Improving the spectrophotometric procedure for vitamin A analysis - Stability of the Carr-Price product

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Abstract

Anhydroretinylic cation is a product of the reaction between Vitamin A and a chromogenic reagent. The reaction is referred as the Carr-Price reaction. Anhydroretinylic cation is also referred to as the Carr-Price product or the blue colour product because its formation is remarked by the blue colour. The colour is the basic fundamental in vitamin A assaying in various food carriers. Studies have shown that the blue colour fades so rapidly within 5 seconds upon formation due to instability of the cation. The instability is caused by different factors. The colour's rapid fading makes assaying of vitamin A to be more challenging and affects the precision and accuracy of the results. Therefore, this study investigated the effects of different factors such as mass of oil sample, temperature, light brightness, kind of solvent, reacting volume ratio of sample aliquot to chromogenic reagent, type and concentration of chromogenic reagent on the stability of the blue colour product. In order to achieve the objective, the study used observation to qualitatively record colour fading time and spectrophotometric method was used to monitor changes in the concentration of the blue colour product with reference to time taken for the concentration to drop.

The study found that the Carr-Price reaction done outside the spectrophotometer in a 25 ºC water bath with 5.00 g oil sample and a 20 % TCA dissolved in DCM under darkness stabilized the blue colour for 160±10 seconds. Statistical regression indicated that the concentration of the blue colour product ($p\ value = 0.917$) was the same within the said period. The optimum conditions attained in the study showed that 160 seconds stable cation had a recovery of 98.36 %. The percentage recovery is within recommended standard range of between 80 and 110. Spectrophotometric procedure for vitamin A assaying that includes 25 ºC water bath has potential to achieve stability of the blue colour product and be able to help analyst to obtain accurate and precise results with minimal human error as 160 seconds is long enough to do the measurements.

Keywords: Anhydroretinylic cation; Carr-Price; Spectrophotometric procedure; blue colour product; Vitamin A

1. Introduction

Daily consumption of foods with vital vitamins is important in safeguarding the good health of man-kind. Most vitamins are obtained from the food that people eat, except vitamin D which the human body can synthesize in the presence of sunlight. However, daily intake of most vitamins such as A, B, C, E and K has decreased due to high cost of foods rich in these vital micronutrients which most indigenous Malawians cannot afford to purchase, loss of the vitamins in food processing procedure and low content of the same in most food carriers. Most African governments including Malawi engaged in food fortification to ensure that most food that are consumed by local natives is rich in vital vitamins. Vitamin A is the most commonly micronutrient which is added in most locally consumed foodstuff as compared to other vitamins. This is because vitamin A plays a crucial role in many physiological processes including growth and differentiation of target tissues, reproduction, proper functioning of the retina and modulation of the

immune system (Marzęda & Łuszczki, 2019). Therefore, vitamin A assaying in food carriers requires an appropriate method that give precise, accurate and true vales of vitamin content.

Appropriate and conventional methods that do give precise, accurate and true values of vitamin A content in food carriers are generally based on the Carr-Price reaction. Such commonly known methods are fluorometry, iCheck Chroma, high pressure liquid chromatography (HPLC) and spectrophotometry. Fluorometry measures the intensity of fluorescence emitted from a solution of fluorescent substance irradiated with an exciting light in a certain wavelength range. Retinol and retinyl esters absorb light at 325 nm and emit fluorescent radiation at approximately 480 nm. The method is a rapid means of quantitative determination of vitamin A in applications such as small blood samples (called Futterman assay) and in fortified food products such as cooking oil if interferences are not present (Craft & Furr, 2019). ICheck Chroma assesses vitamin A concentration using the colorimetric Carr-Price reaction between vitamin A and antimony trichloride which produces a blue colour proportional to the vitamin A content. The device also tests a range of oil types which include darker oils or unrefined oils. The method's limitations includes waste materials (antimony trichloride) requires implementing an appropriate waste management system and measures vitamin A content in oils at a range of 3-30mg of RE/kg (Omega, 2022). High performance liquid chromatography or High-pressure liquid chromatography (HPLC) is a specific form of column chromatography used in science laboratories. The instrument is used to separate, identify and quantify the active compounds. HPLC is usually the primary technique to determine vitamin A (Hasan et al., 2023). The method has high separation capacity, enables the batch analysis of multiple components, superiority quantitative capability and reproducibility, high sensitivity and low sample consumption. The major disadvantage of this method is the high cost of buying and running such an instrument (Thammana, 2016) and involvement of complicated and time-consuming sample preparation steps such as extraction and purification. Spectrophotometry is the most widely applied physico-chemical technique which is remarkable for its sensitivity and precision. Spectrophotometric technique is popular because the instrumentation is fairly easy to operate and widely available. The chief advantage of spectrophotometric method is that it provides a simple means for determining minute quantities of substances (Taha et al., 2017). Spectrophotometry in the visible region of electromagnetic spectrum which extend from 380 to 780nm is called colorimetry. Colorimetry is based on comparing the colour intensity of the unknown with that of standard solution. The intensity of the blue colour formed as a result of the Carr-Price reaction (fig.1) of a sample is compared with the intensity of the standard solution. The blue colour fades rapidly within few seconds which renders it very difficult to quantitatively measure its content in food carriers.

