Verification of 2D Gaussian Model of Concentration on TLC Plate for Image-Based Quantification of Carbohydrates[*]

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Abstract: - Thin-layer chromatography (TLC) is a well-known and commonly used technique in synthetic chemistry for identifying compounds and measuring the concentration of a solution, and it is applied not only in basic chemist but also in the fields of biochemists, food analysis and so on. On this technique, operators obtain much information from the results on the plate by evaluating positions and the color of the sample components of the solutions. However, the results depend on the operator because the information is usually evaluated by just only visual assessments. So the accurate measurement for concentrations of solutions can no longer be expected. In order to solve the problem, some authors of this paper proposed a quantification method of the carbohydrate based on the scanned image analysis and its efficacy was confirmed by experiments comparing with the result of the high performance liquid chromatography (HPLC). In this method, color density of each spot was modeled by the 2-dimensional (2D) Gaussian function. In this paper, a dynamical model of the TLC is introduced and the reasonableness of this Gaussian model is verified by both numerical and experimental results.

Key-Words: - TLC, quantification of carbohydrates, chemical image analysis, dynamics of chromatography, advection-diffusion equation.

1 Introduction

Thin-layer chromatography (TLC) is a well-known and commonly used technique in synthetic chemistry for identifying compounds and measuring the concentration of a solution, and it is applied not only in basic chemist but also in the fields of biochemists, food analysis and so on [1], [2]. On this technique, a glass plate coated with a thin layer of a material, usually silica gel, alumina, or cellulose, is used. The sample is spotted on this plate, by the micropipette, and solvents are drawn up the plate as blurred dots on different positions corresponding to components of the mixture through several processes. Operators obtain much information about the material from the results by watching positions and the color of the sample components. Since analyzing results depend on the operator, the accurate measurement of concentration can no longer be expected. In order to solve this problem, methods for calculating the cumulative color density of the spot using a flatbed color scanner was reported in [3]. In the report, micro

samples of the Mercury, Arsenic and Lead were investigated for quantifying the concentrations.

On the other hand, carbohydrates play important roles in numerous physiological processes [4]. For analyzing carbohydrates, several technologies have been reported, *i.e.* the nuclear magnetic resonance (NMR), the high performance liquid chromatography (HPLC), the gas chromatography (GC), the mass spectrum (MS) and so on, but these technologies are only available in specialized laboratories. For glycobiology to advance, widely applicable simple analytical methods like as the TLC are needed. So, some authors of this paper proposed a method for quantifying of carbohydrates from the TLC results by using image analysis compensating lack of spot overlaps and , in order to confirm the proposed method, experiments results are investigate comparing with HPLC results [5], [6]. On this method, the color density concerned with the concentration of the solution was modeled by 2-dimensional (2D) Gaussian function, and the concentration was quantified by calculating the

volume of the function. This idea was inspired by the quantification method of the result for the HPLC such that the concentration of the solution is determined by the electrical peaks of the output current curve, and a method that the curve is modeled by the one dimensional Gaussian function [7]. However, for investigating that the shapes of the results of the chemical analysis can be modeled by Gaussian or not, few literature exist.

On the other hand, as simple model explaining the dynamics of the chromatography, the one-dimensional advection-diffusion equation was introduced by Giddings [8] as follows:

$$\frac{\partial c(t,y)}{\partial t} = -R_v \frac{\partial c(t,y)}{\partial y} + D \frac{\partial^2 c(t,y)}{\partial y^2}, \qquad (1)$$

where $c(t,y)$ is the concentration of solution for the spatial position y at time t, R_v and D are the velocity and the diffusion coefficient of the component, respectively.

This equation can be explain of the dynamics of the gas or the liquid chromatography under the condition that the column in which solution flows is very narrow with respect to the length. So, this equation is no longer applicable for the TLC because the solution spreads widely on the TLC plate during the chromatographic process.

In this paper, dynamics of the TLC is proposed by extending this model and reasonability of the density model (2D Gaussian) for the image-based quantification is verified by numerical and experimental results.

The remainder of the paper is organized as follows: section 2 introduce the dynamical model of the solution on the TLC plate and propose the identification method of the model coefficients. Section 3 deals with the verification method of Gaussian and we confirm it by simulations and experiments.

