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Digital movie-based on automatic titrations

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ABSTRACT

This study proposes the use of digital movies (DMs) in a flow–batch analyzer (FBA) to perform automatic, fast and accurate titrations. The term used for this process is "Digital movie-based on automatic titrations" (DMB-AT). A webcam records the DM during the addition of the titrant to the mixing chamber (MC). While the DM is recorded, it is decompiled into frames ordered sequentially at a constant rate of 26 frames per second (FPS). The first frame is used as a reference to define the region of interest (ROI) of 28×13 pixels and the *R*, *G* and *B* values, which are used to calculate the Hue (*H*) values for each frame. The Pearson's correlation coefficient (*r*) is calculated between the *H* values of the initial frame and each subsequent frame. The titration curves are plotted in real time using the *r* values and the opening time of the titrant valve. The end point is estimated by the second derivative method. A software written in C language manages all analytical steps and data treatment in real time. The feasibility of the method was attested by application in acid/base test samples and edible oils. Results were compared with classical titration and did not present statistically significant differences when the paired *t*-test at the 95% confidence level was applied. The proposed method is able to process about 117–128 samples per hour for the test and edible oil samples, respectively, and its precision was confirmed by overall relative standard deviation (RSD) values, always less than 1.0%.

1. Introduction

Determination of the free fatty acids (FFA) in edible oils is a measure of the extent to which hydrolysis has liberated fatty acids from the ester linkages to their parent triglyceride molecule [1]. It is one of the most common processes used to evaluate and classify oils and to control the quality of oils during production, storage and marketing. Organizations such as the Association of Official Analytical Chemists (AOAC), the American Oil Chemists Society (AOCS), the British Standard Institution (BSI), and the European Committee for Standardization (CEN), etc., have been involved in the standardization of analytical methods applied to oils and fats. Official methods for determination of the acidity of edible oils are based on non-aqueous visual titration methods [2].

Visual titrations for determination of both major and minor components in samples [3] have played a fundamental role in the development of chemistry and the chemical industry [4] since the 18th century. They are considered the primary method of analysis and continue to be used for the validation of secondary methods [5] due to their precision, convenience and affordability [3]. Despite these advantages, visual titration procedures are generally confined to a batch operation with:

(1) Subjectivity in the identification of the end point, requiring an experienced analyst;
(2) Significant amounts of sample and titrant;
(3) Poor throughput rate.

The two first problems were overcome by the introduction of techniques instrumental to the detection of end points in titration procedures. However, the throughput rate remained limited due to the manual addition of titrant and samples. By using several flow-based approaches for several analytes [3,6–15], the development of automatic and miniaturized systems for carrying out titrations solved these problems, especially for determination of acidity in edible oils [2,16,17].

Even with the successful implementation of automatic titration methods, research on innovative techniques, which are more affordable and are independent of analytical system detection methods, led to the emergence of alternative methods using digital images (DIs) [18–21]. The main motivation for the use of DI treatment for end point detection is its ability to overcome the great dificulty with which traditional methods handle the formation of the intermediary colors of the indicator (dichroism) [18]

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and other color samples [20] in obtaining accurate visual identifications of end points. Another attractive feature of the DI method is the wide availability of imaging devices based on CMOS (complementary metal oxide semiconductor) and CCD (coupled charge device) technologies. Digital imaging methods have demonstrated accuracy and sensitivity to titration procedures, however DIs continue to be manually obtained, which results in throughput rates as poor as other instrumental titration procedures.

This study proposes the use of digital movies (DMs) to acquire the sequence of frames necessary to perform faster titrations using DIs. By using a suitable software, DMs can be decompiled into individual frames at a constant rate, thereby generating a time-based titration curve. The analytical response of this curve is obtained by calculating the Pearson's coefficient correlation [22] for the color of each frame. This approach involves simultaneous titrant addition and data acquisition and enables the detectection of slight changes in the RGB (red–green–blue) [23] properties of the recorded frames. It requires the use of a flow–batch analyzer (FBA), which is universal in nature and easily automates most analytical procedures. Furthermore, the FBA attaches to practically any analytical instrument, as demonstrated in the literature [24].

This study proposes the use of the FBA in association with DMs obtained from a webcam to perform automatic, fast and accurate titrations in real time. This technique was applied to alkaline and acidic test samples and to edible oil samples for determination of total acidity in the edible oils.

