Detection of Total and A1c-Glycosylated Hemoglobin in Human Whole Blood Using Sandwich Immunoassays on Polydimethylsiloxane-Based Antibody Microarrays

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Of the top ten causes of death, five causes of death are related to metabolic syndromes in 2010, in Taiwan. Hyperglycemia is the major criteria for defining metabolic syndromes. In 2005, American Diabetes Association even announces that random detection of A1c-Glycosylated hemoglobin (GHbA1c) becomes a better method of diagnosing diabetes mellitus (DM) and a reliable tool for early detection of DM with GHbA1c ≤ 6.5% [1-3]. Conventionally, high performance liquid chromatography (HPLC) is used to detect GHbA1c. However, due to the complexity of HPLC, the measurement of GHbA1c is limited at University Hospitals. With development of small detectors by Bio-Rad Diagnostic Group, GHbA1c can be measured at local clinics. However, it is still inconvenient for the patients in suburban areas and not suitable for personal point-care. Therefore, either AccuBase A1c Test Kit or A1c AtHome Testing Kit is developed. A1cNow INView, Metrika, Inc invests a portable device to detect GHbA1c at home. To make the detection of GHbA1c level easier, we aim to develop a polydimethylsiloxane-based antibody microarrays chip which can detect total and A1c-Glycosylated hemoglobin (GHbA1c) in human whole blood using sandwich immunoassays.

**Sandwich immunoassay of PDMS Substrate detection scheme**

In this study, we co-polymerized the fluorinated compound 1H,1H,2H-Perfluoro-1-decene (FD) with acrylic acid (AA) and bonded the resulting co-polymer with protein G on the surface of polyelectrolyte coated polydimethylsiloxane (PDMS) to form an antibody microarrays chip [4-5]. Then PDMS antibody microarrays chip was applied for sandwich immunoassay. Figure 1 showed workflow of the sandwich immunoassay. At first, a common probe, polyclonal Hb antibody that can capture total hemoglobin (tHb) including non-glycosylated and glycosylated Hb(GHbA1c), is bound to the surface of the modified PDMS substrate, and any unbound species, including many glycan-containing molecules, are washed away. Subsequently, the detection probes, i.e. the monoclonal antibodies against the Hb (anti-Hb) and GHbA1c motifs (anti-GHbA1c), are added to individual spots to bind to the captured Hb and GHbA1c, respectively. Finally, anti-mouse IgG-HRPs are added to individual spots to bind to the captured anti-Hb or anti-GHbA1c for chemiluminescence reaction and detection.
Figure 1. Workflow of the sandwich immunoassay for tHb and GHbA1c detection using the antibody microarray immobilized with the common capture probe, anti-Hb.

The PDMS antibody microarray chip was used to measure tHb and GHbA1c of Level 1 and Level 2 standard sample. As shown in Figure 2A and 2B, the spot intensity for tHb and GHbA1c decreases as Standard Level 1 and Level 2 solution are serially diluted by an order of magnitude from 1/10^2, 1/10^3, 1/10^4 to 1/10^5 (n=3), indicating the proposed scheme can quantitatively detect and differentiate tHb and HbA1c proteins over a wide dynamic range up to three orders of magnitude. The detection limit was estimated to be approximately 3.58 ng/mL (S/N=4.41) and 0.20 ng/mL (S/N=3.41) for tHb and GHbA1c, respectively.

Figure 2. Detection of tHb and GHbA1c proteins from serial dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and blank) of (A) Level 1 and (B) Level 2 standards with chemiluminescence detection

Whole blood analysis

A cohort of 28 clinical blood samples from 13 normal individuals and 15 diabetic patients was collected and analyzed by our method using the modified PDMS antibody microarray, the commercial enzyme-linked immunosorbent assay (ELISA) kits, and the standard method using TOSOH G7 ion exchange HPLC for comparison. As shown in Figure 4(A), the calculated %GHbA1c PDMS values are correlate well with the values determined by the standard method using HPLC (%GHbA1c HPLC =1.06*%GHbA1c PDMS - 0.46, R^2=0.98). In contrast, as shown in Figure 4(B), no correlation was observed between the values obtained from
the commercial ELISA kits and the HPLC method for diabetic samples. Thus, we concluded that our method is superior to the commercial ELISA kits in sensitivity, reproducibility, and the ease of use. The good correlation of our results with the certified HPLC values (Figure 4(A)) also demonstrates that our sandwich immunoassays using antibody microarrays on the modified PDMS substrate have great potential for clinical applications.

CONCLUSION

By combining a detection scheme using a common capture probe and specific detection probes with antibody microarray on the fluoro-modified PDMS substrate, serum tHb and GHbA1c levels can be accurately and reproducibly determined without sample pretreatment and a tedious blocking step. Moreover, the determined %GHbA1c values can be correlated with the values determined by the standard HPLC method. In view of the low cost of PDMS soft material, we believe the reported method can be further developed for point-of-care diagnostics.

