc.)

Time (sec.)	% T	L_{620}
Start	58.25	0.235
15	59.00	0.229
30	60.25	0.220
60	61.75	0.209
90	63.00	0.200
120	63.75	0.196
150	64.25	0.192
180	64.25	0.192

Table 95. CCl_3COOH in $CHCl_3$[d] (Ch.)

Time (sec.)	% T	L_{620}
Start	59.00	0.229
15	59.25	0.227
30	59.75	0.224
60	60.75	0.216
90	61.25	0.213
120	62.00	0.208
150	62.50	0.204
180	63.00	0.201

Table 96. CCl_3COOH in $CHCl_3$[d] (Ch.Ac.)

Time (sec.)	% T	L_{620}
Start	59.00	0.229
15	59.25	0.227
30	59.50	0.226
60	59.75	0.224
90	60.00	0.222
120	60.50	0.218
150	61.00	0.215
180	61.25	0.213

Table 97. CCl_3COOH in $CHCl_3$[d] (DES)

Time (sec.)	% T	L_{620}
Start	58.25	0.235
15	58.50	0.233
30	58.75	0.231
60	59.00	0.229
90	59.50	0.226
120	60.25	0.220
150	60.75	0.216
180	61.25	0.213

Table 98. CCl_3COOH in $C_2H_4Cl_2$[d] (No sterol)

Time (sec.)	% T	L_{620}
Start	54.25	0.266
15	54.50	0.264
30	54.75	0.262
60	55.75	0.254
90	57.00	0.244
120	58.00	0.237
150	59.00	0.229
180	59.75	0.224

Appendix Tables 63-107 (contd)

Table 99. CCl_3COOH in $C_2H_4Cl_2$[d] (Toc.)

Time (sec.)	% T	L_{620}
Start	55.00	0.260
15	55.50	0.256
30	56.25	0.250
60	58.00	0.237
90	59.50	0.226
120	61.00	0.215
150	62.25	0.206
180	63.50	0.197

Table 100. CCl_3COOH in $C_2H_4Cl_2$[d] (Ch.)

Time (sec.)	% T	L_{620}
Start	55.75	0.254
15	56.00	0.252
30	57.25	0.242
60	58.50	0.233
90	59.75	0.224
120	61.25	0.213
150	62.50	0.204
180	63.75	0.196

Table 101. CCl_3COOH in $C_2H_4Cl_2$[d] (Ch.Ac.)

Time (sec.)	% T	L_{620}
Start	55.75	0.254
15	56.50	0.248
30	57.25	0.242
60	58.75	0.231
90	60.50	0.218
120	61.75	0.209
150	63.00	0.201
180	64.25	0.192

Table 102. CCl_3COOH in $C_2H_4Cl_2$[d] (DES)

Time (sec.)	% T	L_{620}
Start	55.75	0.254
15	56.25	0.250
30	57.25	0.242
60	58.75	0.231
90	60.25	0.220
120	61.75	0.209
150	63.25	0.199
180	64.75	0.189

Table 103. CCl_3COOH in CH_2Cl_2[d] (No^3sterol)

Time (sec.)	% T	L_{620}
Start	55.75	0.254
15	56.25	0.250
30	57.00	0.244
60	58.00	0.234
90	59.25	0.227
120	60.25	0.220
150	61.25	0.213
180	62.25	0.206

Table 104. CCl_3COOH in CH_2Cl_2[d] (Toc.)

Time (sec.)	% T	L_{620}
Start	55.75	0.254
15	56.25	0.250
30	57.00	0.244
60	59.25	0.227
90	61.25	0.213
120	63.25	0.199
150	65.25	0.190
180	66.75	0.176

Appendix Tables 63-107 (contd)

Table 105. CCl_3COOH in CH_2Cl_2[d] (Ch.)

Time (sec.)	% T	L_{620}
Start	55.75	0.254
15	56.25	0.250
30	57.25	0.242
60	59.00	0.229
90	60.25	0.220
120	61.75	0.209
150	63.00	0.201
180	64.25	0.192

Table 106. CCl_3COOH in CH_2Cl_2[d] (Ch.Ac.)

Time (sec.)	% T	L_{620}
Start	56.25	0.250
15	56.75	0.246
30	52.50	0.280
60	59.00	0.229
90	60.75	0.216
120	62.25	0.206
150	64.00	0.194
180	65.25	0.186

Table 107. CCl_3COOH in CH_2Cl_2[d] (DES)

Time (sec.)	% T	L_{620}
Start	55.75	0.254
15	56.25	0.250
30	57.25	0.242
60	59.25	0.227
90	61.25	0.213
120	63.25	0.199
150	65.00	0.187
180	66.25	0.179

Appendix Tables 108–125. Studies with carotenoids in combination with vitamin A. Carotenes and vitamin A[g] (C-A)[h]. Monohydroxycarotenoids and vitamin A (MHC-A). Dihydroxycarotenoids and vitamin A (DHC-A).

Table 108. C-A: $SbCl_3$ in $CHCl_3$[a,b]

Time (sec.)	% T	L_{620}
Start	44.75	0.349
15	45.25	0.344
30	46.50	0.332
60	49.75	0.303
90	52.50	0.280
120	--	--
150	55.00	0.260
180	55.50	0.256

Table 109. MHC-A: $SbCl_3$ in $CHCl_3$[a,b]

Time (sec.)	% T	L_{620}
Start	43.75	0.359
15	44.50	0.352
30	46.75	0.330
60	50.75	0.295
90	53.75	0.270
120	55.50	0.256
150	56.75	0.246
180	57.50	0.243

Table 110. DHC-A: $SbCl_3$ in $CHCl_3$[a,b]

Time (sec.)	% T	L_{620}
Start	38.25	0.417
15	39.00	0.410
30	41.25	0.385
60	45.50	0.342
90	48.25	0.320
120	50.00	0.301
150	51.00	0.290
180	51.75	0.286

Table 111. C-A: $SbCl_3$ in CH_2Cl_2[a,c]

Time (sec.)	% T	L_{620}
Start	41.00	0.387
15	40.50	0.392
30	41.25	0.385
60	43.50	0.362
90	46.00	0.337
120	48.00	0.319
150	50.00	0.301
180	51.50	0.288

Table 112. MHC-A: $SbCl_3$ in CH_2Cl_2[a,c]

Time (sec.)	% T	L_{620}
Start	40.00	0.398
15	36.25	0.441
30	36.50	0.438
60	39.25	0.406
90	41.00	0.387
120	44.75	0.349
150	47.50	0.323
180	49.25	0.308

Table 113. DHC-A: $SbCl_3$ in CH_2Cl_2[a,c]

Time (sec.)	% T	L_{620}
Start	39.00	0.409
15	27.00	0.569
30	28.00	0.553
60	31.50	0.502
90	34.25	0.465
120	37.00	0.432
150	39.25	0.406
180	41.25	0.385

[g] Vitamin A concn in same solvent as reagent: $CHCl_3$, 2.90 μg/ml; CH_2Cl_2, 2.78 μg/ml.

[h] Symbols indicate vitamin-carotenoid mixture used.

