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DEVELOPMENT OF STABILITY MONITORING TECHNIQUE FOR VITAMIN C TABLETS USING GRAYSCALE ANALYSIS

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Abstract: The objective of this research is to investigate the instability of vitamin C tablets by monitoring their color change using grayscale analysis. Vitamin C tablets were prepared by direct compression. The tablets composed of vitamin C as an active ingredient, talcum and Aerosil® as glidants, and Avicel PH® 102 as a diluent and disintegrant, while polyvinylpyrrolidone K30 was used as a dry binder. The instability of the tablets was accelerated by keeping the tablets without any packaging at 40±2°C, 75±5% RH. The tablet discoloration was observed and the color recorded as an RGB (Red Green Blue) image, and then transformed to grayscale value. The tablets were also determined for remaining ascorbic acid content using high performance liquid chromatography (HPLC). The results showed that when the storage time increased, the tablet color changed from white to brown, with a decrease in grayscale value, and a reduction in ascorbic acid content was observed. The results indicated that the change in grayscale value correlated with the instability of vitamin C in the tablets. This research showed that grayscale analysis could be a useful technique for monitoring the instability of vitamin C tablets.

Keywords: Instability, vitamin C tablets, grayscale analysis, grayscale value, tablet discoloration

INTRODUCTION

The applications of color measurement were used with various drugs in quality control and stability studies. In the quality control study, the efficiency of a coating process on the pellets was determined by color uniformity in the pellet coating. The stability of various drugs was investigated by monitoring the change in color, such as nifedipine, carbamazepine, mefloquine hydrochloride, and ofloxacin, including vitamin C (Subert and Cizmarik, 2008; Jutkus *et al*., 2015; Choi *et al*., 2002).

Vitamin C (ascorbic acid) is an essential substance in maintaining normal health and growth in humans. Vitamin C is mostly found in food and can also be taken as a food supplement. The degradation of vitamin C is induced by moisture, heat, and oxygen (Shephard *et al*., 1999$_a$; Matei *et al*., 2008; Pavlovska and Tanevska, 2013). Vitamin C is reversibly oxidized to dehydroascorbic acid and irreversibly hydrolyzed to 2, 3-diketogulonic acid (Lima *et al*., 2010; Shephard *et al*., 1999a). The discoloration to a brown color is related to many degradation compounds. Shephard, A.B. *et al*. (1999) reported that eight different compounds were detected in severely discolored solid-state ascorbic acid (Shephard *et al*., 1999b). Instability of vitamin C was investigated by the detection of brown discoloration by the tristimulus colorimetry technique using the L*a*b scale (Shephard *et al*., 1999a).

In this research, grayscale analysis was applied for the detection of tablet discoloration to monitor the instability of vitamin C tablets. Vitamin C was formulated as tablets by direct compression and subjected to accelerated treatment (40±2°C, 75±5% RH). The discoloration of the tablets was recorded as a digital RGB image. The RGB color levels were transformed into

grayscale values that represent the measurement of intensity of the tablets' image. The drug content retained in the tablets was analyzed by HPLC, and the relationship between grayscale values and instability of vitamin C was investigated.

MATERIALS AND METHODS

Materials

L-ascorbic acid, monobasic potassium phosphate, dibasic potassium phosphate, and ethylene diamine tetra-acetic acid disodium salt were purchased from Ajax Finechem, Auckland, New Zealand. Methanol (HPLC grade) was purchased from VWR Chemicals, France. Talcum, Avicel PH®102, Aerosil® and polyvinylpyrrolidone K30 were of pharmaceutical grade. Ascorbic acid reference standard was purchased from Sigma-Aldrich, MO, USA.

Development of Vitamin C Tablets

Vitamin C tablets were prepared by direct compression. Their formulation is shown in Table 1.

All powder was sieved through a sieve, mesh size 60. A powder blend of l-ascorbic acid, Avicel PH®102, and polyvinylpyrrolidone K30 was uniformly mixed for 6 min. Talcum was then added and mixed for 3 min. Aerosil® was subsequently added and mixed for another 3 min. A rotary punch tableting machine (UET-6D, Unity Equipment co., LTD, Thailand) was used, with 9 mm round-shaped concave punches.

Table 1. Formula of vitamin C tablets.

