Three-dimensional Representation Method Using Pressure, Time, and Number of Theoretical Plates to Analyze Separation Conditions in HPLC Columns

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There has been considerable discussion of the speed performance of HPLC separation, especially regarding the relationship between theoretical plates and hold-up time. The fundamental discussion focuses on the optimal velocity, $u_{0,\text{opt}}$, which gives a minimal height equivalent to a theoretical plate of the van Deemter plot. On the other hand, Desmet’s method, using the kinetic performance limit (KPL), calculates the highest performance with a constant pressure drop, without focusing solely on the optimal velocity. In this paper, a precise method based on the KPL is proposed, to understand how increasing pressure enhances both theoretical plates and hold-up time. A three-dimensional representation method that combines the pressure drop with two axes of time and theoretical plates will be useful for discussing the effect of pressure in pressure-driven chromatography. Using three dimensions, the methods based on $u_{0,\text{opt}}$ and the KPL can be combined, because $u_{0,\text{opt}}$ can be visualized three-dimensionally, including the neighbor of $u_{0,\text{opt}}$; and the question of whether the KPL is an asymptotic or effective limit can be investigated. Three performances of high resolution, high speed, and low pressure can be understood on different packing supports at a glance.

Keywords Kinetic performance limit, pressure drop, number of theoretical plates, hold-up time, optimal velocity, column length, pressure-driven strength, velocity-length product, separation impedance, impedance time

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Introduction

Various types of packing support, such as particle and monolithic columns, are widely used especially for reversed-phase chromatography in HPLC, to obtain high resolution or rapid analytical time. However, it is difficult to select the optimal packing support, and to find suitable separation conditions, given a specific pressure in an HPLC system; and peripheral studies on the theory have thus far been limited. In 2005, Desmet et al. invented kinetic performance methods by expanding both Bristow and Knox’s and Poppe’s methods. At that time, van Deemter plots of height equivalent to a theoretical plate ($H$) and linear velocity ($u_0$) were used to analyze the minimal height ($H_{\text{min}}$) and optimal velocity ($u_{0,\text{opt}}$). From the viewpoint of pressure-driven chromatography, the kinetic plot methods use column permeability ($K_v$) to find the minimal hold-up time ($t_0$) and maximal number of theoretical plates ($N$), given a specified pressure drop ($\Delta P$). $K_v$ is a physical constant that depends on the diameter of the packing particles and the specific surface area, and is inversely proportional to the flow resistance against $\Delta P$. The methods give us a kinetic performance limit (KPL) of $t_0$ and $N$, incorporating the length of the column ($L$), uniquely calculated with $u_0$ as an operating parameter. $L$ is the other operating parameter, and the largest limit to be automatically obtained at $u_0$ with the specified $\Delta P$.

Given a chosen packing support, the methods can assess the performance of the packing support as the KPL at a given $\Delta P$, where the KPL shows the maximal performance that can be achieved by the packing support under the specific conditions of $u_0$ and $L$. However, the KPL conditions of $u_0$ and $L$ are not always adopted as conditions of practical analytical methods. In the present paper, we first investigate the three-dimensional space of $N$, $t_0$, and $\Delta P$ by scanning the parameter of $\Delta P$ to show the curved surface generated by KPL in the space. Then we demonstrate a method to find practical conditions of $u_0$ and $L$ by investigating the three-dimensional relationship among $N$, $t_0$, and $\Delta P$. In addition, $u_{0,\text{opt}}$ enables the determination of optimal performance sets of $t_0$ ($t_{0,\text{opt}}$) and $N$ ($N_{\text{opt}}$), by calculating $L$ and $\Delta P$ automatically; and the optimal performance is shown as a straight line through the original point in the three-dimensional space.

Experimental

Acetonitrile of HPLC grade was purchased from Wako Pure Chemicals as a mobile phase. The UHPLC system (Hitachi High-Technologies) consisted of an L-2160U Binary pump with high pressure gradient elution, L-2200U autosampler, L-2300 column oven, L-2400U UV detector, and CDS of EZChrom.
Elite™ for Hitachi. The columns, also manufactured by Hitachi High-Technologies, included a LaChromUltra C18 (2 μm) column (50 × 2 mm), LaChrom C18 (3 μm) column (100 × 4.6 mm), and LaChrom C18 (5 μm) column (150 × 4.6 mm). The packing supports consisted of fully porous octadeacylsilyl silica gel. A monolithic column of octadeacylsilyl silica (75 × 2.3 mm), a prototype manufactured by Hitachi High-Technologies, was used for comparison. Figure S1 (Supporting Information) shows an electron microscope photograph of the monolithic column.