Appendix Tables 108–125 (contd)

Table 114. C–A: CF_3COOH in $CHCl_3$[d]

Time (sec.)	% T	L_{620}
Start	46.00	0.337
15	44.25	0.354
30	44.50	0.352
60	46.00	0.337
90	48.00	0.319
120	49.75	0.303
150	51.25	0.290
180	52.25	0.282

Table 115. MHC–A: CF_3COOH in $CHCl_3$[d]

Time (sec.)	% T	L_{620}
Start	47.00	0.328
15	47.25	0.325
30	48.25	0.317
60	50.50	0.297
90	53.25	0.274
120	56.00	0.252
150	58.00	0.237
180	60.00	0.222

Table 116. DHC–A: CF_3COOH in $CHCl_3$[d]

Time (sec.)	% T	L_{620}
Start	44.00	0.357
15	42.25	0.374
30	42.75	0.369
60	44.75	0.349
90	47.75	0.321
120	50.00	0.301
150	52.00	0.284
180	53.75	0.270

Table 117. C–A: CF_3COOH in CH_2Cl_2[d]

Time (sec.)	% T	L_{620}
Start	44.00	0.357
15	41.75	0.380
30	40.25	0.395
60	39.50	0.403
90	41.50	0.382
120	43.75	0.359
150	45.50	0.342
180	47.00	0.328

Table 118. MHC–A: CF_3COOH in CH_2Cl_2[d]

Time (sec.)	% T	L_{620}
Start	45.00	0.347
15	44.50	0.352
30	43.25	0.364
60	44.50	0.352
90	46.00	0.337
120	47.75	0.321
150	49.25	0.308
180	51.00	0.292

Table 119. DHC–A: CF_3COOH in CH_2Cl_2[d]

Time (sec.)	% T	L_{620}
Start	41.25	0.385
15	39.00	0.406
30	38.25	0.417
60	39.50	0.403
90	41.75	0.380
120	44.00	0.357
150	46.00	0.337
180	48.00	0.319

Appendix Tables 108–125 (contd)

Table 120. C–A: CCl_3COOH in $CHCl_3$[d]

Time (sec.)	% T	L_{620}
Start	55.00	0.260
15	57.25	0.242
30	57.00	0.244
60	57.25	0.242
90	58.75	0.231
120	60.00	0.222
150	61.25	0.213
180	62.25	0.206

Table 121. MHC–A: CCl_3COOH in $CHCl_3$[d]

Time (sec.)	% T	L_{620}
Start	56.00	0.252
15	63.25	0.199
30	64.50	0.191
60	65.00	0.187
90	66.50	0.177
120	68.00	0.168
150	69.00	0.161
180	70.00	0.155

Table 122. DHC–A: CCl_3COOH in $CHCl_3$[d]

Time (sec.)	% T	L_{620}
Start	51.75	0.286
15	55.50	0.256
30	55.50	0.256
60	55.50	0.256
90	56.50	0.248
120	58.00	0.237
150	59.50	0.226
180	60.75	0.216

Table 123. C–A: CCl_3COOH in CH_2Cl_2[c]

Time (sec.)	% T	L_{620}
Start	41.25	0.385
15	40.50	0.392
30	40.75	0.390
60	42.25	0.374
90	44.25	0.354
120	46.00	0.337
150	47.25	0.325
180	48.25	0.317

Table 124. MHC–A: CCl_3COOH in CH_2Cl_2[c]

Time (sec.)	% T	L_{620}
Start	41.50	0.382
15	41.25	0.385
30	41.50	0.382
60	45.00	0.347
90	48.00	0.319
120	50.75	0.295
150	53.00	0.276
180	55.00	0.260

Table 125. DHC–A: CCl_3COOH in CH_2Cl_2[c]

Time (sec.)	% T	L_{620}
Start	39.00	0.409
15	38.50	0.414
30	39.50	0.403
60	42.75	0.369
90	46.50	0.332
120	49.50	0.305
150	52.00	0.284
180	54.00	0.268

Appendix Tables 126-133. Comparison of hydrocarbon sample solvents which might be used both for extraction procedures and analysis of vitamin A[1]. Hexane (C_6H_{14}); cyclohexane (C_6H_{12}); cyclohexene (C_6H_{10}); chloroform ($CHCl_3$)[j].

Table 126. $SbCl_3$ in $CHCl_3$-(C_6H_{14})

Time (sec.)	% T	L_{620}
Start	67.00	0.174
15	68.75	0.163
30	71.50	0.146
60	77.00	0.114
90	81.00	0.092
120	84.75	0.072
150	87.75	0.057
180	90.00	0.046

Table 127. CF_3COOH in $CHCl_3$-(C_6H_{14})

Time (sec.)	% T	L_{620}
Start	60.75	0.216
15	61.25	0.213
30	62.25	0.206
60	64.25	0.192
90	66.25	0.179
120	68.00	0.166
150	69.75	0.157
180	70.75	0.150

Table 128. $SbCl_3$ in $CHCl_3$-($CHCl_3$)

Time (sec.)	% T	L_{620}
Start	66.25	0.179
15	69.75	0.157
30	73.50	0.134
60	81.50	0.089
90	88.00	0.056
120	92.25	0.035
150	95.00	0.022
180	96.50	0.016

Table 129. CF_3COOH in $CHCl_3$-($CHCl_3$)

Time (sec.)	% T	L_{620}
Start	59.50	0.226
15	60.25	0.220
30	61.25	0.213
60	62.50	0.204
90	63.75	0.196
120	65.25	0.186
150	66.75	0.176
180	68.00	0.168

Table 130. $SbCl_3$ in $CHCl_3$-(C_6H_{12})

Time (sec.)	% T	L_{620}
Start	67.00	0.174
15	68.75	0.163
30	71.50	0.146
60	77.50	0.111
90	82.75	0.082
120	87.25	0.059
150	91.00	0.041
180	93.50	0.029

Table 131. CF_3COOH in $CHCl_3$-(C_6H_{12})

Time (sec.)	% T	L_{620}
Start	59.50	0.226
15	60.25	0.220
30	61.00	0.215
60	62.00	0.208
90	63.50	0.197
120	65.00	0.187
150	66.50	0.177
180	67.26	0.172

[i] Vitamin A Concn: 2.84 μg/ml.

[j] Vitamin A extracted in solvent indicated in parentheses and reacted with colorimetric reagent shown without transfer to $CHCl_3$.

Appendix Tables 126–133 (contd)

Table 132. $SbCl_3$ in $CHCl_3$-(C_6H_{10})

Time (sec.)	% T	L_{620}
Start	70.50	0.152
15	75.25	0.124
30	81.00	0.092
60	87.50	0.058
90	90.25	0.045
120	91.25	0.040
150	91.75	0.037
180	91.75	0.037

Table 133. CF_3COOH in $CHCl_3$-(C_6H_{10})

Time (sec.)	% T	L_{620}
Start	63.50	0.197
15	66.50	0.177
30	69.00	0.161
60	72.25	0.141
90	74.50	0.128
120	76.50	0.116
150	77.50	0.111
180	78.50	0.105

Appendix Tables 134-143. Studies on the effect of radiation in the visible region on some of the reagents used for the analysis of vitamin A

Table 134. CCl_3COOH in $CHCl_3$ (For initial photometric reading, reagent was kept in amber colored bottle and under subdued light)

Table 135. CCl_3COOH in $CHCl_3$ (Reagent kept in dark, photometric reading after 5½ hours)

Table 136. CCl_3COOH in $CHCl_3$ (Reagent kept under laboratory light, photometric reading after 5½ hours)

Table 137. CCl_3COOH in $CHCl_3$ (Reagent kept under laboratory light, photometric reading after 26 hours)

Table 138. CCl_3COOH in $CHCl_3$ (Reagent kept in dark, photometric reading after 26 hours)

Appendix Tables 134-143. Studies on the effect of radiation in the visible region on some of the reagents used for the analysis of vitamin A.