References

Hyperuricemia after exposure to polychlorinated dibenzo-p-dioxins and dibenzofurans near a highly contaminated area

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Hyperuricemia means too much uric acid in the blood. Uric acid is a metabolic product resulting from the metabolism of purines (found in many foods and in human tissue). 1,2 Hyperuricemia is associated with hypertriglyceridemia, diabetes mellitus, 3 and coronary artery disease. 4 Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) cause renal dysfunction and elevate uric acid. 5,6 The aim of this work was to study the relationship between exposure to PCDD/Fs and serum uric acid levels, and to examine whether the risk of hyperuricemia is different between males and females.

This cross-sectional study recruited 1531 healthy participants living near a deserted pentachlorophenol factory. We measured seventeen 2,3,7,8-substituted PCDD/Fs, then examined associations between the main predictor variable, serum TEQDF-2005, and dependent variables, e.g., uric acid, estimated glomerular filtration rates (eGFR), and hyperuricemia risk.

The average age of the 1531 participants including 715 men and 816 women was 37.0 years. The median serum TEQDF-2005 level was 12.3 pg WHO2005-TEQDF/g lipid (range: 0.1-482.8 pg WHO2005-TEQDF/g lipid). In general, men had higher creatinine (men: 0.9; women: 0.7 mg/dL, p < 0.001), serum urea nitrogen (men: 14.0; women: 12.0 mg/dL, p < 0.001), uric acid (men: 6.3; women: 4.6 mg/dL, p < 0.001), and lower TEQDF-2005 (men: 10.9; women: 13.9 pg WHO2005-TEQDF/g lipid, p < 0.001) than women did. There was a strong monotonic inverse relationship between serum TEQDF-2005 quartiles and eGFR after adjusting for smoking, drinking, body fat, and insulin resistance (Men: β: 0, −4.7, −6.2, and −14.8, P for trend < 0.001; Women: β: 0, −6.7, −12.9, and −21.5, P for trend < 0.001) (Figure 1). A 1% increase in serum ln-TEQDF-2005 resulted in a decrease in eGFR of 7.73 × 10^{-2} mL/min/1.73 m² in men (p < 0.001) and 9.50 × 10^{-2} mL/min/1.73 m² in women (p < 0.001), respectively, which shows kidney function could be impaired. There was also a positive trend between serum TEQDF-2005 quartiles and uric acid only in men after adjusting for age, smoking, drinking, body fat, eGFR, and insulin resistance (Men: β: 0, 0.40, 0.36, and 0.59) (Figure 2). Moreover, a 1% increase in serum ln-TEQDF-2005 resulted in an increase in blood uric acid levels of 2.8 × 10^{-3} mg/dL in men (p = 0.001), but no significant change in women. Men with serum TEQDF-2005 higher than the reference group’s (< 7.4 pg WHO2005-TEQDF/g lipid) had a higher hyperuricemia risk after adjusting for confounding factors (25th to < 50th percentile, adjusted odds ratio (AOR) = 2.20 [95% CI: 1.30-3.73]; 50th to < 75th percentile, AOR = 1.86 [95%
CI: 1.08-3.22); ≥ 75th percentile, AOR = 3.00 [95% CI: 1.69-5.31]). These data show that serum PCDD/Fs affected the risk of hyperuricemia in apparently healthy men.

FIGURE 1. Adjusted regression coefficients (β [95% CI]) for change in eGFR in relation to serum TEQDF-2005 quartiles for (A) men and (B) women. Values are adjusted for smoking, drinking, body fat, and insulin resistance.

FIGURE 2. Adjusted regression coefficients (β [95% CI]) for change in uric acid in relation to serum TEQDF-2005 quartiles for (A) men and (B) women. Values are adjusted for age, smoking, drinking, body fat, eGFR, and insulin resistance.

We conclude that in populations with moderate exposure to environmental PCDD/Fs, serum PCDD/F levels are an important independent determinant of serum uric acid levels. Men with higher PCDD/F exposure had a significantly higher hyperuricemia risk than did women with higher PCDD/F exposure. Our results support efforts to reduce potential sources of environmental exposure to PCDD/Fs and to offer possibilities for decreasing the risk of hyperuricemia in the general population.

References:

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Use of the Gaussian hypergeometric function to solve the equation of gradually-varied flow

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1. Introduction

The gradually-varied flow (GVF) is a steady non-uniform flow in a prismatic channel with gradual changes in its water surface elevation. The drawdown produced at a sudden drop in a channel and the backwater produced by a dam or weir across a river are few typical examples of GVF. The evaluation of steady one dimensional gradually-varied flow (GVF) profiles under a specific flow discharge is very important in open-channel hydraulic engineering. Almost all major hydraulic-engineering activities in free surface flow involve the computation of GVF profiles. The various available procedures for computing GVF profiles can be classified as: the graphical-integration method, the direct integration, and the numerical method, as shown in Chow (1959). This paper presents an innovative direct-integration method for analytically solving the GVF equation by using Gaussian hypergeometric functions.