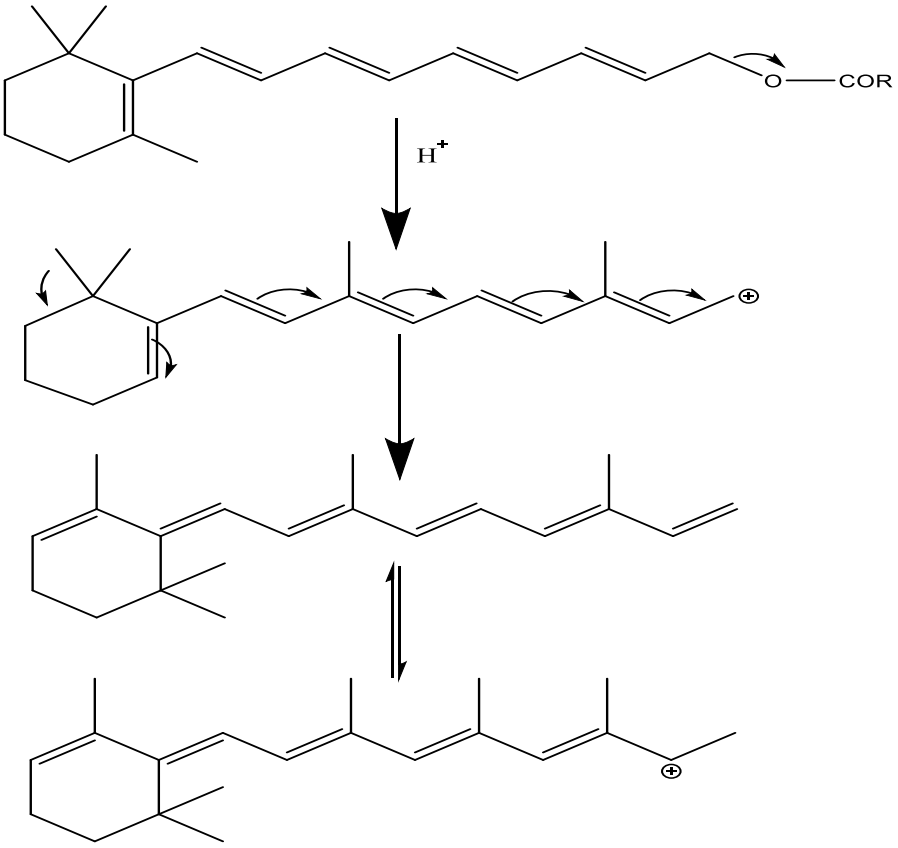

Fig. 1. The Carr-Price Reaction

In view of this, attempts have been made to stabilise the blue colour product so that it takes long before its colour completely fades. Investigations were made on the effects of temperature, light intensity, type of solvent and chromogenic reagent used in the reaction.

Caldwell and Hughes (1947a) explained that the blue colour of the Carr-Price reaction is affected by temperature. The increase in temperature leads to decrease in the stability of the product resulting to rapid fading of the colour. Caldwell and Parrish (1944) further observed that with low temperature, the fading is so slow that one may obtain satisfactory readings over a period of seconds.

The brightness of light influences the rate of fading of the blue colour developed in the Carr-Price reaction. Light affects the anhydroretinylic cation much more rapidly than it affects vitamin A itself, hence rapid disappearance of the blue colour. Less intense light would cause less rapid fading (Caldwell & Parrish, 1944; Subramanyam & Parrish, 1976).

The type of chromogenic reagents and solvents also affect the stability of the carbocation. Subramanyam and Parrish's 1976 study on the effects of reagents and solvents suitable for blue colour development and stability, concluded that CF_3COOH produced slightly more stable colour than $SbCl_3$ in CH_2Cl_2 and the blue colour produced with CCl_3COOH in $CHCl_3$ was unsatisfactory because the colour faded so rapidly.

2. Materials and Methods

2.1. Sample collection and Storage

Fortified, unfortified (blank) oil and pure amorphous retinyl palmitate was obtained from Capital Oil Refining Industries (CORI) Ltd, manufacturer of Kukoma cooking oil in Chilimba, Blantyre. The samples were kept in sealed bottles of 1000ml, 500ml and 250ml respectively. The bottles were wrapped with aluminium foil to protect them from direct light and refrigerated.

2.2. Preparation of sample

Fortified oil (2.00g) was diluted in 25ml volumetric flask with chloroform. The same procedure was repeated with DCM and a mixture of both solvents. The flasks were wrapped with aluminium foil to protect the dissolved vitamin A from light.

2.3. Preparation of standard concentration of retinyl palmitate

2.3.1. Stock solution

Vitamin A (0.1972g) with a purity of 1.7 mio IU/g contained 184.382mg retinyl palmitate since 1.7mio IU/g contains 935mg retinyl palmitate per gram. The measured mass was completely dissolved in 50mL beaker before quantitatively transferring into 1000ml volumetric flask where DCM was added to the mark. The solution produced had a concentration of 184.382mg/L retinyl palmitate.

2.3.2. Working solutions

The standard content of Vitamin A in cooking oil is between 30 and 40mg/kg which is 3mg/100g of oil at market level. Considering the standard content of vitamin A in fortified cooking oil and under fortification of the same, the study prepared two sets of standard solutions. One set of standards ranged from 2.0 to 40 mg/L that includes the standard content of vitamin A in cooking oil while the second set ranged from 0.2 to 1.0 mg/L. The first set of standard solutions (2.0 mg/L ,5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L, 25 mg/L, 30 mg/L, 35 mg/L, 40 mg/L) was prepared by pipetting 271 μL, 678 μL, 1356 μL, 2034 μL, 2712 μL, 3340 μL, 4068 μL and 5424 μL from the stock solution into different volumetric flasks of 25mL. DCM was then added to each flask up to the mark and shaken for homogeneity. The second set of standard solutions (0.2 mg/L, 0.4 mg/L, 0.6 mg/L, 0.8 mg/L, 1.0 mg/L) was similarly prepared by pipetting 27 μL, 54 μL, 81 μL, 108 μL and 136 μL from the stock solution.

2.4. Preparation of solvent

A mixture of chloroform to DCM was prepared in the ratio, 1:1, 1:2, 1:3, 1:4 and 1:5 volume by volume. A 1:1 v/v mixture was prepared by mixing chloroform (500ml) with DCM (500ml) in a 1000ml volumetric flask. Similarly, chloroform (250ml) with DCM (500ml), $CHCl_3$ (250ml) with DCM (750ml), $CHCl_3$ (200ml) with DCM (800ml) in 1000 ml volumetric flask and finally $CHCl_3$ (200ml) with DCM (1000ml) in 2000 ml volumetric flask. These mixtures had produced a solvent mixture of 1:2, 1:3, 1:4 and 1:5 v/v respectively.