Theoretical

Fundamental principle of KPL

The KPL is related to a number of basic equations, as shown below:3,12 $t_0$ is proportional to $L$, and $u_0$ comes from Eq. (1):

$$ t_0 = \frac{L}{u_0}. \tag{1} $$

It can also be regarded as a definition of $t_0$, made from $u_0$ and $L$. The column efficiency, $N$, is also proportional to $L$, and $H(u_0)$ comes from Eq. (2):

$$ N = \frac{L}{H(u_0)}. \tag{2} $$

$H(u_0)$ is a specific function of $u_0$, the so-called van Deemter equation. Equation (2) shows that $N$ can also be regarded as an expression of $H(u_0)$, which is an essential property of separative performance. $N(u_0, L)$ is also a function of $u_0$ and $L$.

$H(u_0)$ may have a minimum value. Although $H(u_0)$ is originally an arbitrary function of $u_0$, Eq. (3) is empirically adopted as a fitting function of $H(u_0)$ to discuss the results in this paper. Equation (3) shows the van Deemter equation, one of the most representative expressions:11

$$ H(u_0) = A + B \frac{1}{u_0} + C u_0, \tag{3} $$

where $A$, $B$, and $C$ are constants depending on the packing supports.

The KPL generates a $t_0$-$N$ curve at a certain $\Delta P$ by scanning the operating parameter of $u_0$ through Eqs. (1) and (2). It does not require Eq. (3), but does need Eq. (4). It is an advantage for the KPL that the other operating parameter, $L$, can be determined automatically by Eq. (4):12

$$ \Delta P = \frac{\eta u_0 L}{K_v}. \tag{4} $$

where $\eta$ is the dynamic viscosity of the mobile phase. In pressure-driven chromatography, the mathematical product of $u_0$ and $L$ is bonded, because $\Delta P$ is constricted. $K_v$ is a kind of fitting constant experimentally. In this paper, Eq. (4) is the most important equation that is a fundamental principle to generate the product of $u_0$ and $L$. It can be said that the pressure-driven chromatography obeying Eq. (4) is the scope of this paper. On the other hand, Eq. (3) is a characteristic function to give the diversity of chromatographic separation. Equations (1) and (2) are positioned as secondary functions of $u_0$ and $L$ to stretch the $t_0$-axis and $N$-axis of the three-dimensional graph. Especially Eq. (1) can uniquely determine $u_0$ and $L$, respectively by using Eq. (4) simultaneously.

Each of the three performance parameters, $t_0$, $N$, and $\Delta P$, is proportional to $L$, as shown in Eqs. (1), (2), and (4), respectively. In other words, the KPL converts the van Deemter $H$-$t_0$ curve to a $\Delta P$-$N$ curve for each $L$, which is automatically determined as a limit based on the specific value of $\Delta P$. The dependency of $L$ is an important feature of pressure-driven chromatography.

Optimal velocity

In addition, assuming Eq. (3) holds, $u_{0,\text{opt}}$ can be estimated to obtain the minimum of $H(u_0)$, $H_{\text{min}}$, and the optimal $N$, $N_{\text{opt}}$, by differential calculation of Eq. (3), using Eq. (5):

$$ H_{\text{min}} = H(u_{0,\text{opt}}) \left( u_{0,\text{opt}} = \frac{B}{\sqrt{C}} \right). \tag{5} $$

$u_{0,\text{opt}}$ is useful and convenient for traditional determination of the optimal conditions.