2 Dynamical Model of the Thin-Layer Chromatography

2.1 Dynamics of Concentration

The left figure of Fig. 1 shows a sample of scanned image of 6 TLC plates developing the components of glucose solutions for different time $t = 300,\ 600,\ 1200,\ 2400,\ 3600,\ 4200$ sec. The experimental setup and the scanning condition are shown in Appendices. The test solution spreads widely on the TLC plate during the chromatographic process. Furthermore, the solution is transported by the developing solvent caused by the capillary action [2]. Considering above, in this paper, the dynamical model of the concentration of the solution on the TLC plate is assumed to be the 2-dimensional advection-diffusion equation with time varying velocity $R_v(t)$ and diffusion coefficient D extending the model proposed by Giddings given by equ. (1) as follows:

$$\frac{\partial c(t,x,y)}{\partial t} = -R_v(t) \frac{\partial c(t,x,y)}{\partial y}$$
$$+ D \left(\frac{\partial^2 c(t,x,y)}{\partial x^2} + \frac{\partial^2 c(t,x,y)}{\partial y^2} \right), \qquad (2)$$

where $c(t,x,y)$ is the concentration on a position (x,y) on the TLC plate by coordinating a system shown in the right figure of Fig. 1. The 1st and the 2nd term of RHS of equ. (2) indicate the advection and the diffusion of the component, respectively. The TLC plate having the length Y and the width X. The test solution is spotted around (x_0, y_0) on the TLC plate. Then the plate is dipped upright into the developing solvent. So the spotted solution is transported in the vertical direction (y direction). The time when the TLC plate is dipped into the solvent is set to the initial time $t = 0$. To investigate dynamics of the concentration for fixed time t, $c(\cdot, x, y)$ is conveniently assumed to be equivalent to the color density of the scanned image.

2.2 Identification of Coefficients

The relation between the diffusion coefficient D in equ. (2) and the variance of molecular $\sigma^2(t)$ is well known as the Einstein diffusion equation given by

$$\sigma^2(t) = 2Dt. \qquad (3)$$

This relation is also shown in [7], and in this paper, $\sigma^2(t)$ is obtained by calculating the 2nd moment of color density for fixed $t = t_i$ ($i = 1, \cdots, 6$) and $y = m_1$ i.e., $c(t_i, x, m_1)$, where m_1 is the 1st moment of the marginal density of x of color density. Moment functions and the marginal distribution calculating by (A1)-(A3) in Appendices. Then the coefficient D was identified by the least squares (LS) method. From experimental results, D was identified as 1.6×10^{-10} m^2/s.
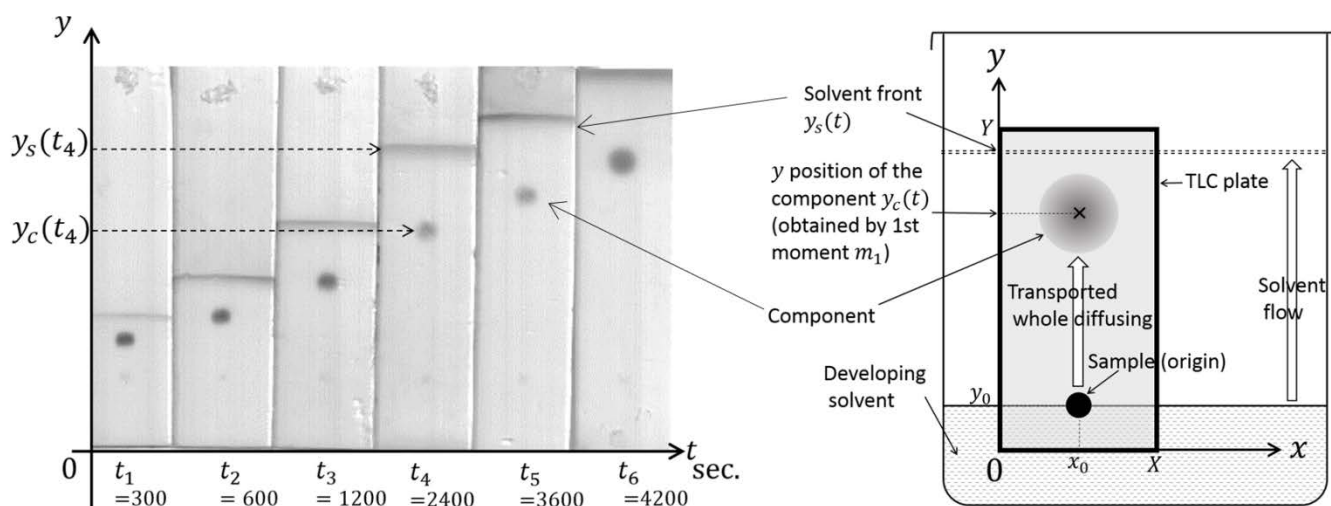
Fig. 1. A sample of the scanned image of 6 TLC plates ordered by the time evolution (left) and the diagram of the development of a TLC (right).