2.5. Preparation of chromogenic reagent

Antimony (III) chloride (20.0g) was dissolved in a 10 ml beaker. The solution was quantitatively transferred into 100ml volumetric flask which was diluted with chloroform to the mark and then shaken to mix thoroughly. The same mass of antimony was dissolved in 100ml volumetric flask with DCM as solvent. Different mass of trichloroacetic acid (12.50, 8.00 and 5.00g) were dissolved in separate 25.0ml volumetric flask and then diluted with DCM to the mark. The solutions had TCA concentrations of 50, 30 and 20% w/v. Triflic or trifluoromethane sulphonic acid (20.0mL) was dissolved in 100mL DCM which was diluted to a concentration of 10, 5 and 0.5 %. Trifluoroacetic acid (5mL) was dissolved in 100ml DCM then diluted to a concentration of 0.5%. The flasks of the acids were wrapped with aluminium foil and refrigerated.

2.6. Qualitative method of determining suitable conditions for the blue colour stability

2.6.1. Effect of solvent on the stability of the blue colour

Sample aliquot (2.00mL) was pipetted into a test tube. Followed by the addition of antimony trichloride solution (2.00ml). The blue colour formed was observed as it fades and the time taken from colour development to partial fading was recorded. The procedure was repeated with all the solvents under section 2.4 and the solvent in which the colour faded longer than the rest was determined.

2.6.2. Effect of chromogenic reagent on the stability of the blue colour

Sample aliquot (2.00ml) was reacted with a solution of antimony trichloride (2.00 ml). The blue colour was observed and colour fading time was recorded. The procedure was repeated with triflouroacetic acid, trichloroacetic acid and trifluoromethane sulphonic acid.

2.6.3. Effect of concentration of chromogenic reagent on stability of the colour

The prepared reagent (2.0ml) was pipetted and added to sample aliquot (2.00ml) using a dropper. The period at which blue colour faded was recorded. The procedure was repeated for the rest of the concentration (50%, 30% and 20%) and the period of fading was compared.

2.6.4. Effect of the reacting volumes on the stability of the colour

Initially, the volume of sample aliquot was kept constant and that of the reagent (TCA) was changing in the ratio one to one (1:1), one to two (1:2), one to three (1:3), one to four (1:4) and one to five (1:5). 20% TCA (2.00ml) was pipetted into a test tube and sample aliquot (2.00ml) was added. These reacting volumes were in the ratio one to one (1:1 v/v). The colour fading time was recorded. This procedure was repeated with ratios of one to two (1: 2 v/v), one to three (1:3 v/v), one to four (1: 4 v/v) and (1: 5 v/v).

2.6.5. Effect of light brightness on the stability of the blue colour

20% TCA (2.00ml) was reacted with sample aliquot (2.00ml) at low light intensity (when sunlight is not bright), high light intensity (when sunlight's brightness was at pick) and in total darkness. The time taken for the blue colour to fade was recorded.

2.6.6. Effect of temperature on the stability of the blue colour

TCA (2.00ml, 20%) was added into a sample aliquot (2.00ml) in a test tube. The test tube was emersed in a water bath whose temperature reading was at 15ºC and the period at which the blue colour faded was recorded. The procedure was repeated with temperatures at 20, 25, 30, 35 and 40ºC.

2.6.7. Effect of the amount of sample on the stability of the blue colour

Different masses of sample oil (25.0, 12.5, 8.00, 5.00 and 2.00g) were dissolved in a beaker. Individual solution was quantitatively transferred into a 25 ml volumetric flask which was further diluted with DCM to the mark. Each sample aliquot (2.00ml) was pipetted into test tube and TCA (2.00ml) was added and the stop watch was set instantly. Then fading time of the colour was recorded.

2.7. Monitoring concentrations of the blue colour product

A sample aliquot (2.00mL) was placed into a cuvette using a dropper. Then the cuvette was half way dipped into a water bath. TCA (2.00mL) was forcibly added into the cuvette by Pasteur pipette and concurrently started the stopwatch. When the watch reading was close to the required reaction time, the cuvette was taken out of the water bath, water droplets were removed using toilet paper. Then a cuvette was inserted into the UV/VIS machine to measure its absorbance as well as concentration. The concentration of the sample and standards were recorded when time 0, 5, 10 and then at intervals of 10 seconds to 200 seconds reaction time (0, 5, 10 , up to 200 s).

3. Results and discussion

3.1. Effect of solvent on the stability of the blue colour

Solvents serve as medium for chemical reactions and play two crucial roles namely non-participatory role and participatory role. Solvent plays a non-participatory role by dissolving the reactants only and a participatory role by acting as a source of acid, base or nucleophiles (Mondal, 2020). The effects of solvent; dichloromethane (DCM) and chloroform on the stability of the Carr-Price product were investigated (fig. 2).

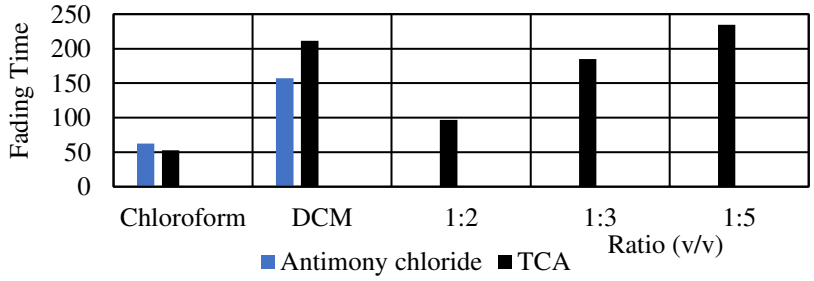

Fig. 2. Effects of solvent and reagents in darkness at 25 ºC, reacting ratio of 1:1, 5.0g of sample, 20%TCA and 20% $SnCl_3$

DCM gave approximately 200 seconds fading time while chloroform showed short fading time (~160 seconds). The results shown is in agreement to the findings of Subramanyam and Parrish (1976). Chloroform and DCM were mixed in different ratios shown in fig. 2 and mixing ratios of 1 to 5 (Chloroform: DCM v/v) took about 180 seconds

fading time more than when Chloroform was used alone. The solvation properties of the mixture improved as blue colour could persist longer in the mixture than in chloroform and DCM alone.