From five dimensions to three dimensions

The five essential variables of $u_0$, $N$, $u_0$, $L$, and $\Delta P$ should be utilized to study separation performance in detail. However, 5-dimensional space is extremely difficult to visualize. In considering the aspects of the five variables, $u_0$ and $L$ are measurement condition inputs, $t_0$ and $N$ are performance outputs obtained as results, and the last variable, $\Delta P$, is a type of undesirable ramification factor that may reach a boundary condition $\Delta P_{\text{max}}$. A new mathematical variable, $\Pi$, may be introduced to simplify the following discussion. Given Eq. (4),

$$ \Pi = \frac{K_v \Delta P}{\eta}. \tag{6} $$

$\Pi$ (m^{2}/s) is a kind of extensive parameter to normalize $\Delta P$ with $K_v$ and $\eta$. $\Pi$ may be seen as expressing both pressure-driven strength and velocity-length product, as seen in Eq. (7), given Eqs. (1) and (4):

$$ \Pi = u_0 L = u_0^2 t_0. \tag{7} $$

When $\Pi$ is given, any $u_0$ and any $L$ can be chosen under the condition of the constant, $\Pi = u_0 L$. In other words, when a chosen $u_0$ and $L$ are used, $\Pi$ will be yielded automatically, which in turn means that $\Delta P$ can be calculated by Eq. (6). For rapid analysis, any large $u_0$ can be set under the upper-limit condition, while considering $\Pi$. Then an $L$ is automatically determined by $\Pi$. For high-resolution performance, any large $L$ can be set, along with $u_0$, while considering $\Pi$ as well. $\Pi$ can be equally applied to $u_0$ or $L$, because $u_0$ and $L$ form a pair. In addition, Ohm’s law is a good analogy to explain the structure of pressure-driven chromatography, with $\Pi$ corresponding to the voltage, with current $u_0$ and electrical resistance $L$ in Eq. (7). $\Pi$ is a good index for demonstrating the kinetic strength of a given analytical method, including not only column permeability in the stationary phase, but also dynamic viscosity in the mobile phase, instead of the allowable pressure drop.

The aforementioned five dimensions can be reduced to three by introducing a third axis, of $\Pi$. $\Pi$ can unify $u_0$, $L$, and $\Delta P$ in one dimension, because $\Pi$ is the velocity-length product $u_0 L$, and proportional to $\Delta P$. $\Pi$ is thus effective in simplifying the mathematics of chromatographic theories.

$L$ can be expressed simply as the function $L(u_0, u_0)$ by Eq. (1). $u_0$ might be considered more important than $L$, because $H(u_0)$ is essentially a function not of $L$, but of $u_0$ by Eq. (3). However, $L$ is still useful in discussing $N$ because of its proportional
properties. \( L \) can be regarded as a kind of extensive scaling factor, because \( N, \Pi, \) and \( t_0 \) are all proportional to \( L \), by Eqs. (2), (7), and (1), respectively. This is partly why the respective variables have good correlations with each other. In the case of \( u_{0,\text{opt}} \), \( N \) becomes a straight line, based on the scaling factor \( L \), in the three-dimensional space, because of the constant \( H (= H_{\text{mm}}) \) with \( u_{0,\text{opt}} \).

**Introduction of impedance time**

Inverted impedance time \( (t_E^{-1}) \) is introduced to provide a simpler three-dimensional representation, by Eq. (8):\(^{32}\)

\[
t_E^{-1} = \frac{N^2}{t_0}.
\]

\( t_E^{-1} \) is proportional to \( \Pi \) in Eq. (9) through Eqs. (2), (7), and (8):

\[
\frac{1}{t_E(u_0)} = \frac{u_0^2 t_0}{(H(u_0))^3} = \frac{\Pi}{(H(u_0))^3}.
\]

It is clear that a three-dimensional representation with \( t_E^{-1} \) is characterized by \( H(u_0) \). \( \Pi \) is expressed in Eq. (10) through Eqs. (8) and (9):

\[
\Pi = (H(u_0))^3 \frac{N^2}{t_0}.
\]

On the other hand, plate time \( t_P \) is a function of \( u_0 \) as Eq. (11) through Eqs. (1) and (2):

\[
t_P = \frac{t_0}{N} = \frac{H(u_0)}{u_0}.
\]

\( t_P \) has a feature not to be influenced by \( \Pi \) directly, when compared to \( t_E \).