2. Theoretical

2.1. Digital movies

Digital movies obtained from CMOS or CCD devices are comprised of a series of orthogonal bitmap digital images called frames. When displayed in rapid succession at a constant rate, these frames generate a sense of movement for the viewer. This phenomenon, called “persistence of vision,” is responsible for the fact that humans continue to see the image of a static picture for a fraction of a second (approximately 1/12 of a second) after it disappears. If, before this time, another image reaches the retina, both images are combined. Frame rate, or frame frequency, expressed in frames per second (FPS), is the ratio at which an imaging device yields unique consecutive images. This technical term is applied to computer graphics, video cameras, film cameras, and motion capture systems.

Since every frame is an orthogonal bitmap DI comprised of a raster of pixels, it is possible to decompile the frame sequence of the DM using a suitable software. Once the DM is decompiled, it becomes possible to obtain chemical information based on the color changes between frames.

Data treatment of frames is based on the RGB color model, in which the values of each component vary from 0-255 integers (8 bits) [25,26]. Parameters of other color models, such as the HSI (hue–saturation–intensity), HSV (hue–saturation–value) and the HSB (hue–saturation–brightness) models, are obtained as starting variables through mathematical equations using the RGB data acquired from the DIs [25].

2.2. Pearson's correlation coefficient

The Pearson's correlation coefficient, denoted as r, is widely used in image processing, pattern recognition and statistical analysis [27–29]. For monochromatic frames, the Pearson's correlation coefficient can be defined as the following equation:

$$r = \sum_{i,j} (x_{i,j} - \bar{x})(y_{i,j} - \bar{y}) / \left(\sqrt{\sum_{i} (x_{i,j} - \bar{x})^2} \right) \left(\sqrt{\sum_{i,j} (y_{i,j} - \bar{y})^2} \right) \quad (1)$$

Where $x_{i,j}$ is the color component of the ith pixel located at line i and column j of frame 1; y_i is the color component of the ith pixel located at line i and column j of frame 2; $\bar{x}$ is the mean color component of all pixels of frame 1; and $\bar{y}$ is the mean color component of all pixels of frame 2.

This coefficient presents three notable values. If $r=1$, the compared frames are absolutely identical. If $r=0$, they are completely uncorrelated. If $r=-1$, they are completely anti-correlated (frames are negative of each other). In DI analysis, r is typically used to compare two images taken of the same object at different times. The r value indicates whether the object has been somehow altered. In theory, an $r=1$ value is obtained only if the object is intact, that is, without alteration. However, in practice some distortions may occur in the monitored system due to pixel noises (pictorial variations caused by environmental lighting) and other factors which yield r values <1. Typical r values for two digital images of the same scene recorded sequentially by using the same imaging system, position and illumination range from 0.95 to 0.98. Typical r values used to detect significant changes vary from 0.30 to 0.85. This study examines the optimal r values for indicating significant changes in a sequence of images obtained from a webcam operating in movie mode.

2.3. Digital movie-based on automatic titration mathematical model

The term used for the proposed method is “Digital movie-based on automatic titrations” (DMB-AT). Considering the generic reaction: $xT+yS \leftrightarrow$ products, in which x mols of titrant (T) and y mols of titrate (or analyte) (S) react in completeness, the mathematical model for a manually performed titration is shown in the following equation, where C_S is the analyte concentration in the sample; C_T is the titrant concentration; V_S and V_T are the respective volumes of sample and titrant; and x and y are the stoichiometric coefficients of the reaction between the titrant and titrate.

$$x\, C_S\, V_S = y\, C_T\, V_T \quad (2)$$

In the analyzer of the propsed method, $V_i = Q_i\, t_i$ (where Q_i is the flow rate in the i channel), and the valve timing courses, t_i, define the volumes, V_i, which are delivered to the MC. At the end point of titration, the volume of titrant that is proportional to t_T is now t_{EP} and the volume of analyte is now t_S. Therefore, Eq. (2) can be rewritten with regards to time rather than volume by using t_S and t_{EP} instead of V_S and V_T:

$$x\, C_S\, Q_S\, t_S = y\, C_T\, Q_T\, t_{EP} \quad (3)$$

The analyte concentration is then calculated by the following equation:

$$C_S = yC_TQ_T\, t_{EP}/xQ_S\, t_S \quad (4)$$

The ratio Q_T/Q_S must be identified due to the differences that may exist between the flow rates of each channel. Eq. (4) assumes the following form, where the ratio Q_T/Q_S is labeled $\rho_{T/S}$:

$$C_S = (y\, t_{EP}/x\, t_S) C_T\, \rho_{T/S} \quad (5)$$

The concentrations of titrant and analyte must be expressed in mmol L^{-1} or mol L^{-1}. This equation is valid for any chemical reaction.