3.2. Effect of chromogenic reagent on the stability of the blue colour

Effect of chromogenic reagents such as $SbCl_3$, TCA, TFA and TfOH were investigated in the study with DCM as solvent. The first reagents to be compared were $SbCl_3$ and TCA because $SbCl_3$ is the basic reagent used by Carr and Price in the early 1900's and TCA is the most commonly found reagent. The results suggest that TCA is a better chromogenic reagent as fading time was approximately 50 seconds more than $SbCl_3$ (fig. 2). The effect of TCA on colour stability was also compared to TFA and TFMAS or TfOH in both sample aliquot and standards. Their comparisons were done at a concentration of 0.5% because TFA and TfOH gave very much low fading time at a concentration of 20% (fig. 3).

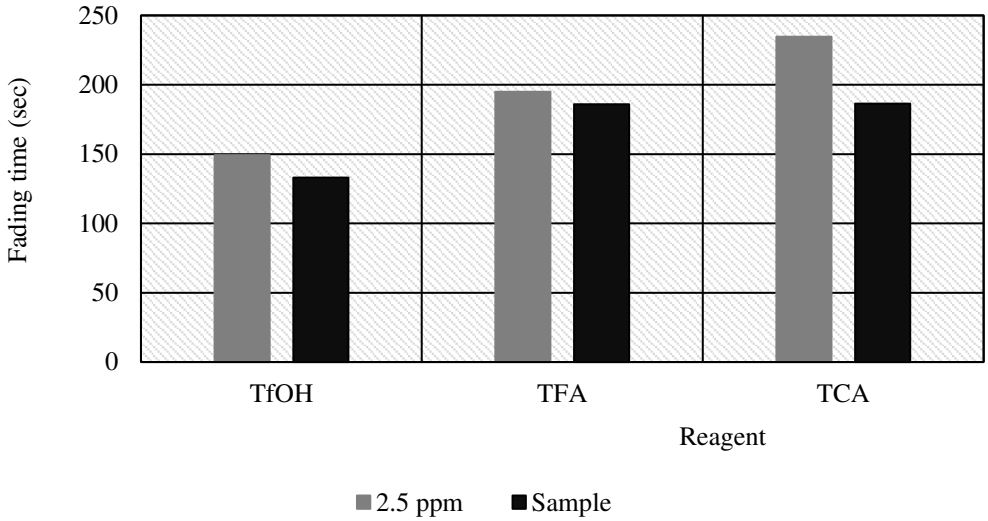

Fig. 3. Effect of reagents in darkness at 25 ºC, reacting ratio of 1:1, in 2.5mg/L standard, 5.00g sample, at a reagent's concentration of 0.5% with DCM as solvent

TCA and TFA (0.5%) gave comparable fading time in sample aliquot but differed greatly in standard solutions (fig. 3). The difference in fading time in the analyte (standard solution and sample aliquot) could be due to the differences in reaction matrix in the two solutions which might have considerable effects on the reaction and such effects are known as matrix effects (Thorne, 2010). This suggest that the components in the reaction matrix of the standard solutions were not exactly the same as that of the sample aliquot. The concentration of the blue colour product formed by these three chromogenic reagents (TfOH, TFA and TCA) were spectrophotometrically compared with reference to time taken for the concentrations to drop significantly (fig. 4).

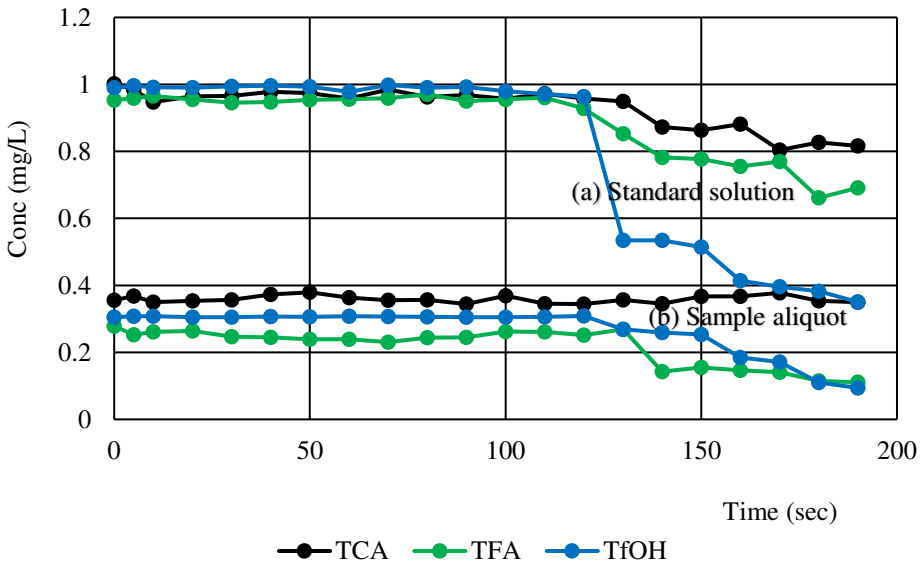

Fig. 4. Effects of reagents (0.5%) on concentration of the cation in standard (1.0 mg/L) and oil sample, 25ºC, darkness

Analysing the concentrations drop for 190 seconds using one-Way ANOVA, statistic F was 3.624, degree of freedom was 65 and P value was 0.032. There was significant difference on their effects in terms of fading time in sample aliquot than in standard concentration. By further analysis using Tuckey test, TfOH produced unstable cation that could fade in the shortest time than the two reagents (TFA and TCA). The stability is shown by constant concentration values of the cation for a specified time since the brightness of the blue colour is proportion to the concentration of the blue colour product. TFA and TCA produced a comparable stable cation, according to Tuckey test, as P value was 0.628 > 0.05 implying that there was no significant difference in the concentration of the cation produced by the two reagents.