Table 134. CCl_3COOH in $CHCl_3$

Time (sec.)	% T	L_{620}
Start	62.50	0.204
15	69.00	0.161
30	70.25	0.153
60	71.75	0.144
90	73.00	0.137
120	74.25	0.129
150	75.25	0.124
180	76.50	0.116

Table 135. CCl_3COOH in $CHCl_3$

Time (sec.)	% T	L_{620}
Start	65.25	0.186
15	66.25	0.179
30	67.50	0.171
60	68.25	0.166
90	69.25	0.160
120	70.00	0.155
150	70.50	0.152
180	71.25	0.147

Table 136. CCl_3COOH in $CHCl_3$

Time (sec.)	% T	L_{620}
Start	65.50	0.184
15	68.25	0.166
30	69.00	0.161
60	70.25	0.153
90	71.75	0.144
120	72.75	0.138
150	74.00	0.131
180	75.00	0.125

Table 137. CCl_3COOH in $CHCl_3$

Time (sec.)	% T	L_{620}
Start	91.00	0.041
15	97.25	0.012
30	99.00	0.004
60	99.25	0.003

Table 138. CCl_3COOH in $CHCl_3$

Time (sec.)	% T	L_{620}
Start	66.00	0.181
15	71.25	0.147
30	73.00	0.137
60	73.75	0.132
90	74.50	0.128
120	75.00	0.125
150	75.75	0.121
180	76.25	0.118

Appendix Tables 134-143 (contd)

Table 139. CCl_3COOH in CH_2Cl_2 (For initial photometric reading, reagent kept in dark)

Table 140. CCl_3COOH in CH_2Cl_2 (Reagent kept in dark, photometric reading after 5 hours)

Table 141. CCl_3COOH in CH_2Cl_2 (Reagent kept under laboratory light, photometric reading after 20 hours)

Table 142. CCl_3COOH in CH_2Cl_2 (Reagent kept under laboratory light, photometric reading after 24 hours)

Table 143. CCl_3COOH in CH_2Cl_2 (Reagent kept under subdued laboratory light, photometric reading after a month)

Appendix Tables 134-143 (contd)

Table 139. CCl_3COOH in CH_2Cl_2

Time (sec.)	% T	L_{620}
Start	60.50	0.218
15	61.25	0.213
30	62.00	0.208
60	63.75	0.196
90	65.50	0.184
120	67.50	0.171
150	68.75	0.163
180	70.25	0.153

Table 140. CCl_3COOH in CH_2Cl_2

Time (sec.)	% T	L_{620}
Start	60.00	0.222
15	60.25	0.220
30	60.75	0.216
60	62.00	0.208
90	63.25	0.199
120	64.25	0.192
150	65.25	0.186
180	66.25	0.179

Table 141. CCl_3COOH in CH_2Cl_2

Time (sec.)	% T	L_{620}
Start	58.50	0.233
15	59.25	0.227
30	60.00	0.222
60	61.25	0.213
90	62.50	0.204
120	63.50	0.197
150	64.50	0.191
180	65.25	0.186

Table 142. CCl_3COOH in CH_2Cl_2

Time (sec.)	% T	L_{620}
Start	58.50	0.233
15	59.00	0.229
30	59.50	0.226
60	60.50	0.218
90	61.25	0.213
120	62.25	0.206
150	63.00	0.201
180	63.75	0.196

Table 143. CCl_3COOH in CH_2Cl_2

Time (sec.)	% T	L_{620}
Start	58.75	0.231
15	59.25	0.227
30	59.75	0.224
60	61.25	0.213
90	62.50	0.204
120	63.50	0.197
150	64.50	0.191
180	65.50	0.184

Appendix Tables 144 and 145. Studies on the effect of moisture on some of the reagents used for the analysis of vitamin A

Table 144. CCl_3COOH in $CHCl_3$ (Without moisture)

Table 145. CCl_3COOH in $CHCl_3$ (With one drop of water added prior to estimation)

Appendix Tables 144 and 145. Studies on the effect of moisture on some of the reagents used for the analysis of vitamin A.

Table 144. CCl_3COOH in $CHCl_3$

Time (sec.)	% T	L_{620}
Start	65.25	0.186
15	66.25	0.179
30	67.50	0.171
60	68.25	0.166
90	69.25	0.160
120	70.00	0.155
150	70.50	0.152
180	71.25	0.147

Table 145. CCl_3COOH in $CHCl_3$

Time (sec.)	% T	L_{620}
Start	65.25	0.186
15	67.50	0.171
30	70.00	0.155
60	71.75	0.144
90	73.00	0.137
120	74.00	0.131
150	74.75	0.126
180	75.50	0.122

Plates 1 and 2. Standard curves for vitamin A using different color-developing reagents