### Results and Discussion

**Basic performance of packing supports**

\( H(u_0) \) can represent a major feature of chromatography in Fig. 1. \( H(u_0) \) or \( N \) includes the extra column dispersion without specific calculations in this experiment. \( u_0 \) is simply calculated with \( L \) divided by the hold-up time of uracil as \( t_0 \) (Eq. (1)). For convenience, Eq. (3) is used as a fitting function of \( H(u_0) \). The experimental parameters \( A, B, \) and \( C \) of the different supports are shown in Table 1. For simplicity, \( \eta \) is fixed as 0.001 Pa·s in this paper. The \( K_V \) values of different supports are also shown as hydrodynamic constants in Table 1. \( K_V \) is also an experimental coefficient in Eq. (4) to demonstrate the three-dimensional representation.

**A precise method, based on the KPL, for determining column conditions**

The KPL is expressed as a curved line on the \( t_P-N \) plane, at a specific \( \Delta P \). It indicates a performance limit of the packing support, which can be reached by the boundary condition of \( \Delta P \) with specific \( u_0 \) and \( L \). This offers a good method for assessing the packings. However, the KPL does not clearly illustrate the importance of pressure drop. And the actual conditions in the HPLC cannot be directly determined by the KPL. Three-dimensional representations of \( N, t_0, \) and \( \Delta P \) or \( \Pi \) will be useful for finding an appropriate analytical method that includes the effect of pressure drop.\(^{33}\) Figure 2 shows the curved surface of the KPL with 2-\( \mu \)m packings, in a three-dimensional representation. To generate the figure, first a basal plane was defined based on the input parameters of \( \Delta P \) and \( t_0 \). Then, as a third axis, the \( u_0 \) of each point \( \Delta P \) and \( t_0 \) was calculated using Eqs. (1) and (4). \( N \) was calculated using \( u_0 \) with Eqs. (1) and (2). Finally, \( N \) was built at each point of \( \Delta P \) and \( t_0 \) on the basal plane, in the manner of a topographical map. Any \( N \) is available under the KPL surface, because the KPL is an upper limit on the basal plane, and \( u_0 \) and \( L \) should be adjustable for the \( N \). \( \Pi \) is also shown, beside the \( \Delta P \)-axis. \( \Pi \) is proportional to \( P \), and \( \Pi = 350 \text{ mm}^2/\text{s} \) corresponds to \( \Delta P = 50 \text{ MPa} \), with a 2-\( \mu \)m particle and \( \eta = 0.001 \text{ Pa·s} \). \( \Pi = 350 \text{ mm}^2/\text{s} \) may be achieved when \( L = 50 \text{ mm} \) and \( u_0 = 7 \text{ mm/s} \), for instance.

To take an example, in the case of \( t_0 = 5 \text{ s} \), when \( \Delta P \) increases 33% from 15 to 20 MPa, \( N \) increases 14% from 3250 to 3700. And when \( \Delta P \) again increases 33% from 45 to 60 MPa, \( N \) increases 11% from 5180 to 5780. Therefore, pressure increase is not as effective for the plates at 60 MPa, as at 20 MPa.

**Table 1 Experimental values for different packing supports**

| Packing | \( A/10^4 \text{ m} \) | \( B/10^4 \text{ m}^2/\text{s} \) | \( C/10^4 \text{ s} \) | \( K_V/10^{-12} \text{ m}^2/\text{Pa} \) |
|---------|-----------------|-----------------|-----------------|-----------------|
| 2-\( \mu \)m particle | 5.1 | 3.3 | 0.27 | 0.007 |
| 3-\( \mu \)m particle | 4.5 | 4.7 | 1.3 | 0.011 |
| 5-\( \mu \)m particle | 6.8 | 3.9 | 2.7 | 0.018 |
| Monolith | 3.4 | 4.1 | 0.52 | 0.025 |

a. The parameters \( A, B, \) and \( C \) were estimated by the method of least squares through Eq. (3) from Fig. 1. The column permeability \( K_V \) was determined using Eq. (4).
There is a straight line, \( \{E(u_{0,\text{opt}})\}^{-1} \), on the KPL surface of \( \{E(u_{0})\}^{-1} \), because \( u_{0,\text{opt}} \) gives a constant of \( \{E(u_{0,\text{opt}})\}^{-1} (=K_{V}/H_{\text{min}}) \). The maximum of \( E^{-1} \) is \( 1.4 \times 10^{-4} \), as the minimum of \( E \) is \( 7.0 \times 10^{-3} \).