Statistical analysis using regression, both TFA and TfOH produced a 120 seconds stable cation in standard concentration (fig. 4a) with F = 0.020 and p value of 0.889 which suggested that there was no significant relationship between time and concentration for the first 120 sec. This suggested that concentration of the blue colour product was constant in standard aliquot within 120 seconds of reaction indicating that the cation was stable within this period. TFA and TfOH produced a blue colour product which was stable for 130 sec with P = 0.964 which is great at 5% level in sample aliquot (fig. 4b). The differences in the fading time of the blue colour product in both sample and standards produced by the two chromogenic reagents (TFA and TfOH) could be due to the differences in their chemical-composition matrix that specifies the number of atoms of each chemical element which make up each of the reactants and products in a given reaction equation (Thorne, 2010). TCA gave a more stable blue colour product as compared to the two chromogenic reagents (TFA and TfOH) in both sample aliquot and standard concentrations. Statistic F for TCA regression was 0.011 and p value was 0.917 > 0.05. This indicated that time had no significant effects at 5% level in the concentration of the blue colour product for about 150 seconds in sample aliquot. The effects of the reagents (TCA, TFA and TfOH) on the stability of the cation could be summarised as **TCA > TFA ≫ TfOH ≫$SbCl_3$.**

3.3. Effect of the concentration of chromogenic reagent

Different concentrations of TCA (10%, 20%, 30% and 50%) were reacted with different standards and sample aliquot. The results in fig. 5, clearly shows that a 20% TCA gave the highest fading time (~140 seconds) of the blue colour than the rest of the concentrations of TCA. Any increase in the concentration of TCA beyond 20% resulted in low fading time which implied to rapid depletion of the reactants. The rate of chemical reaction increases with an increase in concentration of reactants (Phimmavong, 2020). The higher the concentration the faster the rate of chemical reaction and the faster the depletion of the reactants.

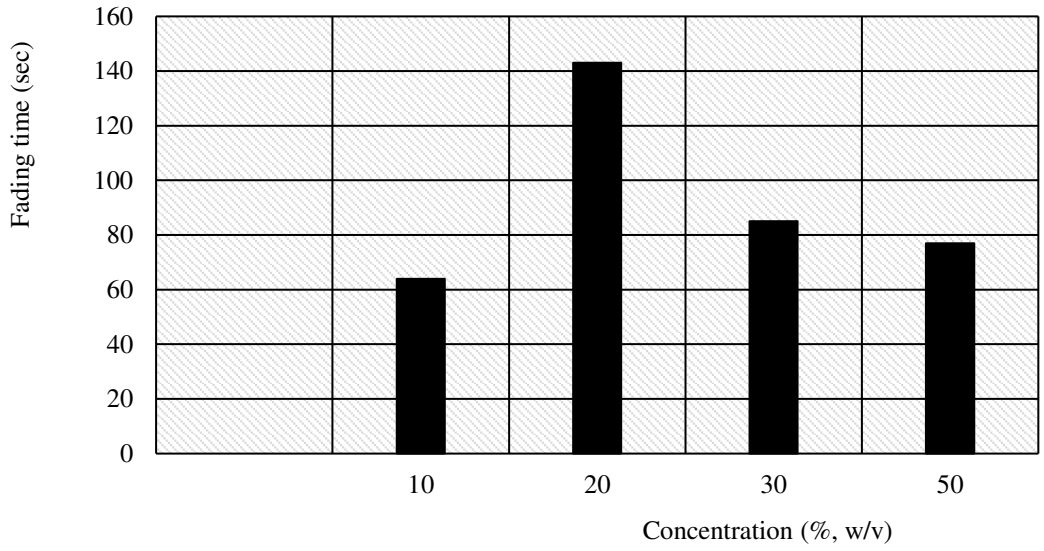

Fig. 5. Effect of concentration of TCA on fading time in low light brightness,1:1 reacting ratios at 25ºC, DCM as solvent,5.00g sample

A concentration of 20% of both TFA and TFMSA (TfOH) was used in both sample and standards but could get very low fading time. TFA and TfOH are stronger Bronsted acids with TfOH having a pKa value of -13.6 and these two chromogenic reagents are among the small group of acids known as 'super acids' which are used in various protonation reactions (Rakita, 2020). The supper acids donate a proton more quickly than ordinary acids such as TfOH hence the colour faded rapidly.

3.4. Effect of reacting volumes

The study conducted several experiments to find out if the volume of reactants (TCA and sample) had effects on fading of the blue colour product. The study investigated their effects in terms of ratios of acid to sample (v/v) as well as sample to acid and the results were shown in fig.6 and 7.