PLATE 1

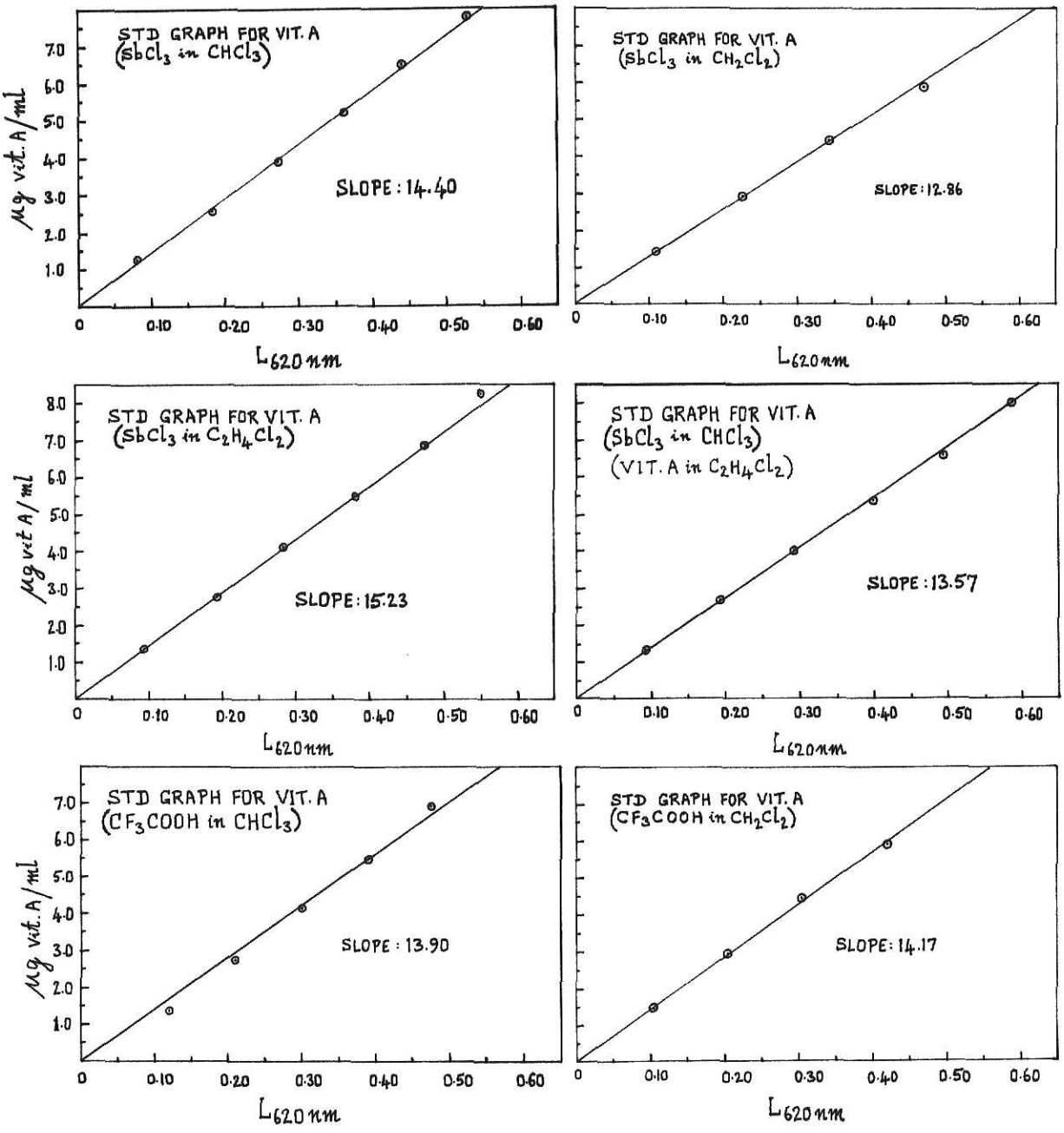
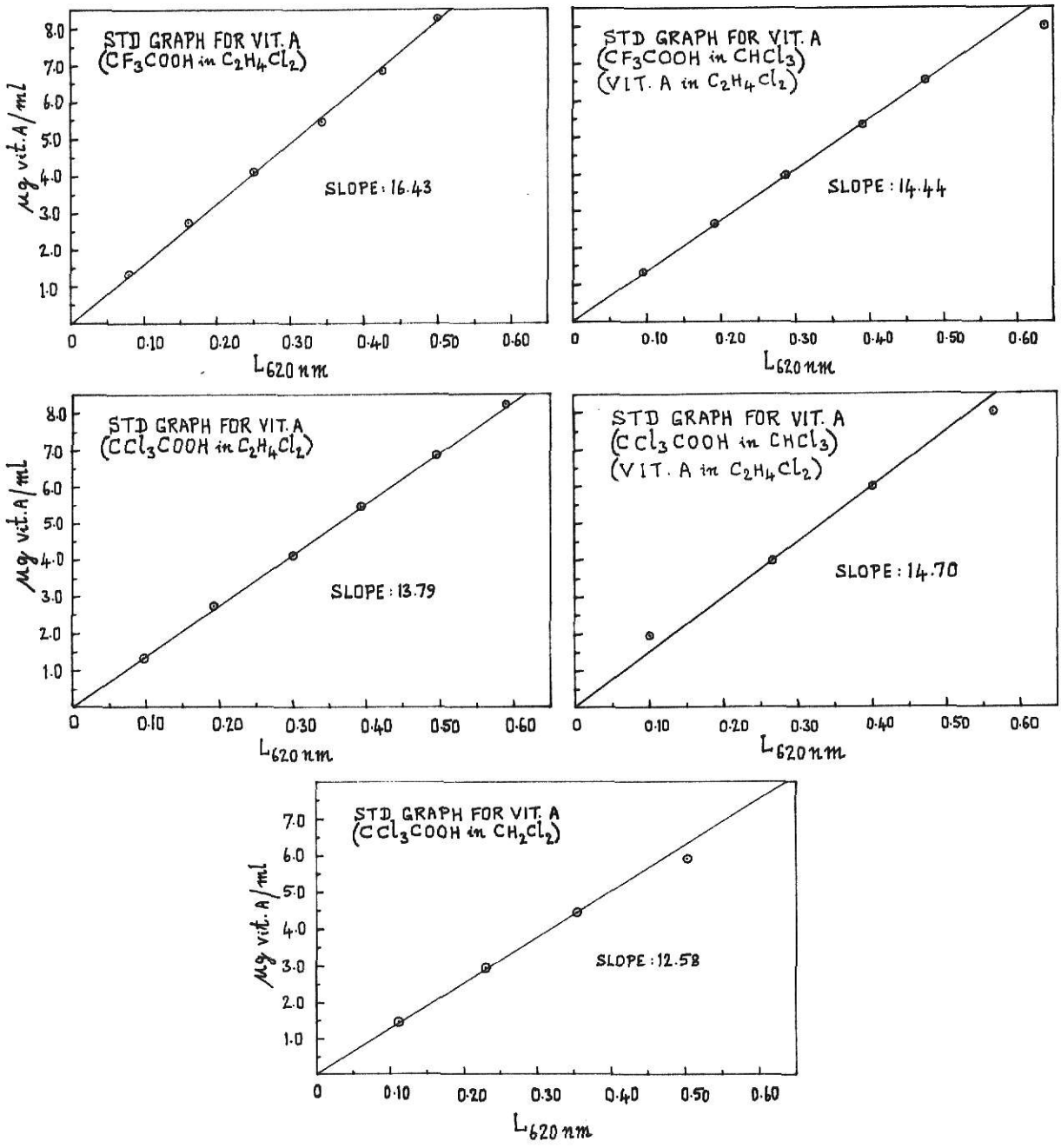

STD GRAPH FOR VIT. A
($SbCl_3$ in $CHCl_3$)
SLOPE : 14.40
STD GRAPH FOR VIT. A
($SbCl_3$ in CH_2Cl_2)
SLOPE : 12.86
STD GRAPH FOR VIT. A
($SbCl_3$ in $C_2H_4Cl_2$)
SLOPE : 15.23
STD GRAPH FOR VIT. A
($SbCl_3$ in $CHCl_3$)
(VIT. A in $C_2H_4Cl_2$)
SLOPE : 13.57
STD GRAPH FOR VIT. A
(CF_3COOH in $CHCl_3$)
SLOPE : 13.90
STD GRAPH FOR VIT. A
(CF_3COOH in CH_2Cl_2)
SLOPE : 14.17
mg vit. A/ml
$L_{620\,nm}$

PLATE 2


STD GRAPH FOR VIT. A
(CF_3COOH in $C_2H_4Cl_2$)
SLOPE: 16.43
STD GRAPH FOR VIT. A
(CF_3COOH in $CHCl_3$)
(VIT. A in $C_2H_4Cl_2$)
SLOPE: 14.44
STD GRAPH FOR VIT. A
(CCl_3COOH in $C_2H_4Cl_2$)
SLOPE: 13.79
STD GRAPH FOR VIT. A
(CCl_3COOH in $CHCl_3$)
(VIT. A in $C_2H_4Cl_2$)
SLOPE: 14.70
STD. GRAPH FOR VIT. A
(CCl_3COOH in CH_2Cl_2)
SLOPE: 12.58
mg vit. A/ml
$L_{620\,nm}$
0 0·10 0·20 0·30 0·40 0·50 0·60
1·0 2·0 3·0 4·0 5·0 6·0 7·0 8·0

Plates 3 - 5. Fading curves for the blue-color reaction of vitamin A with reagents and solvents indicated