Ingredients	Amount (%)
Ascorbic acid	66.7
Avicel PH®102	23.3
Polyvinylpyrrolidone K30	4.0
Talcum	5.0
Aerosil®	1.0
Total	100.0

Evaluation of Vitamin C Tablets

The formulation blend was weighed and transferred into a graduated cylinder. The powder blend was tapped until the final volume was constant by using a tap density tester (Labindia, TD1025, India). The compressibility index (CI) and Hausner ratio were determined according to Equations 1 and 2, respectively, as follows:

$$\% \text{ Compressibility Index} = [(\text{Tapped density} - \text{Bulk density}) / \text{Tapped density}] \times 100 \quad \text{Eq. 1}$$

$$\text{Hausner ratio} = \text{Tapped density} / \text{Bulk density} \quad \text{Eq. 2}$$

The weights of 20 vitamin C tablets were individually measured using an electronic balance (Mettler Toledo, MS204, Switzerland) for determination of uniformity of weight. Twenty tablets were investigated for their hardness using a hardness tester (Erweka, TBH325TD, Germany). Friability was tested using a friability tester (Erweka, TAR220, Germany).

Disintegration times of six tablets were determined using a disintegration test apparatus (Erweka, ZT322, Germany). The dissolution test was performed using a dissolution apparatus

2 (paddle) (Erweka, DT720, Germany) in 900 ml of water at 37 ± 0.5°C. Six tablets were used for dissolution test. The amount of drug released was measured at 45 min. by HPLC.

Determination of Ascorbic Acid Content in Vitamin C Tablets

Sample Preparation

Twenty vitamin C tablets were grounded and the sample precisely weighed. The sample was then dissolved in diluent and the volume adjusted to 50 ml in a volumetric flask. One liter of diluent was composed of 0.56 g of ethylene diamine tetra-acetic acid disodium salt and 2.04 g of monobasic potassium phosphate with pH 3. The sample was filtered by a syringe filter with 0.45 µm membrane and analyzed by HPLC.

HPLC Analysis

The ascorbic acid content in the formulated tablets was analyzed using HPLC (a Shimadzu instrument with a UV detector, Shimadzu, Japan). Reversed phase C-18 Hypersil® (250 x 4.6 mm i.d., 5 µm particle size) with a C-18 guard column was used. The mobile phase system consisted of solvents A and B. Solvent A was a solution of 0.204 %(w/v) monobasic potassium phosphate solution, pH 3, while Solvent B was methanol.

In gradient elution, at 0 to 3 min, solvent A was constant at 100%. At 3 to 5 min, solvent B gradually increased to 100%. At 5 to 6 min, solvent B gradually reduced to 50%. At 6 to 7 min, solvent A returned to 100% and then remained constant for 3 min. The flow rate was 0.8 ml/min. The injection volume was 5 µl. A UV detector was set at a wavelength of 245 nm (The United States Pharmacopeia, 2012). The calibration curve of ascorbic acid was constructed in the concentrations of 50, 150, 250, 350, and 500 µg/ml.

Stability Study

The formulated tablets, without packaging, were kept in an environmental test chamber (Memmert; CTC256, Germany) under accelerated treatment (40±2°C, 75±5% RH) for 123 days. Twenty tablets were tested on days 0, 3, 5 and 7, at weekly and monthly intervals. The images of the formulated tablets were captured using a digital camera, and the ascorbic acid content in the tablets was analyzed by HPLC.

Grayscale Analysis by Image Setting Technique

Twenty vitamin C tablets were photographed in a lightbox using a digital camera (DSLR camera Nikon D750 equipped with Nikon AF-S VR 60 mm F2.8G IF-ED MICRO Ratio 1:1, Nikon, Japan). The lightbox contained a 22-watt light source with a color temperature of 6,500 K. The power of the light was 1,110 ± 10 lux, as calibrated by using a luminometer (UNI-T; UT383). The shutter speed of the camera was set at 200, with an aperture number of 22. ISO value was set at 400, and the F value was 5.6. White balance was set to auto mode.

The digital images were analyzed by transforming the RGB color system to grayscale, and the grayscale level of the tablets' image was analyzed on Day 0. After the tablets were subjected to accelerated treatment (40±2°C, 75±5% RH), the tablets were sampled at various time intervals for grayscale analysis and HPLC assay.