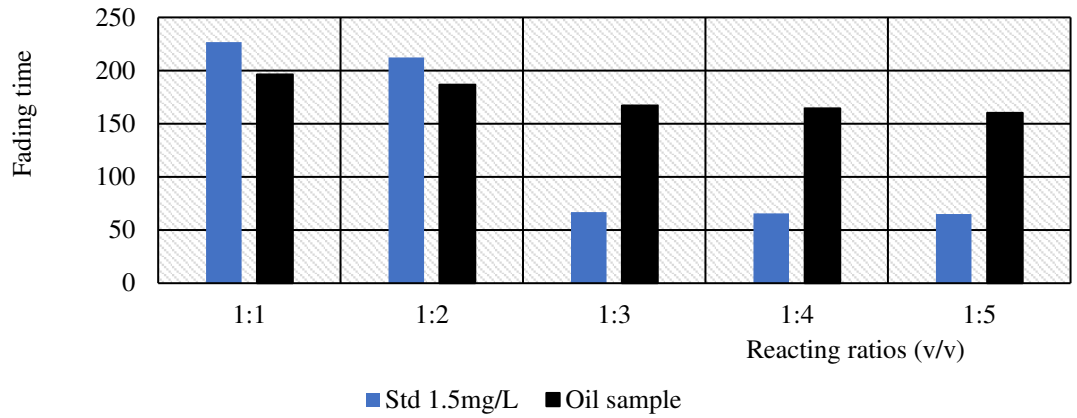

Fig.6. Effects of reacting ratio of acid to aliquot (v/v) in 1.5 mg/L standard and sample oil, 25ºC, 20%TCA under darkness

Volume of acid was kept constant while the volume of sample aliquot was changing. The increase in the volume of sample aliquot led to a decrease in the fading time of the blue colour. The ratio of one part of acid to one part of sample aliquot (1:1, v/v) gave the highest fading time in both standard solution and sample aliquot but the rate of fading was different. Time taken for the blue colour to fade in sample aliquot was decreasing slowly and there was a drastic decrease of the colour in standard concentration. The difference in the rate of fading could be due to difference in the reaction matrix of sample and standard solution. In fig.7, the volume of acid varied and that of sample aliquot remained constant. An increase in the volume of acid also led to a decrease in fading time of the blue colour and the trend was the same as in fig.6.

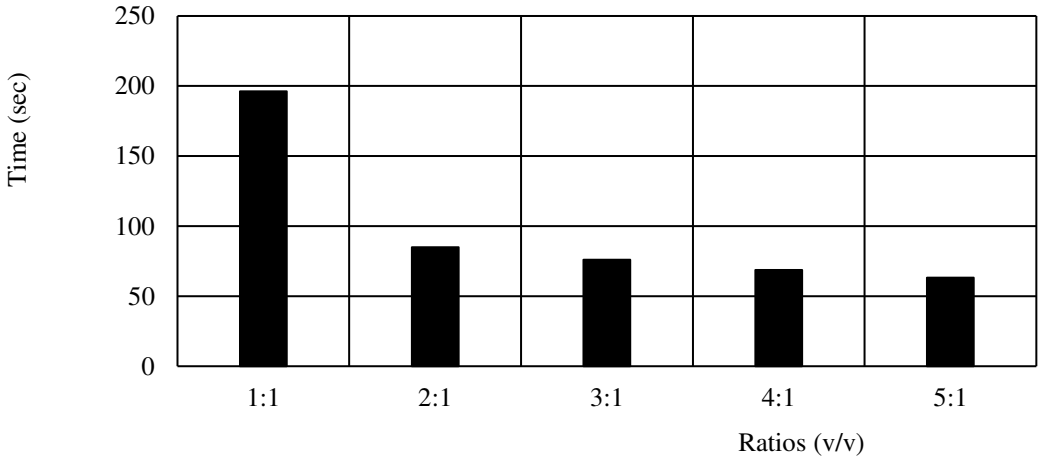

Figure 7: Effects of increase in acid volume sample oil at 25ºC, 20% TCA, in 1.00mL sample aliquot under darkness

3.5. Effect of light brightness

Caldwell and Parrish (1944) mentioned that light affects the blue colour product, anhydroretinylic cation, more greatly than vitamin A itself. The study investigated the effects of light brightness as well as UV/VIS's radiation on

fading time of the blue colour by monitoring brightness of blue colour with time and drop in concentration of the anhydroretinylic cation by checking its concentration over a period of time inside the spectrophotometer. The effects were considered in both standards and sample aliquot (fig. 8).

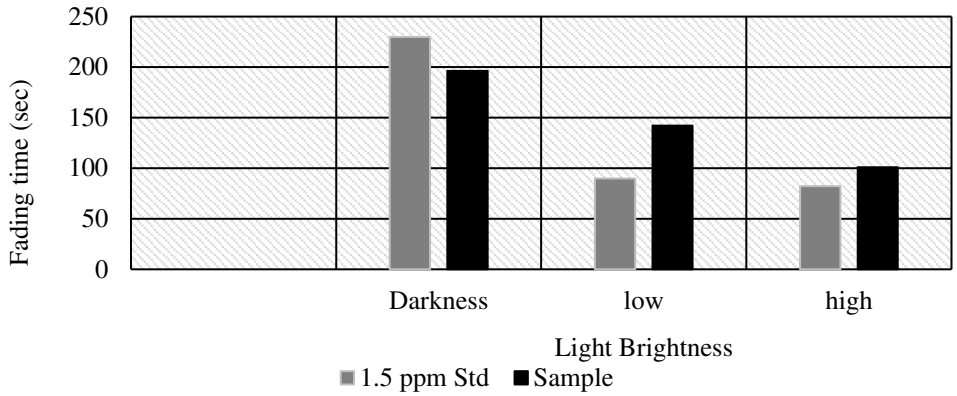

Fig. 8: Effects of brightness of light in standard Vitamin A and 5.00g sample at 25ºC, 1:1 reacting ratio

Fig. 8 shows that vitamin A assaying done in darkness obtain good results with minimal loss of the vitamin which is in agreement with the fact that light exert powerful effects in the fading of blue colour (Parrish, 1944). It is important to carry out the analysis in darkness as the blue colour took much more time to fade in darkness ~190 seconds.

The effect of light of the UV-VIS spectrophotometer was investigated by continuous monitor of concentrations of retinyl palmitate and the blue colour product. The blue colour's concentration in standard solutions and sample aliquot was monitored for 200 seconds while the cuvette remained inside the instrument and the results showed that there was a drastic decrease in concentrations of the blue colour product in both standard and sample aliquot (fig. 9).