PLATE 3

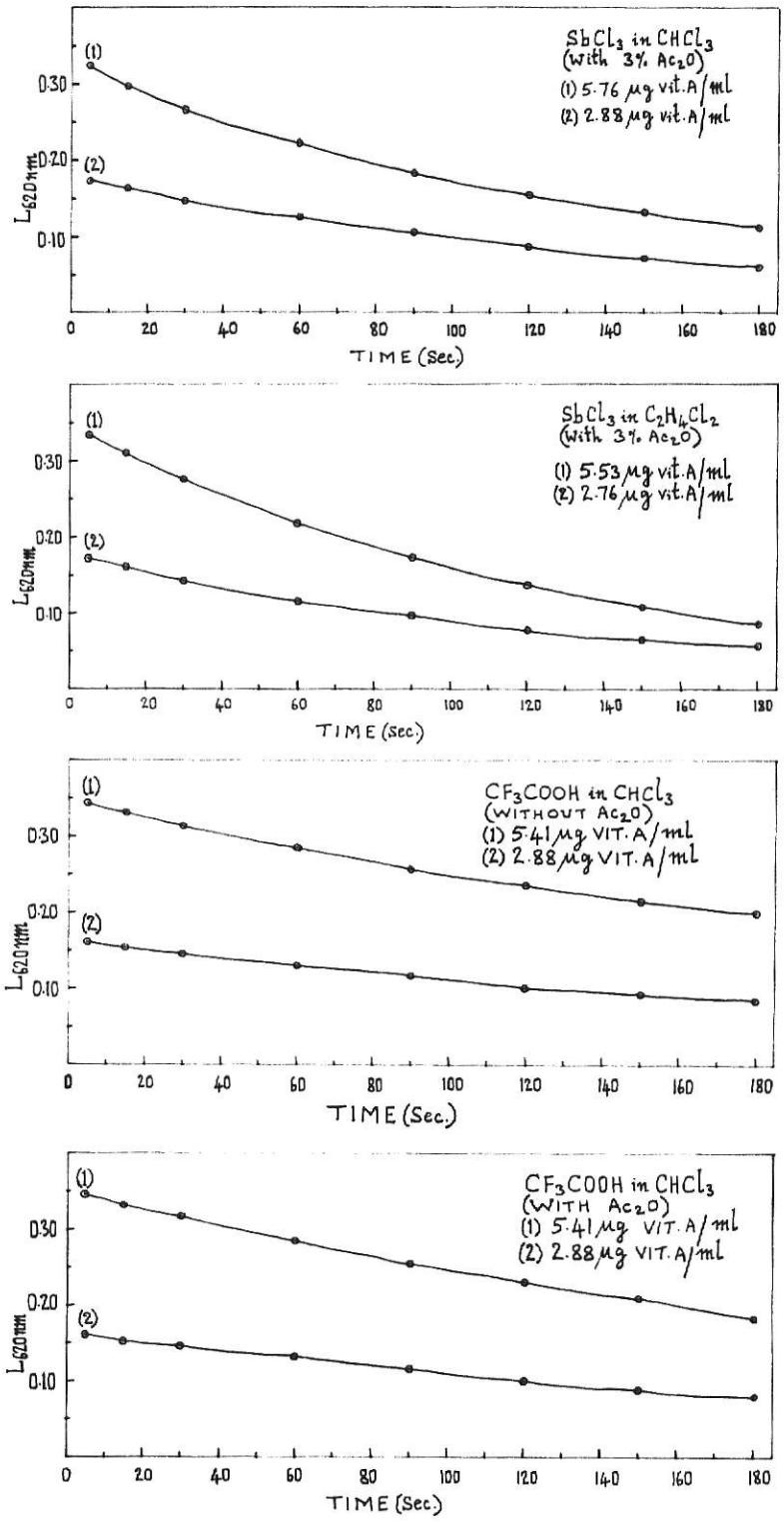

SbCl3 in CHCl3
(With 3% Ac2O)
(1) 5.76 µg vit.A/ml
(2) 2.88 µg vit.A/ml
L620 nm
TIME (Sec.)
SbCl3 in C2H4Cl2
(With 3% Ac2O)
(1) 5.53 µg vit.A/ml
(2) 2.76 µg vit.A/ml
CF3COOH in CHCl3
(WITHOUT Ac2O)
(1) 5.41 µg VIT. A/ml
(2) 2.88 µg VIT. A/ml
CF3COOH in CHCl3
(WITH Ac2O)
(1) 5.41 µg VIT. A/ml
(2) 2.88 µg VIT. A/ml

PLATE 4

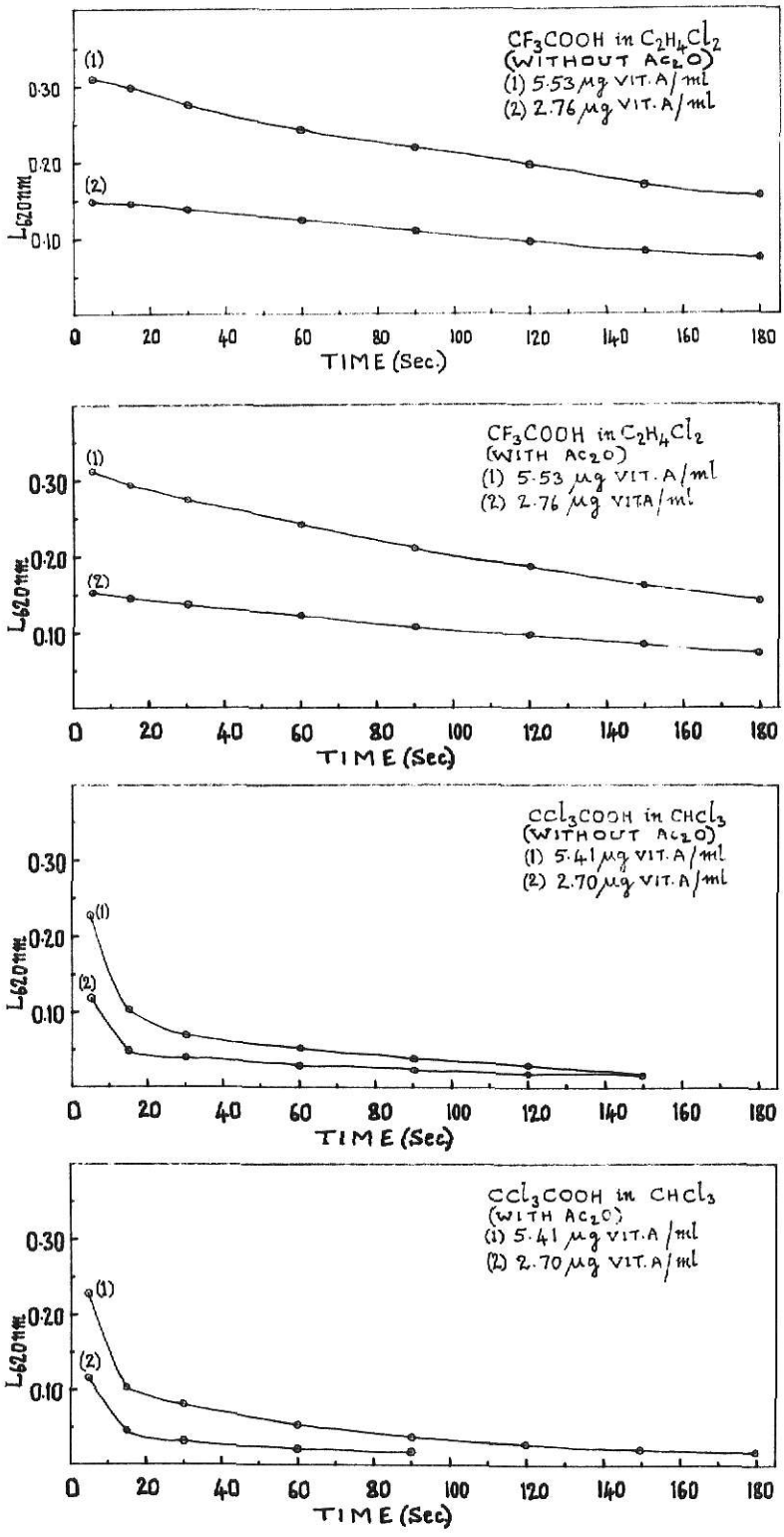
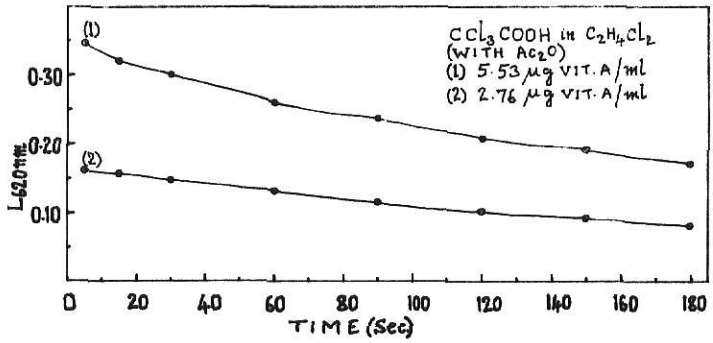
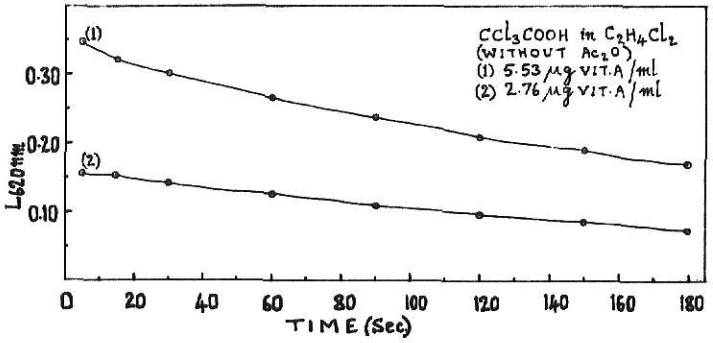
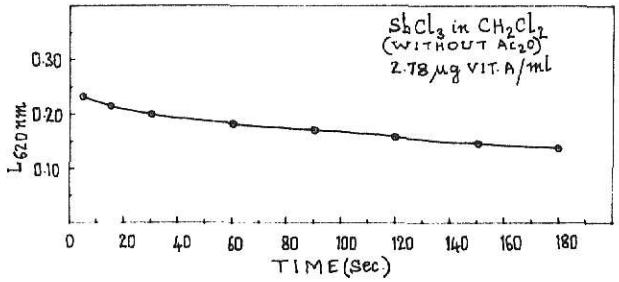
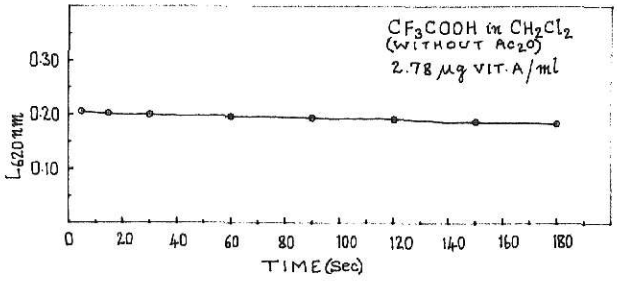

