Rapid Communications

Inner Product of RGB Unit Vectors for Simple and Versatile Detection of Color Transition

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We propose a novel concept for detecting color transition by the inner product (IP) of RGB unit vectors. A digital microscope-based detector and a Visual Basic program were developed in-house. The concept is applied to indicator-based flow titration. The IP is 1 or < 1 if the vector's direction is the same or different, respectively. The IP's change can be used as a criterion for the indicator's color transition. The present IP-based approach is simple, economical, and versatile because it is applicable to any color transition without selecting an analytical wavelength.

Keywords Image-based colorimetry, digital microscope, inner product, RGB, flow titration, flow ratiometry

(Received November 20, 2020; Accepted November 29, 2020; Advance Publication Released Online by J-STAGE December 4, 2020)

Introduction

Since the advent of commercial digital cameras in the 1990s, studies on analysis using digital images[1,2] have been reported. Recently, not a few studies using general photographing equipment, especially smartphones,[3-5] have been published for quantitation. Applying image-based colorimetry to flow analysis, Gaiao *et al.*[6,7] sampled the titrand from a reaction vessel on-line to determine the indicator's color transition based on RGB values. Digital movie-based titrations by flow-batch methods[8,9] employed the RGB and Hue values for the equivalence point detection. However, selecting an appropriate tristimulus value (*i.e.*, *R*, *G*, or *B*-value) is needed in advance, depending on the indicators' color.

In the present paper, we propose a novel concept of RGB-based detection for the indicator's color transition. The color image obtained with a digital microscope is expressed as an RGB unit vector. The inner product of the vectors before and during titration is used as a criterion for the color transition. The concept was demonstrated by applying to a fixed triangular wave-controlled flow ratiometric titration.[10]

Digital Microscope-based Flow-through Detector

Figure 1 shows a photograph of a detector fabricated in house. A digital microscope having ten white LEDs, a 1-mm i.d. quartz optical flow cell, and three white LED modules were set in a black plastic box. The fabricated detector was set in a flow system, shown in Fig. S1 (Supporting Information), where the titrand/titrant flow ratio is continuously varied in accordance with the controller output voltage (V_c). Image data (example: Fig. S2 in Supporting Information) were acquired in a PC using the Open Computer Vision Library (Ver. 2.4.13) at a sampling frequency of 10 Hz. The image's color was expressed as tristimulus values (*i.e.*, *R*, *G*, and *B*-values) for each pixel in a preset range (6 × 6 pixels). The values were respectively averaged and used for further analysis. All operations, except

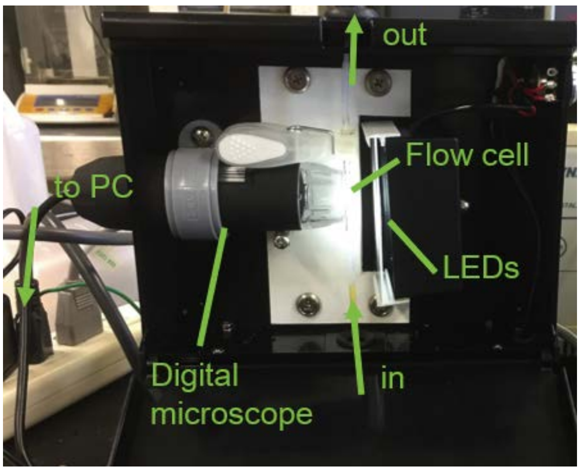

Fig. 1 Side view of the inside of the digital microscope-based detector. Digital microscope: YPC-X02 digital microscope (640 × 480 pixels, UMTELE Co., China). The dimension of the black plastic box: 18.0 (W) × 5.5 (D) × 14.3 (H) cm. The cell was illuminated by the 10 LEDs arranged around the microscope's lens and the 3 LEDs set behind the cell. The solution was allowed to flow upwards in the cell so that accidentally introduced air bubbles could be easily flushed away.

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DOI:10.2116/analsci.20C015

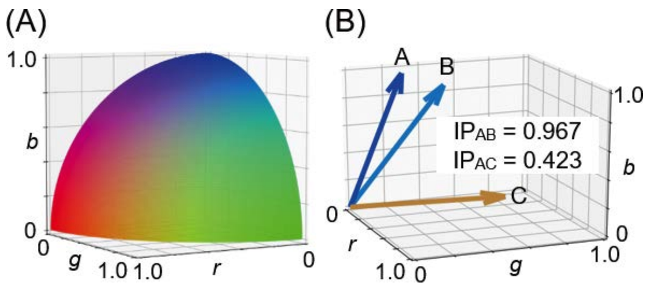

Fig. 2 (A) Three-dimensional color space; (B) two types of blue and orange vectors. The color of the arrows (Web version of this paper) corresponds to the images' color.