The zone velocity $R_v(t)$ depends on the motion of the developing solvent caused by the capillary action. However, its motion equation is complicated and it is not reasonable to identify $R_v(t)$ from the motion equation. So the curve approximation for the component displacement

$$y_c(t) - y_0$$

is employed. In order to achieve this way from data directly, many experimental results are needed for short time interval but it is not reasonable because several processes are required for obtaining one result on the TLC technique. Conversely, the evolution of the solvent front $y_s(t)$ can be measured almost continuously. On the other hand, a parameter R_f is used in the chromatographic field such as the displacement ratio of the component to the solvent front given by

$$R_f = \frac{y_c(t) - y_0}{y_s(t) - y_0}. \tag{4}$$

This ratio R_f is a well-known as almost constant during the developing, then the significant value of R_f can be obtained from samples of experiments easily. Based on the above, the velocity $R_v(t)$ is identified by following procedures:

1) Deciding the fitting curve (specific function) of $y_s(t)$ from experimental results.

2) Calculating the average of the R_f value from experimental results.

3) Substituting results of 1) and 2) into equ (4), estimate $y_c(t)$.

4) Compute the theoretical derivation of $y_c(t)$ w.r.t. t, *i.e.,*

$$R_v(t) = \frac{dy_c(t)}{dt}.$$

Using the procedures, in this paper, $y_s(t)$ was approximated by

$$y_s(t) = at^b \ (b < 1),$$

and a and b were estimated as 1.33 and 0.47 by using the LS method, respectively. R_f was also obtained by 0.65 from experimental results. Then the time-depending velocity $R_v(t)$ was computed as

$$R_v(t) = \frac{dy_c(t)}{dt} = 0.41t^{-0.53}.$$

Approximated curve and experimental results of $y_s(t)$ are illustrated in Fig. 2. Almost of experimental results are confirmed to fit well to the curve.
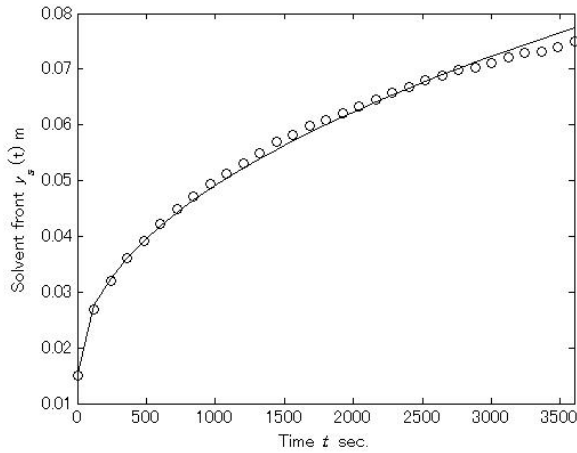
Fig. 2. Experimental results of the evolution of the solvent front $y_s(t)$ (the solid line indicates the approximated curve $y_s(t) = 1.33t^{0.47}$).

3 Verification of the 2D Gaussian Model for the Component

3.1 Simulations

Solving the dynamical equation (2) numerically, the periodic boundary conditions

$$c(t, 0, y) = c(t, X, y)$$

and

$$c(t, x, 0) = c(t, x, Y)$$

are employed, and the finite-difference method (FDM) is used with uniform grid. The spatial and time partition are set to be $\Delta x = \Delta y = 4.0 \times 10^{-4}$ m, and $\Delta t = 1.0$ sec., respectively. Based on the experimental results, initial condition $c(0, x, y)$ is set to the cylindrical shape having the normalized height

$$c(0, x, y) = 1$$
$$\text{for} \quad (x - x_0)^2 + (y - y_0)^2 \leq r^2$$

and, $x_0 = 0.01$ m, $y_0 = 0.015$ m and $r = 1 \times 10^{-3}$ m. For the approximation of the advection and the diffusion term in (2), the upwind and the central difference are used, respectively. The time-derivative term is approximated by the forward difference. Figure 3 shows the y position of the solution component $y_c(t)$ obtained by the numerical results comparing with experimental results.
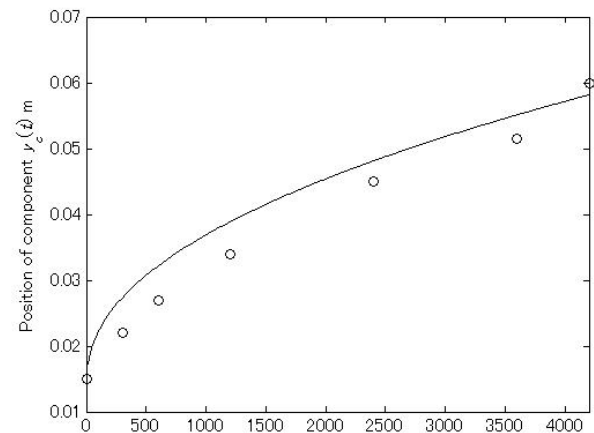