CF_3COOH in $C_2H_4Cl_2$
(WITHOUT Ac_2O)
(1) 5.53 μg VIT. A/ml
(2) 2.76 μg VIT. A/ml
$L_{620\,m\mu}$
TIME (Sec.)
CF_3COOH in $C_2H_4Cl_2$
(WITH Ac_2O)
(1) 5.53 μg VIT. A/ml
(2) 2.76 μg VIT A/ml
$L_{620\,m\mu}$
TIME (Sec)
CCl_3COOH in $CHCl_3$
(WITHOUT Ac_2O)
(1) 5.41 μg VIT. A/ml
(2) 2.70 μg VIT. A/ml
$L_{620\,m\mu}$
TIME (Sec)
CCl_3COOH in $CHCl_3$
(WITH Ac_2O)
(1) 5.41 μg VIT. A/ml
(2) 2.70 μg VIT. A/ml
$L_{620\,m\mu}$
TIME (Sec)

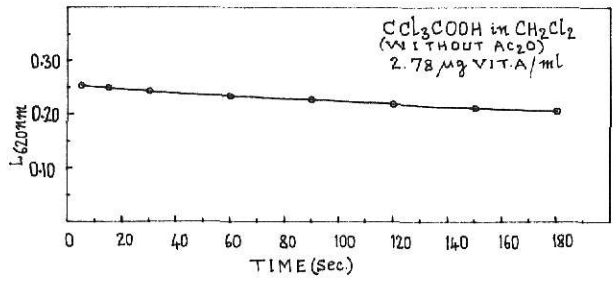
PLATE 5


CCl_3COOH in $C_2H_4Cl_2$
(WITH Ac_2O)
(1) 5.53 μg VIT. A/ml
(2) 2.76 μg VIT. A/ml
(1)
(2)
0·30
0·20
0·10
$L_{620\,nm}$
0 20 40 60 80 100 120 140 160 180
TIME (Sec)


CCl_3COOH in $C_2H_4Cl_2$
(WITHOUT Ac_2O)
(1) 5.53 μg VIT. A/ml
(2) 2.76 μg VIT. A/ml
(1)
(2)
0·30
0·20
0·10
$L_{620\,nm}$
0 20 40 60 80 100 120 140 160 180
TIME (Sec)


$SbCl_3$ in CH_2Cl_2
(WITHOUT Ac_2O)
2.78 μg VIT. A/ml
0·30
0·20
0·10
$L_{620\,nm}$
0 20 40 60 80 100 120 140 160 180
TIME (Sec)


CF_3COOH in CH_2Cl_2
(WITHOUT Ac_2O)
2.78 μg VIT. A/ml
0·30
0·20
0·10
$L_{620\,nm}$
0 20 40 60 80 100 120 140 160 180
TIME (Sec)


CCl_3COOH in CH_2Cl_2
(WITHOUT Ac_2O)
2.78 μg VIT. A/ml
0·30
0·20
0·10
$L_{620\,nm}$
0 20 40 60 80 100 120 140 160 180
TIME (Sec.)

Plates 6 and 7. Changes of absorbances with time for the blue-color reactions of carotenoids with reagents and solvents indicated