Analysis of Images

The methods of pattern-finding algorithms and color recognition of the tablets by image thresholding were applied.

Image segmentation is a common technique to partition objects and boundaries in images. This technique separates an image of a tablet from the background (Priya and Kavitha,

2015; Senthilkumaran and Vaithegi, 2016; Sengur *et al*., 2019). The RGB image was transformed into a grayscale image containing only gray tones.

The RGB value of each pixel was transformed into a single value reflecting the brightness of the pixels, where 0 = black (dark) and 1 = white (bright). Therefore, the intensity of the 8-bit grayscale image was 256 shades of gray (0-255), varying from black (grayscale value 0) to white (grayscale value 255). A lower grayscale level denotes a darker color, while a higher value denotes a brighter one (Buyuksahin, 2014). The discoloration of vitamin C tablets from white to brown was expressed as a grayscale level.

Calculation of Average Grayscale Level

The color level of the histogram was 256 shades. Each pixel composed of R (Red), G (Green) and B (Blue) colors. Each RGB pixel value at location (i, j) was transformed to a grayscale value by a weighted sum of the R, G and B components allocated into corresponding locations (i, j), as shown in Equation 3.

The coefficients are identical to those used for calculating luminance of ITU-R BT.601-7 (as recommended by the radio-communication sector of ITU for Broadcasting Service (Television). The grayscale value of the tablets' image was averaged from all pixels (Buyuksahin, 2014; Okama, 2015).

$$\text{Grayscale values at } (i, j) = 0.2989*R(i, j) + 0.5870*G(i, j) + 0.1140*B(i, j) \quad \text{Eq. 3}$$

Where:

(i, j) = the location,
R = red value,
G = green value, and
B = blue value.

Statistical Analysis

The results were expressed as mean ± standard deviation (SD). The statistical analysis of data was performed using one-way ANOVA (SPSS software version 21). The level of significance was $p < 0.05$.

RESULTS AND DISCUSSION

The discoloration of dosage forms is considered as physical instability that may result from chemical degradation (Yoshioka and Stella, 2002). The discoloration of vitamin C was associated with ascorbic acid retention (Choi *et al*., 2002; Lima *et al*., 2010). The degradation of l-ascorbic acid caused a brown discoloration (Shephard *et al*., 1999a).

In this study, discoloration of vitamin C tablets was monitored after storing the formulated vitamin C tablets in an accelerated condition. The vitamin C tablets were prepared to monitor the discoloration of the tablets by using grayscale analysis of digital images of the tablets. Digital image processing is a useful technique in the pharmaceutical field as a method often used for detecting defective pills and pill identification (Prajwala, 2018; Semnani and Ghayoor, 2009).

In this research, grayscale analysis with digital image processing was used for monitoring the instability of vitamin C tablets, as shown by their discoloration. The vitamin C tablets composed of ascorbic acid as an active ingredient, talcum as a glidant, and Avicel PH® 102 as a diluent and disintegrant. Polyvinylpyrrolidone K30 was used as dry binder and Aerosil® as a glidant. The tablets were prepared by direct compression, but were not coated with coating materials because it could influence the stability of ascorbic acid and the discoloration of the tablets (Ogbonna *et al*., 2016).

The formulation blend had a compressibility index of 22.00% and a Hausner ratio of 1.28, indicating passable flow property and showing the blend was flowable for direct compression. The vitamin C tablets were white in color, round shaped, with a concave surface.

The tablets' properties at Day 0 are shown in Table 2. The tablets contained 99.18% of a labeled amount that was within the acceptance range of 90-110%. The percentage of weight loss after the friability test was < 1%. The tablets had a disintegration time of 59 seconds. Ascorbic acid content dissolved from the tablet from the dissolution test was not < 80% within 45 min., passing the USP criteria of: not < Q + 5%; Q = 75 % for ascorbic acid tablets (The United States Pharmacopeia, 2012).

Table 2. Properties of vitamin C tablets (Day 0).