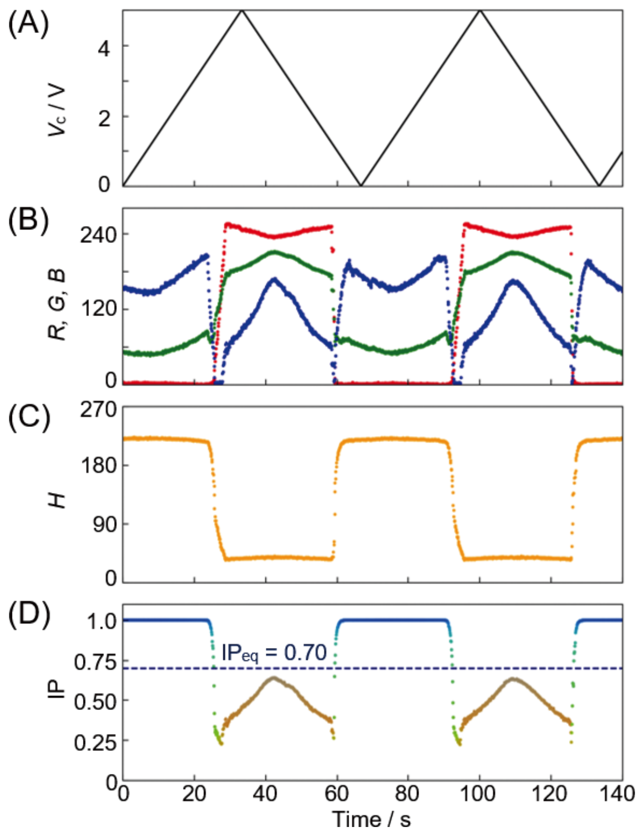

Fig. 3 Control and analytical signals. Titrand, 0.10 mol dm^{-3} HCl; titrant, 0.10 mol dm^{-3} NaOH containing 2.0 mmol dm^{-3} BTB. The scan range of V_c was fixed (0 – 5 V). (A) Temporal profile of V_c. (B) Temporal profiles of R (red), G (green), and B (blue) values. (C) Temporal profile of H value. (D) Temporal profile of IP. The dotted line shows the equivalence IP (IP_{eq}). The sampling frequency was 10 Hz.

for changing liquids, were automated with an in-house program developed in Microsoft Visual Basic .NET.

The principle of fixed triangular wave-controlled flow ratiometry[10] is shown and described in detail in Fig. S3 and its caption (Supporting Information). Briefly, while the total flow rate (F_T) is held constant, the titrand is pumped at a flow rate of F_B, which is in proportion to V_c; the titrant containing an indicator is aspirated at a flow rate of ($F_T - F_B$) and merged with the titrand (also see Fig. S1 in Supporting Information). A microscope set downstream captures a digital video image of a merged solution. The RGB values are obtained from the image data and then converted to a unit vector. The inner products (IP) of the first and the ith image vectors are calculated. Even if V_c reaches V_E, which gives the equivalence point, the equivalence signal (IP_{eq}) is not detected because of the lag time (t_{lag}) between the upstream merging and downstream image taking. V_E is obtained by averaging the most recent two V_c at the sensing of IP_{eq}. This V_E gives the titrand concentration based on Eqs. (S1) and (S2) (Supporting Information).

Inner Product from RGB Values

The tristimulus RGB values obtained were converted to unit vectors $\vec{v}_i$.

$$\vec{v}_i = (r_i, g_i, b_i), \quad (1)$$

where the direction cosine (r_i, g_i, and b_i), expressed by the following equations, satisfy $r_i^2 + g_i^2 + b_i^2 = 1$:

$$r_i = \frac{R_i}{\sqrt{R_i^2 + G_i^2 + B_i^2}}, \quad (2)$$

$$g_i = \frac{G_i}{\sqrt{R_i^2 + G_i^2 + B_i^2}}, \quad (3)$$

$$b_i = \frac{B_i}{\sqrt{R_i^2 + G_i^2 + B_i^2}}, \quad (4)$$

where R_i, G_i, and B_i mean the tristimulus values for the ith image. The ending point of $\vec{v}_i$ is on a sphere with a radius of unity in the octant of $0 \le r_i, g_i, b_i \le 1$. Figure 2A in Web version shows the color that $\vec{v}_i$ expresses in the three-dimensional orthogonal coordinate color space, the axes of which are r, g, and b.

An inner product (dot product), IP_i, of the first and the ith image vectors ($\vec{v}_1$ and $\vec{v}_i$), respectively, was calculated by Eq. (5).

$$IP_i = \vec{v}_1 \cdot \vec{v}_i = |\vec{v}_1||\vec{v}_i| \cos\theta = \cos\theta$$
$$= r_1 r_i + g_1 g_i + b_1 b_i, \quad (5)$$

where $|\vec{v}_1|$, $|\vec{v}_i|$, and θ mean the $\vec{v}_1$ and $\vec{v}_i$ magnitudes and the angle between them. The magnitudes are unity because both vectors are unit vectors. IP_i is unity if the colors are completely the same (*i.e.*, $\vec{v}_1 = \vec{v}_i$); IP_i is lowered from unity if the colors are different from each other. Figure 2B shows an example of vectors. The coordinates of vectors A, B, and C are (0, 0.303, 0.953), (0, 0.537, 0.843), and (0.780, 0.569, 0.262), respectively. Vector A and vector B, which point in the near direction, give an IP value as high as 0.967. On the other hand, vector A and vector C, the vectors in different directions, give an IP value as low as 0.423. The IP of vectors B and C also has a low value of 0.526. Therefore, a drastic change in IP can be used as a measure for detecting any indicator's color transitions.