PLATE 6

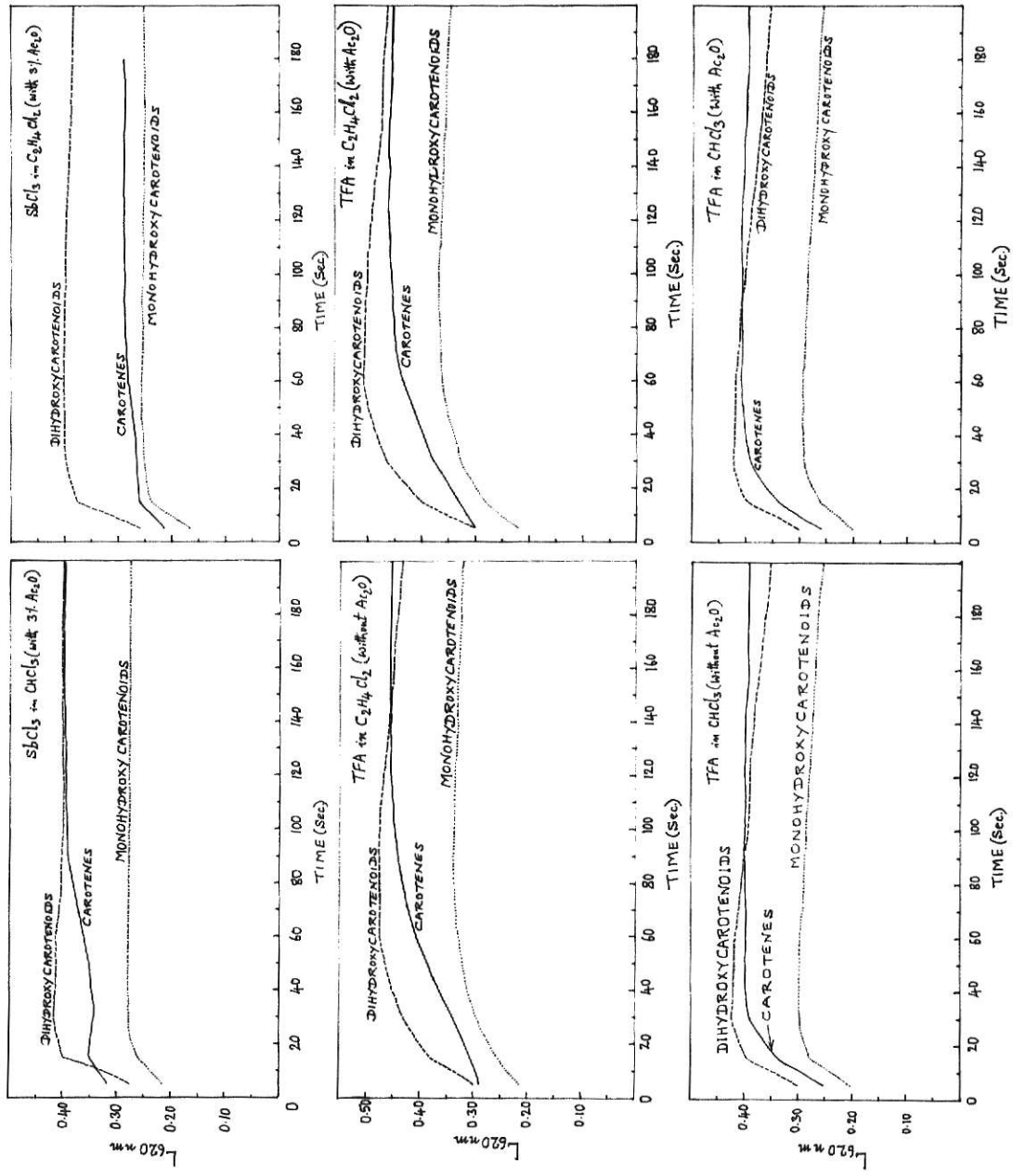

SbCl3 in CHCl3 (with 3% Ac2O)
SbCl3 in C2H4Cl2 (with 3% Ac2O)
TFA in C2H4Cl2 (without Ac2O)
TFA in C2H4Cl2 (with Ac2O)
TFA in CHCl3 (without Ac2O)
TFA in CHCl3 (with Ac2O)
DIHYDROXY CAROTENOIDS
CAROTENES
MONOHYDROXY CAROTENOIDS
L620 mm
TIME (Sec.)

PLATE 7

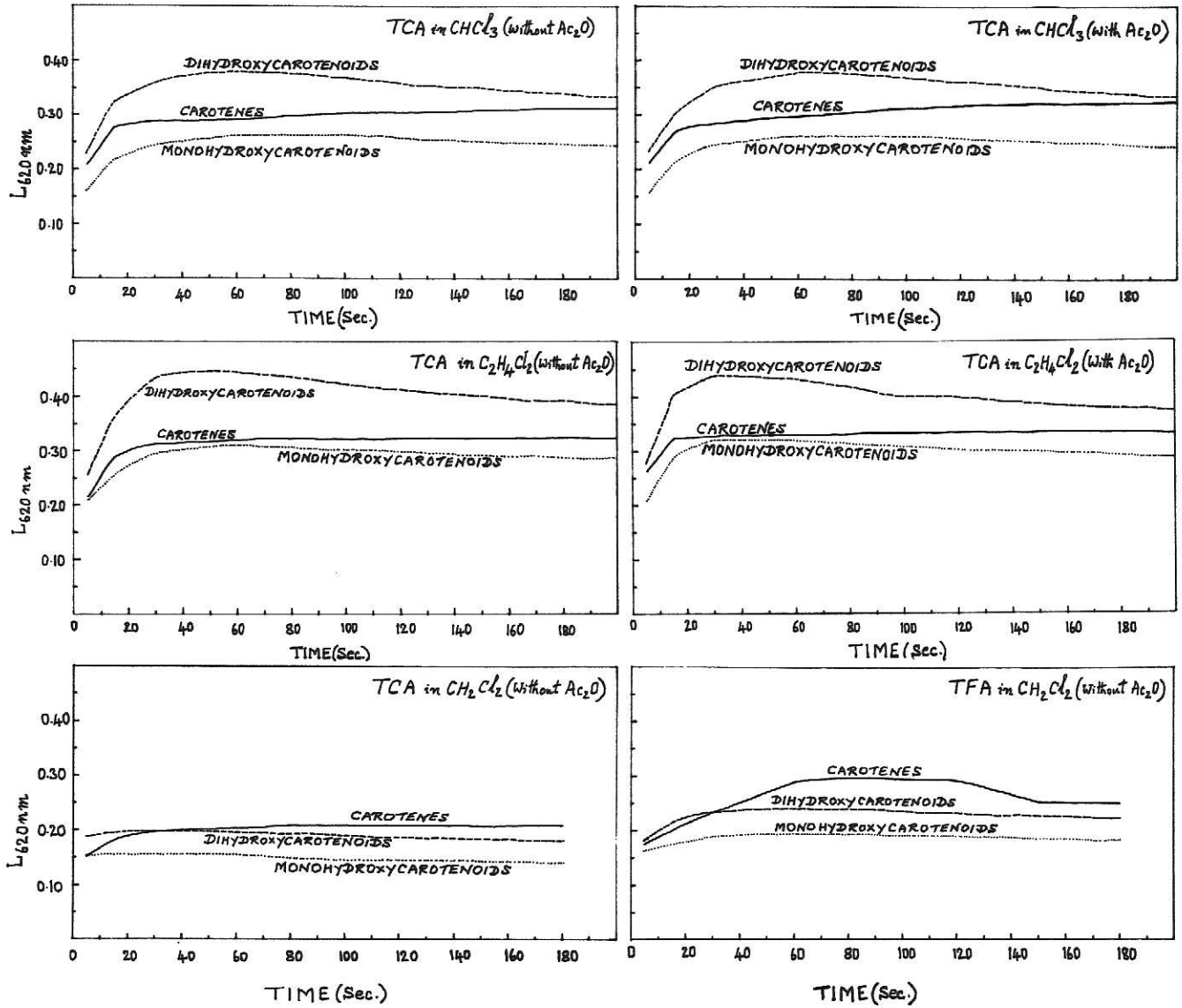
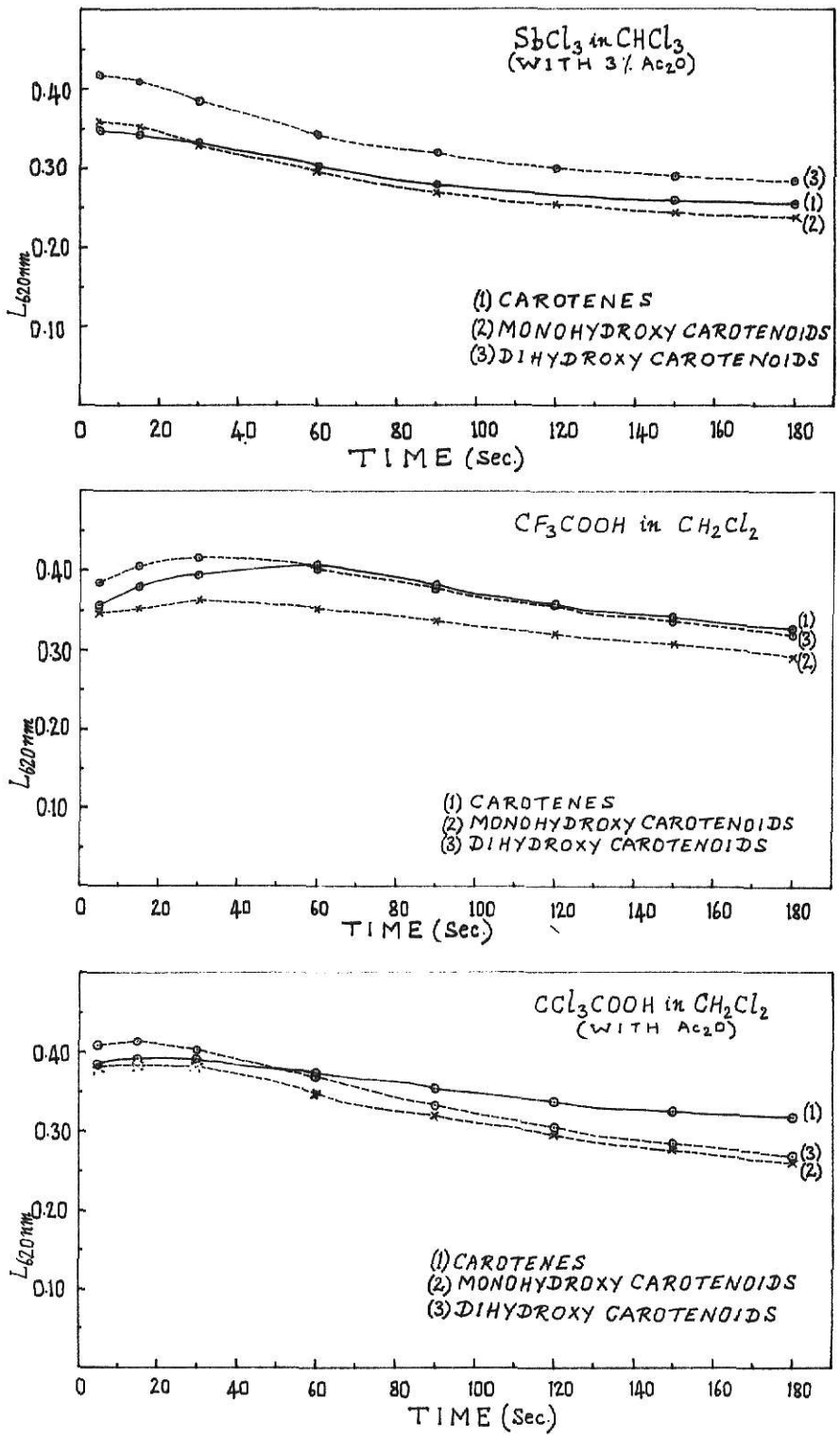