Parameters	
Ascorbic acid content (% labeled amount)	99.18 ± 0.03
Uniformity of weight (mg)	374.90 ± 13.02
Tablet diameter (mm)	9.06 ± 0.03
Tablet thickness (mm)	5.10 ± 0.05
Hardness (N)	73.25 ± 8.30
Friability (%)	0.20
Disintegration time (s)	59 ± 20
Dissolution (45 min) (%)	87.59 ± 3.25

The vitamin C tablets were stored under accelerated conditions (40±2°C, 75±5% RH) to determine the change in their color and the ascorbic acid remaining over storage time. Changes in the color and drug content were examined using grayscale analysis and HPLC analysis, respectively.

The color analysis was performed by taking digital images of the tablets. The tablets' color in RGB and in grayscale is shown in Figure 1. The RGB value was transformed into grayscale value or grayscale level. The RGB value of each pixel was transformed to a grayscale value reflecting the brightness of pixels, where 0 refers to black (0% brightness) and 1 to white (100% brightness). Therefore, the intensity of the 8-bit grayscale image was 2^8 (or 256) shades of gray (0-255) varying from black (grayscale value = 0, 0% of brightness) to white (grayscale value = 255, 100% of brightness). A lower grayscale value means a darker gray, while a higher value means a brighter gray.

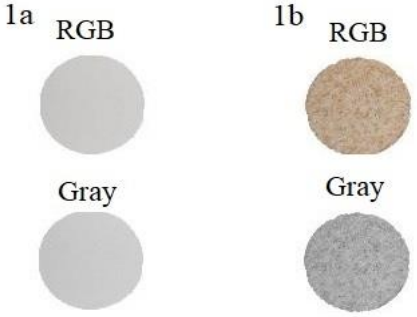

Figure 1. RGB and Grayscale images of vitamin C tablets without discoloration (1a), and discoloration of vitamin C tablets (1b).

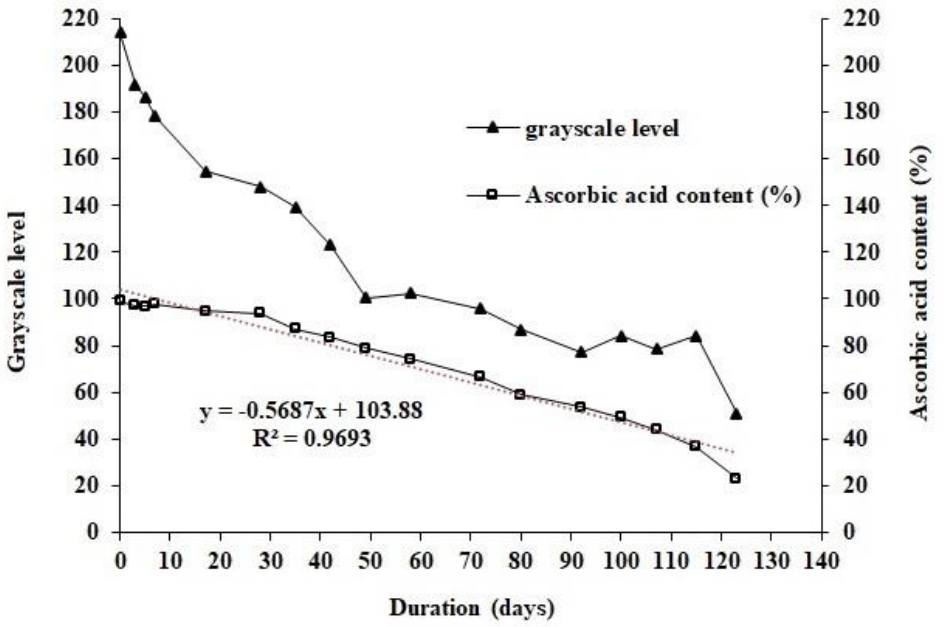

Figure 2. Ascorbic acid content and grayscale levels of the formulated vitamin C tablets over storage time (40±2°C, 75±5% RH).

The results of the change in the tablet grayscale levels over storage time are shown in Figure 2. These show that when the vitamin C tablets were stored under an accelerated condition, the grayscale levels decreased over time. The decrease in drug content over time was determined by HPLC assay.

The results showed that the % labeled amount of ascorbic acid reduced from 99.18% on Day 0 to 97.13% on Day 3, 94.72% on Day 17, and 93.91% on Day 28, within the limit of % labeled amount of vitamin C tablet (90-110%).