Fig. 3. Positions of the solution components $y_c(t_i)$ ($i = 1, \cdots, 6$) on the TLC plates (the solid line indicates simulation results).
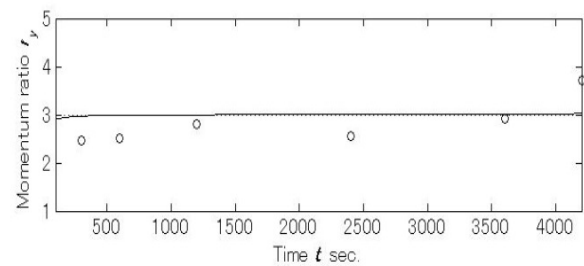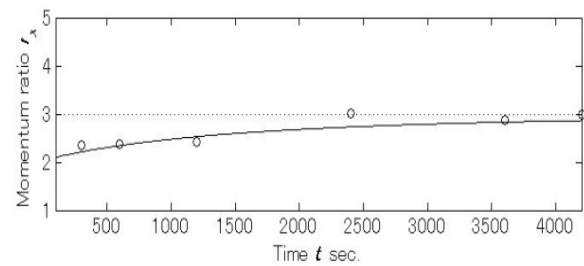

Fig. 4. The momentum ratios r_x (*top*) and r_y (*bottom*). The solid line and markers indicate numerical and experimental results, respectively.

3.2 Verification of Gaussian

In the statistical field, a momentum ratio

$$r = \frac{m_4}{m_2^2}$$

is used for checking similarity that density is Gaussian function, where m_2 and m_4 are the 2nd and 4th moment of the density function given by equ. (A2) in Appendices, respectively [9]. If the function is the Gaussian:

$$Ae^{-\alpha x^2},$$

the ratio r is necessary equal to 3 for any A and α. Based on this condition, 2 values r_x and r_y are evaluated for checking that the density $c(\cdot, x, y)$ is similar to the Gaussian function or not, where r_x and r_y is the momentum ratio for each axis of the marginal density $c_x(x)$ and $c_y(y)$ obtained by (A1) in Appendices, respectively. Figure 4 shows the momentum ratios r_x and r_y for each time calculating from numerical and experimental results. It can be seen that each ratio is close to 3 as time passes.

4 Conclusion

The reasonableness has been verified that the image density model of the TLC plate is the 2D Gaussian function for applying the image-based quantification of carbohydrates by numerical and experimental results. On simulation, as the dynamical model of the concentration of the solution, the 2D advection-diffusion equation with time-varying velocity has been introduced. Reasonability of the Gaussian function has been confirmed by evaluating the momentum ratios. On the synthesis of the carbohydrates, about 30-60 min. are spent for the developing. So the results tell us that the Gaussian function is a reasonable model of the color density for the image-based quantification of the concentration.

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Appendices:

Experimental Setup and preprocessing of the Image

The 0.5 wt. % glucose solution and 6 TLC plates with a size of 0.02×0.08 m were prepared. So $1.0\,\mu L$ solution was spotted at the position of $(x_0, y_0) = (0.02\ \text{m}, 0.015\ \text{m})$ on each plate. Then, the spotted solution was chromatographed with a solvent system of $12:1:4 = 2$-propanol : 1-butanol : H_2O. After that, the TLC plate was dried. Then, glucose was visualized by heating the plate after soaking it into a solution of 10 % H_2SO_4 in EtOH.

Next, the visualized TLC plate was scanned under the conditions of 1200 dpi resolution and 24-bits RGB mode by the Brother scanner-printer DCP-7065DN. The captured image file was digitized to numerical data for using on the computation tool MATLAB. Maximum of each RGB color intensity 255 was set to values where non-colored region in the plate. We magnified each RGB color intensity range between maximum value and saturated low value of image (black) such that limit of analysis using the TLC plate. Next, the black and white part were reversed. Finally, the color density $c(\cdot, x, y)$ was obtained by summing the each color (RGB) density.

Marginal distribution and moments of the density

For a 2-dimmensional density $c(x, y)$ with the finite domain:

$$x_{min} < x \leq x_{max}$$

and

$$y_{min} < y \leq y_{max},$$

marginal density of x and y are respectively given by

$$c_x(x) = \int_{y_{min}}^{y_{max}} c(x,y)dy, \qquad (A1)$$

and

$$c_y(y) = \int_{x_{min}}^{x_{max}} c(x,y)dx. \qquad (A2)$$

The n-th moment m_n $(n > 2)$ of the density $c(\alpha)$ is calculated by

$$m_n = \frac{1}{M} \int_{\alpha_{min}}^{\alpha_{max}} (\alpha - m_1)^n c(\alpha)d\alpha, \qquad (A3)$$

where

$$m_1 = \frac{1}{M} \int_{\alpha_{min}}^{\alpha_{max}} \alpha c(\alpha)d\alpha, \qquad (A4)$$

and

$$M = \int_{\alpha_{min}}^{\alpha_{max}} c(\alpha)d\alpha.$$