**On different packing supports**

Figure 5 shows three-dimensional graphs of a 3-\( \mu \)m particle, 5-\( \mu \)m particle, and monolithic column, respectively. The specification of the column for maximum pressure is 20 MPa in each case. While \( N \) reached 13000 plates at 60 MPa in the case of the 2-\( \mu \)m particle, it could reach 15000 plates in the case of the 3-\( \mu \)m particle, even at 20 MPa. In the case of the 5-\( \mu \)m particle, a still higher \( N \) of 30000 plates or more, can be obtained, because \( t_{0} \) offers another way to increase \( N \), along with \( \Pi \).

The three-dimensional representation method is useful to show the effect of maximum pressure at a glance. For example, in the case of 2-\( \mu \)m particle, although \( N \) of 10000 can be reached at the basal plane of 60 MPa and 13 s, \( N \) of 30000 cannot be reached in Fig. 2. On the other hand, in the case of 5-\( \mu \)m particle, it is found that \( N \) of 30000 can be reached at the basal plane of 20 MPa and 500 s in Fig. 5b. It is found that there are two contributions to reach a required number of theoretical plates. One is the maximum pressure. And the other is long retention time while considering usage of suitable packing supports.

Figure S2 (Supporting Information) shows the \( E^{-1} \) of each packing support. Each \( E^{-1} \), including that of the monolithic column, has a large plateau near the \( u_{0,\text{opt}} \) line, when compared with that of a 2-\( \mu \)m particle (Fig. 4); and the value of \( E^{-1} (=K_{V}/H_{F}) \) on each plateau is roughly the same, because the \( u_{0,\text{opt}} \) line has a common \( H_{\text{min}} \) in each support. The maximums of \( E^{-1} \) for the 3 and 5-\( \mu \)m particles, and the monolith, are \( 1.2 \times 10^{-4} \), \( 1.1 \times 10^{-4} \), and \( 6.3 \times 10^{-4} \) respectively. It is confirmed that the maximal \( E^{-1} \) for each particle diameter \( (d_{p}) \) is almost constant. This means that \( K_{V} \) is inversely proportional to \( d_{p}^{2} \), and \( H_{\text{min}} \) is proportional to \( d_{p} \). It can be said that the \( E^{-1} \) of the monolithic column is significantly larger than that of the particle packing supports.

Regarding the performance of high resolution and high speed, although the optimal velocity has mainly been discussed so far, the concept of KPL was clarified recently. The KPL surface and the ridge line of optimal velocity on the surface can be shown simultaneously at one graph by expanding \( t_{0} \) to \( N \) plains to a three-dimensional graph with another axis of pressure or \( \Pi \). In the three-dimensional representation method, to increase the number of theoretical plates, it can be determined which pressure or time to choose, because pressure and time are independent variables respectively.
A straight line emerging from $u_{\text{opt}}$ can be represented on the KPL surface in three dimensions, and $u_{\text{opt}}$ can appear as a ridge line or plateau by representing the vertical axis of $E^{\perp}$ on the basal plane. And furthermore, a three-dimensional graph of $E^{\perp}$ can be projected into that of $N$ bi-directionally, because $N$ corresponds one-to-one with $E^{\perp}$ on the basal plane of $\Pi$ and $t_0$.

**Conclusions**

A valuable three-dimensional representation for assessing the effect of pressure in pressure-driven chromatography has been designed, using the axes of $\Delta P$, $t_0$, and $N$, or the axes of $\Pi$, $t_0$, and $N$. In addition, a new index based on the velocity-length product $\Pi$ has been defined, using $K_V$ and instead of $\Delta P$; and has been shown to be useful for indicating strength in pressure-driven chromatography. A valuable method has been formulated by considering both ideal values of $u_{\text{opt}}$ and KPL values.