Results and Discussion

Figure 3 shows temporal profiles of V_c (A), RGB (B), Hue (C), and IP (D) for the titration of 0.10 mol dm^{-3} HCl with the same concentration of NaOH containing 2.0 mmol dm^{-3} Bromothymol Blue (BTB). V_c is varied in the 0 – 5 V range at a scan rate of 0.15 V min^{-1} (Fig. 3A). As the titrand/titrant flow ratio increased with V_c, the mixed solution changed from alkaline to acidic. This change resulted in the indicator's color transition from blue to yellow *via* green.

The tristimulus values (R, G, B) of the RGB color system can take 0 – 255 in the 8-bit system. When the indicator's color changed from blue to yellow (24.0 – 28.4 s and 91.0 – 94.9 s), the R and the G-values increased, whereas the B-value decreased significantly (Fig. 3B). These values recovered to the original values when the indicator's color changed from yellow to blue (58.8 – 62.0 s and 125.8 – 128.8 s). Although the R-value showed the most significant change among them, the tristimulus value of which is most optimal depends on the species of the indicator employed.

The Hue (H) value calculated from the tristimulus values by using Eq. (S3) in Table S1 (Supporting Information) can take 0 – 360. The H-value also shows a drastic change from 225 (blue) to 35 (yellow) during the indicator's color transition (Fig. 3C). However, the degree and direction of change in the H-value depend on the indicator used. For example, when *p*-Naphtholbenzein (green and orange in acidic and alkaline media, respectively) was used as an indicator for nonaqueous titration, the amount of the H-value change was 90 (120 to 30). In addition, the H-value has inherent disadvantages in that it is not defined at $R = G = B$,[11] and has no information concerning the saturation and brightness of the color.

In principle, IP can take a value in the range from unity to zero. However, IP = 0 is a rare case because both vectors should be orthogonal (for example, one is on the r-axis, and the other is in the gb-plane). In the present system, IP fell from 1 to 0.22, as shown in Fig. 3D. A significant change in IP was also observed in the indicator's color transition periods mentioned above. Triangular profiles (27.8 – 59.1 s, 94.5 – 125.8 s) having peaks at 43 and 111 s, respectively, are attributed to a decrease in the indicator's concentration. That is, the titrant's flow rate containing the indicator still decreased after the equivalence point until a reversal of the V_c scan. After examining various acid-base titrations using various indicators, we selected 0.7 as the optimum IP for detecting any indicator's color transitions. The mean (0.61) of the maximum and the minimum IP values (*i.e.*, 1 and 0.22, respectively) is not appropriate because IP after the equivalence point increases and may cross this value. It should be noted that IP between the normalized RGB vectors for the (i – 1)th and ith image can also be used to detect the equivalence point. The temporal profile of this IP, as shown in Fig. S4 (Supporting Information), corresponds to the primary differential curve.

V_c that gives the equivalence composition (V_E) can be obtained by averaging the most recent two V_c values at the instant of sensing the equivalence point.[10] Flow ratiometry is an absolute method in principle as long as the flow rate is accurately calibrated. A daily calibration of the flow rate is, however, troublesome and time-consuming. Therefore, the absolute calibration method is adopted, where V_E^{-1} is plotted against the titrand concentration C_B based on Eq. (S2) (Supporting Information). Table S2 (Supporting Information) summarizes the calibration curves for various acid-base titrations. Linear calibration curves were obtained for all titrations with acceptable ranges and linearity ($r^2 > 0.998$).

In conclusion, we first proposed the use of RGB unit vectors' inner product (IP) for analyzing the digital images' color. Our IP-based approach is simple, economical, and versatile because it can be used as a universal criterion to detect any color transition without selecting an analytical wavelength and the optimal color specification value.

Acknowledgements

The present study was partly supported by a Grant-in-Aid for Scientific Research (C) (15K07889 and 18K05173) from the Japan Society for the Promotion of Sciences (JSPS).

Supporting Information

The flow system and principle of fixed triangular wave-controlled flow ratiometry is shown in Figs. S1 and S3, respectively. An example of a microscope image for the colored solution is shown in Fig. S2. The temporal profile of IP of the normalized RGB vectors for the (i – 1)th and the ith image is shown in Fig. S4. Formulae for computing the Hue value from the RGB values are listed in Table S1. Calibration curves of various acids and bases are summarized in Table S2. These materials are available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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