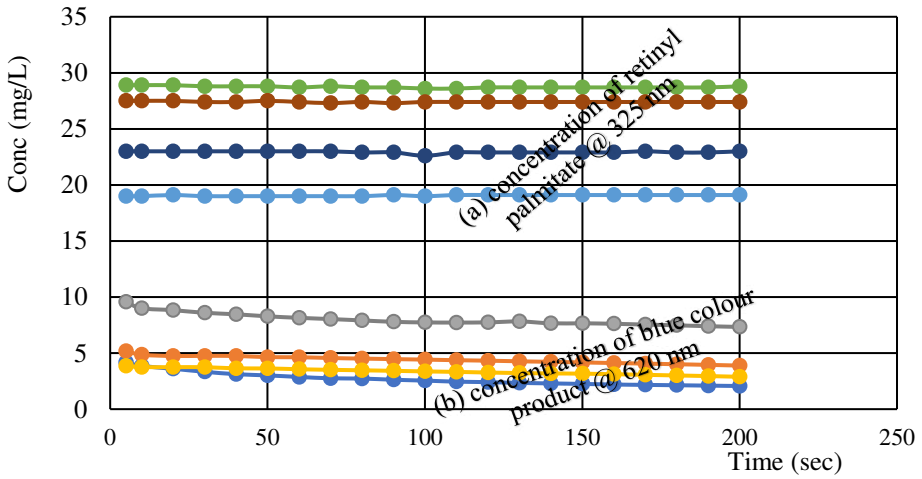

Fig. 9. Comparisons in the concentrations of retinyl palmitate and blue colour product in sample and standard solutions under UV-VIS radiation, at 325nm and 620nm wavelength respectively, 25ºC, 20% TCA with 1:1 reacting ratio

The study investigated the effects of UV-VIS radiation on the concentration of retinyl palmitate itself by monitoring the concentration of the vitamin in both sample and standard solutions at 325nm continuously inside the instrument for 200 seconds. The concentration values of the vitamin were statistically the same throughout 200 seconds. Fig. 9a suggested that UV/VIS's radiation had no effect on the concentration of the vitamin itself at a wavelength of 325nm. To further investigate the drastic drop of the concentration of the cation observed in part (b) of fig. 9, the study modified the procedure on how the concentrations of the cation was measured. The study carried out the Carr-Price reaction (the addition of acid into the cuvette containing sample) in 25°C water bath and the measurement of the concentration of the cation was done instantaneously after the required reaction time instead of continuous monitoring inside the UV/VIS machine with an extended exposure to radiation. The concentration was constant for some time before dropping and the change proved that UV/VIS's radiation had greater effects on the concentration of anhydroretinylic cation. This modification suggested that UV-VIS radiation had negligible effects on the concentration of the Carr-Price product within 150 to170 seconds (fig. 10b). The measured concentration would exactly resemble that of retinyl palmitate in 150 to 170 seconds which is in agreement to Caldwell and Parrish's 1944 findings.

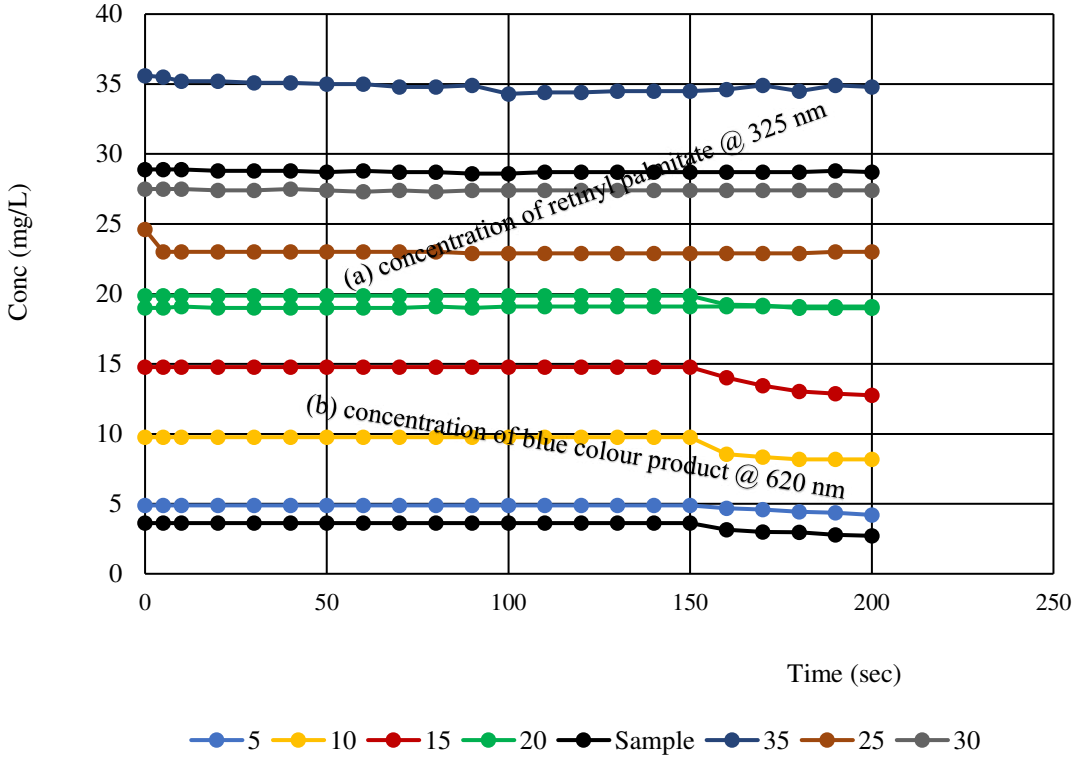

Fig. 10. Comparison of concentrations of retinyl palmitate and blue colour product in sample aliquot and standards monitored under minimal exposure to UV light at 325 nm and 620 nm, 25°C, 1:1 reacting ratio, 20% TCA using T90+UV/VIS's spectrophotometer

3.6. Effect of temperature

Caldwell and Hughes (1947b) studied the effects of temperature on the fading of blue colour product at temperatures of 20, 35 and 50 ºC and they came to a conclusion that room temperature would be insufficient to affect seriously the Carr-Price determination provided that the absorption measurements were made within a few seconds of mixing the reagents. In this study a temperature range between 15 to 40ºC was investigated to ascertain the effects of temperature on the stability of the blue colour. The effects of temperature on fading time were investigated in both standards and sample aliquot (fig. 11).