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**Supporting Information**

The monolithic column (Fig. S1) and inversed separation impedance of different packing supports (Fig. S2) are shown. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

**References**

1. N. Tanaka and D. V. McCalley, *Anal. Chem.*, 2016, 88, 279.
2. Siswoyo, L. W. Lim, and T. Takeuchi, *Anal. Sci.*, 2012, 28, 107.
3. K. Todoroki, T. Nakano, H. Watanabe, J. Z. Min, K. Inoue, Y. Ishikawa, and T. Toyo’oka, *Anal. Sci.*, 2014, 30, 865.
4. Y. Song, K. Takatsuki, T. Sekiguchi, T. Funatsu, S. Shoji, and, M. Tsumoda, *Chromatography*, 2016, 37, 111.
5. C. Okamoto, H. Yoshida, A. Nakayama, S. Kikuchi, N. Ono, H. Miyano, Y. Ino, N. Hiraska, and T. Mizukoshi, *Chromatography*, 2016, 37, 125.
6. H. Kobayashi, M. Sukegawa, K. Fujimura, T. Kubo, and K. Otsuka, *Chromatography*, 2016, 37, 133.
7. T. Toyo’oka, *Anal. Sci.*, 2017, 33, 555.
8. A. Morikawa, H. Fukuoka, K. Uezono, M. Mita, S. Koyanagi, S. Ohdo, K. Zaitsu, and K. Hamase, *Chromatography*, 2017, 38, 53.
9. K. Nojima, M. Niitsu, Y. Kurosawa, T. Izawa, K. Nakayama, and Hisaaki Itoh, *Chromatography*, 2017, 38, 73.
10. Y. Nagatomo, S. Hashimoto, Y. Kishimoto, T. Hayakawa, S. Yamamoto, M. Kinosita, and S. Suzuki, *Chromatography*, 2017, 38, 23.
11. M. Otsubo, T. Motono, S. Kitagawa, and H. Ohtani, *Chromatography*, 2017, 38, 31.
12. J. H. Knox and M. Saleem, *J. Chromatogr. Sci.*, 1969, 7, 614.
13. M. Martin, C. Eon, and G. Guiochon, *J. Chromatogr.*, 1974, 99, 357.
14. P. W. Carr, X. Wang, and D. R. Stoll, *Anal. Chem.*, 2009, 81, 5342.
15. A. J. Matula and P. W. Carr, *Anal. Chem.*, 2015, 87, 6578.
16. H. Kobayashi, T. Ikekami, H. Kimura, T. Haras, D. Tokuda, and N. Tanaka, *Anal. Sci.*, 2006, 22, 491.
17. K. Miyabe and Y. Murata, *Anal. Sci.*, 2014, 30, 277.
18. S. Jespers, K. Broeckhoven, and G. Desmet, *LCGC Europe*, 2017, 30, 284.
19. A. A. Kurganov, A. Yu. Kanat’eva, E. E. Yakubenko, T. P. Popova, and V. E. Shiryaeva, *Russ. J. Phys. Chem. A*, 2017, 91, 182.
20. S. R. Groskreutz and S. G. Weber, *Anal. Chem.*, 2016, 88, 11742.
21. N. Lambert, S. Miyazaki, M. Ohira, N. Tanaka, and A. Felinger, *J. Chromatogr. A*, 2016, 1473, 99.
22. O. H. Ismail, M. Catani, L. Pasti, A. Cavazzini, A. Ciogli, C. Villani, D. Kotoni, F. Gasparrini, and D. S. Bell, *J. Chromatogr. A*, 2016, 1454, 86.
23. A. Kurganov, A. Kanateva, and E. Yakubenko, *J. Sep. Sci.*, 2016, 39, 162.

Fig. 5 KPL surfaces of (a) 3-μm packings, (b) 5-μm packings, and (c) monolithic column.
24. A. Andres, K. Broeckhoven, and G. Desmet, *Anal. Chim. Acta*, 2015, 894, 20.
25. S. Fekete, J. L. Veuthey, and D. Guillarme, *J. Chromatogr. A*, 2015, 1408, 1.
26. K. Miyabe, *Anal. Sci.*, 2009, 25, 219.
27. K. Miyabe, *Anal. Sci.*, 2011, 27, 1007.
28. G. Desmet, D. Clicq, and P. Gzil, *Anal. Chem.*, 2005, 77, 4058.
29. P. A. Bristow and J. H. Knox, *Chromatographia*, 1977, 10, 279.
30. H. Poppe, *J. Chromatogr. A*, 1997, 778, 3.
31. J. J. van Deemter, F. J. Zuiderweg, and A. Klinkenberg, *Chem. Eng. Sci.*, 1956, 5, 271.
32. G. Desmet, D. Cabooter, and K. Broeckhoven, *Anal. Chem.*, 2015, 87, 8593.
33. M. Ito and K. Shimizu, W. O. Patent Application, 2014, 030537.