TCA in $CHCl_3$ (without Ac_2O)
DIHYDROXYCAROTENOIDS
CAROTENES
MONOHYDROXYCAROTENOIDS
$L_{620\,nm}$
0·40
0·30
0·20
0·10
0 20 40 60 80 100 120 140 160 180
TIME (Sec.)
TCA in $CHCl_3$ (with Ac_2O)
DIHYDROXYCAROTENOIDS
CAROTENES
MONOHYDROXYCAROTENOIDS
TIME (Sec.)
TCA in $C_2H_4Cl_2$ (without Ac_2O)
DIHYDROXYCAROTENOIDS
CAROTENES
MONOHYDROXYCAROTENOIDS
$L_{620\,nm}$
TIME (Sec.)
TCA in $C_2H_4Cl_2$ (with Ac_2O)
DIHYDROXYCAROTENOIDS
CAROTENES
MONOHYDROXYCAROTENOIDS
TIME (Sec.)
TCA in CH_2Cl_2 (without Ac_2O)
CAROTENES
DIHYDROXYCAROTENOIDS
MONOHYDROXYCAROTENOIDS
$L_{620\,nm}$
TIME (Sec.)
TFA in CH_2Cl_2 (without Ac_2O)
CAROTENES
DIHYDROXYCAROTENOIDS
MONOHYDROXYCAROTENOIDS
TIME (Sec.)

Plate 8. Changes of absorbances with time for the blue-color reactions of vitamin A - carotenoid mixtures with reagents and solvents indicated

PLATE 8


$SbCl_3$ in $CHCl_3$
(WITH 3% Ac_2O)
0·40
0·30
0·20
0·10
L_{620nm}
(3)
(1)
(2)
(1) CAROTENES
(2) MONOHYDROXY CAROTENOIDS
(3) DIHYDROXY CAROTENOIDS
0 20 40 60 80 100 120 140 160 180
TIME (Sec.)
CF_3COOH in CH_2Cl_2
0·40
0·30
0·20
0·10
L_{620nm}
(1)
(3)
(2)
(1) CAROTENES
(2) MONOHYDROXY CAROTENOIDS
(3) DIHYDROXY CAROTENOIDS
0 20 40 60 80 100 120 140 160 180
TIME (Sec.)
CCl_3COOH in CH_2Cl_2
(WITH Ac_2O)
0·40
0·30
0·20
0·10
L_{620nm}
(1)
(3)
(2)
(1) CAROTENES
(2) MONOHYDROXY CAROTENOIDS
(3) DIHYDROXY CAROTENOIDS
0 20 40 60 80 100 120 140 160 180
TIME (Sec.)

STUDIES OF REAGENTS FOR COLORIMETRIC DETERMINATION OF VITAMIN A IN FOODS AND FEEDS

by

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

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Abstract

Lewis acids such as antimony trichloride, trifluoroacetic acid and trichloroacetic acid produce a blue color with vitamin A. This principle is used in a colorimetric method of estimation of vitamin A. A systematic study of the blue-color reaction for analysis of vitamin A was made using three reagents (antimony trichloride, trifluoroacetic acid and trichloroacetic acid) and three solvents (chloroform, dichloromethane and 1,2-dichloroethane). Observations were made on the effects of carotenoids (carotenes, monohydroxycarotenoids and dihydroxycarotenoids), sterols (alpha-tocopherol, cholesterol, cholesteryl acetate and diethylstilbesterol), moisture and radiation in the visible range on the color reaction under normal assay conditions to find the most suitable colorimetric reagent for the routine determination of vitamin A in foods and feeds.

The carotenoids used were extracted from dehydrated alfalfa leaf and chromatographically separated into three groups (carotenes, monohydroxycarotenoids and dihydroxycarotenoids). The intensity and stability of the blue color produced by various reagent-solvent solutions with vitamin A, carotenoids, vitamin A-carotenoid or vitamin A-sterol mixtures were compared by the absorption maxima and fading curves constructed by measuring the absorbance at time intervals.

In general, trifluoroacetic acid, trichloroacetic acid and antimony trichloride in dichloromethane produced the most stable color. Trifluoroacetic acid in dichloromethane was a relatively good reagent but it

produced a slightly lower intensity of blue color compared to trichloroacetic acid in dichloromethane. Trifluoroacetic acid in any of the three solvents tested (chloroform, dichloromethane and 1,2-dichloroethane) was least affected by moisture. Trichloroacetic acid in dichloromethane was less affected by radiation in the visible region than the same reagent in chloroform.

Trichloroacetic acid and trifluoroacetic acid in dichloromethane, and antimony trichloride in chloroform or dichloromethane were tested for their practical applicability for routine analysis of foods and feeds (a breakfast cereal, instant breakfast drink mix, mixed animal feed, liquid feed supplement and pet food) for vitamin A content. The analytical results obtained were similar regardless of the reagent used - antimony trichloride in either chloroform or dichloromethane, or either trichloroacetic acid or trifluoroacetic acid in dichloromethane.