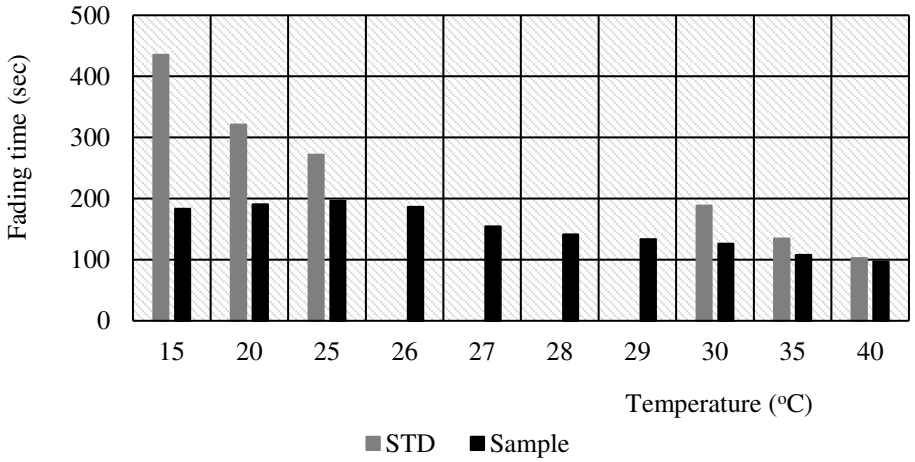

Fig. 11. Effects of temperature on fading time of the blue colour in standard (2 mg/L) and 5.00g sample aliquot under darkness, 20%TCA, 1:1 reacting ratio

A temperature of 15 ºC gave the highest fading time on std aliquot as compared to the other temperatures. The temperature (15ºC) gave low fading time of the blue colour in sample aliquot as compared to 20, 25 and 26 ºC (fig. 11). Temperatures of 20 ºC and 25 ºC gave a comparable fading time of the blue colour in sample aliquot. The two temperatures' (20 ºC and 25 ºC) gave a fading time of 190 and 196 seconds respectively which is not significantly different, their fading time is statistically the same according to T test. Temperatures of between 20 ºC and 25 ºC are considered as room temperature. At this range the differences in fading time are minimal in sample aliquot but great in standard vitamin A solutions. The study was much concerned in determining concentration of retinyl palmitate in samples, therefore 25ºC was taken as ideal temperature because the blue colour's fading time was higher (196 seconds) than the other temperatures. The results obtained here are in agreement to what Caldwell and Hughes (1947b) concluded on the effects of temperature on fading of blue colour.

3.7. Effect of the mass of sample

The study varied the mass of sample on assaying vitamin A to establish their effects on the fading time of the blue colour (fig. 12).

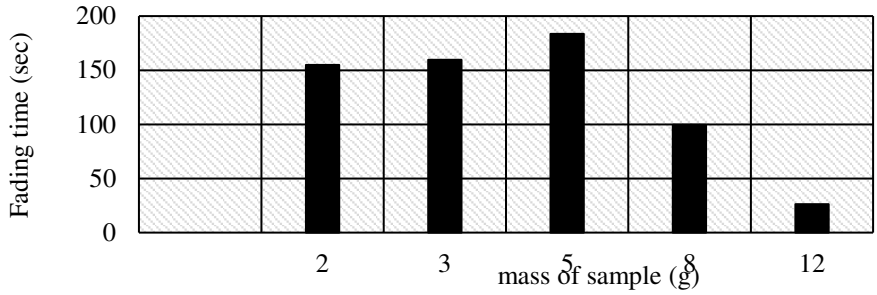

Fig. 12. Effect of mass of sample on fading time under low light intensity, 25°C, 20%TCA and 1:1 reacting ratio of acid to sample

The mass of reactants has effects in any chemical reaction and the Carr-Price reaction is no exception. An increase in the mass of the reactants result in an increase in the mass of the product until an optimal point is reached, a further increase in the mass of any reactants beyond the optimum level leads to constant quantity of products because one reactant acts as the limiting reagent. The limiting reagent is the reactant which is used up first in a reaction (Chang, 2010). In fig. 12, mass of sample was increased from 2.00 to 5.00 g which led to an increase in the fading time and a further increase in mass of sample from 5.00 g led to decrease in fading time which could be due to dimerization of the blue colour product (Kildahl-Andersen et al., 2007). Any increase beyond 5.00 g became excess since the study kept the volume of acid constant as the mass of the reactants increased hence the acid acted as a limiting reagent while the mass of sample was an excess reagent. An excess reagent is defined as a reagent present in quantities greater than necessary to react with quantities of the limiting reagent (Chang, 2010). So, 5.00g was the optimum mass of sample that had reacted with the acid to achieve a maximum fading time of 180 seconds.

4. Conclusion

The spectrophotometric procedure in Vitamin A assaying that applies the Carr-Price reaction should accompany a water bath and be done under suitable optimised mass of oil sample, temperature, light brightness and correct solvent that could give desirable results which are accurate and precise. In this study, the optimised mass of 5.00g of oil sample was dissolved in DCM. The dissolved contents were reacted with 20% TCA in a water bath whose temperature was 25°C under darkness and reacting volumes were one to one (1:1 v/v). Anhydroretinylic cation (blue colour product) got stable for approximately 160±10 seconds with 98.36% recovery when the mentioned conditions were taken into considerations.

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