The degradation of formulated vitamin C tablets followed zero order reaction (r^2 of 0.9693), as shown in Figure 2. The tablets showed light brown spots from Day 3, whereas the ascorbic content was 97.13%. The discoloration developed at a faster rate than the degradation rate of ascorbic acid, as shown by the greater slope of the grayscale level profile.

The result was in accordance with the study of Shephard, A.B. *et al*. (1999). This previous study reported that monitoring of ascorbic acid degradation by color change, using tristimulus colorimetry, was more sensitive in detecting the onset of ascorbic acid degradation than HPLC assay (Shephard *et al*., 1999a).

At Day 92, the formulated tablets showed dark brown sticky gel on their surface, with a grayscale level of 77.13. The ascorbic acid content, analyzed by HPLC, was 53.52% at Day 92, and continued a gradual reduction. In contrast, the color level reduced only slowly after Day 92. The result showed that when the tablets showed severe discoloration and lost physical stability, grayscale analysis was less sensitive than HPLC assay.

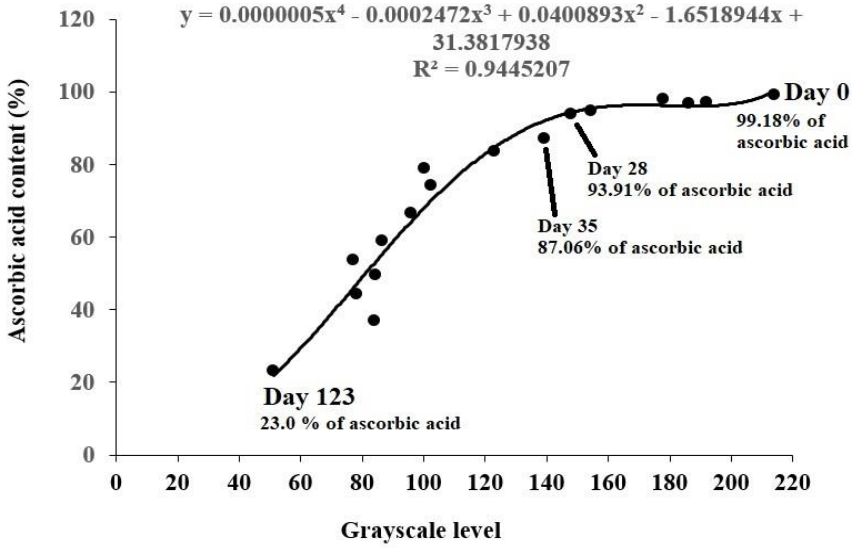

Figure 3. The relationship between grayscale levels and ascorbic acid content of vitamin C tablets (a storage range of Day 0 to Day 123).

Figure 3 shows the polynomial regression model of the relationship between the grayscale levels and ascorbic acid retention of the formulated vitamin C tablets over the storage time. The regression model was fit to fourth order model with a correlation coefficient (r^2) of 0.9445. The result indicated that the grayscale level correlated with the instability of ascorbic acid.

On Day 0, the tablet was white without any brown discoloration, as expressed as the highest grayscale value in Figure 3, and ascorbic acid content was 99.18%. The grayscale value decreased in a faster manner compared with the decrease in ascorbic acid content from Day 0 until Day 28. On Day 28, the tablet contained ascorbic acid content in the acceptable range of 90-110%. The result showed that the discoloration monitored by grayscale analysis which could be observed prior the amount of ascorbic acid remaining in the tablet was lower than 90% of the labeled amount. This result was in accordance with the previous report investigated with solid-state ascorbic acid (Shephard *et al*., 1999a).

As per the routine stability monitoring, the degradation of ascorbic acid could be easily predicted from the grayscale values by using the correlation model. The degradation of ascorbic acid affected by the excipients in the formulation (Vemuri *et al*., 1985) and the different commercial vitamin C tablets had different discoloration profiles (Sukpornsawan and Wannachaiyasit, 2020). Therefore, this polynomial regression model was used only for this specific formulation. This method of monitoring the instability of vitamin C tablets could be useful as a fast, non-destructive method for monitoring the degradation of ascorbic acid.

CONCLUSION

This study showed a significant correlation between the grayscale level and ascorbic acid instability of vitamin C tablets. The relationship between grayscale level and drug retention followed fourth order regression model with r^2 of 0.9445. The grayscale analysis by digital image processing could be a promising technique for monitoring drug stability.

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