2. Dimensionless GVF equation and its solution by using the direct integration method

The differential equation of GVF in open channels with arbitrary cross-sectional shapes can be written as

\[ \frac{dh}{dx} = S_0 \frac{1 - (h_c / h)^M}{1 - (h_c / h)^N} \]  

This equation denotes the relation between the flow depth \( h \) and the axial distance \( x \) along the open channel having a slope \( S_0 \). The subscripts “n” and “c” refer to the normal and critical flow conditions, respectively. In open-channel hydraulics, the exponents \( M \) and \( N \) in (1) are usually called the hydraulic exponents for critical-flow computation and uniform-flow computation, respectively (Chow, 1959). Normalizing \( x \) and \( h \) in (1) based on \( h_n \), and rearranging the equation in terms of the dimensionless variables \( u (= h/h_n) \) and \( \lambda x (= xS_0/h_n) \), we can have the reciprocal of the slope of the GVF profile in the form as

\[ \frac{dx_n}{du} = -u^N + \lambda^M u^{N-M} \frac{1-u^N}{1-u^M} \]  

in which \( \lambda \) is the quotient of peculiar water depth values, \( h_c \) and \( h_n \), i.e., \( \lambda = (h_c/h_n) \). Equation (2) is a nonlinear differential equation, describing a dimensionless GVF profile with \( \lambda, M, \) and \( N \) as parameters. The characteristic length ratio \( \lambda \) is the primary parameter, which classifies GVF under study into those in mild, critical, and steep channels according as \( \lambda \) is less than, equal to, and larger than unity, respectively. By and large, \( M \) and \( N \) are functions not only of \( h \) (or \( u \) in case of the dimensionless form thereof), but also of channel geometry and roughness. However, with the present knowledge in mathematics, it is unlikely that one can solve (2) analytically unless \( M \) and \( N \) are treated as constants. Insomuch that, we henceforth assume the invariance of both \( M \) and \( N \) with \( u \) and confine the solutions of (2) to a case in which the values of \( M \) and \( N \) do not vary with \( u \) within a given channel geometry (i.e., implying the solutions only for GVF in wide channels). The integration of (2) yields (Chow, 1959)
\[ x_\ast = u - \int \frac{1}{1 - u^M} du + \lambda^M \int \frac{u^{N-M}}{1 - u^N} du + \text{Const} \tag{3} \]

in which “Const” is the constant of integration. However, one has not yet successfully integrated directly such a nonlinear differential equation with arbitrarily assumed real numbers of \( M \) and \( N \) due to the difficulty in integrating the two indefinite integrals of a proper fraction in (3). Many attempts have been made by previous investigators to evaluate the hydraulic exponents and the two indefinite integrals. The integration of both indefinite integrals has been performed in two ways by previous researchers. The first way is to have each integrand in the two indefinite integrals expanded into a finite set of partial fractions so that every term of them can be integrated separately, using the elementary transcendental functions (ETF). In the second way, each of the two indefinite integrals was integrated using a number of various infinite series. Bakhmeteff (1932) prepared an integration table of the varied-flow function (VFF), by which such two indefinite integrals for the fixed \( M \)- and \( N \)-values could be approximately evaluated. Chow (1955, 1959) refined and extended the VFF table for computing GVF profiles numerically in both sustaining and adverse channels with all kinds of regular cross-sectional shapes. Unfortunately, the Bakhmeteff-Chow-like procedure based on the VFF table suffers from two major drawbacks, which have impeded the progress of the approach in the GVF profile computation. The first drawback is caused by the imprecise interpolation of the VFF-values for a range of the dimensionless flow depth near unity or between the contiguous \( N \)-values as the VFF parameter. The second drawback has resulted from the incompleteness of a method proposed by Bakhmeteff (1932) and Chow (1955, 1959) to render the exact \( M \)- and \( N \)-values. To overcome the first drawback, this paper presents a novel approach to integrate the two indefinite integrals using the Gaussian hypergeometric functions (GHF) without recourse to the VFF table.

3. Gaussian hypergeometric function (GHF)

The Gaussian hypergeometric function (GHF) can be expressed as an infinite series and symbolized in the form of \( _2F_1(a, b; c; z) \) as shown in the book of Luke (1975).

\[ _2F_1(a, b; c; z) = \frac{\Gamma(c)}{\Gamma(a)\Gamma(b)} \sum_{s=0}^{\infty} \frac{\Gamma(a+s)\Gamma(b+s)}{\Gamma(c+s)s!} z^s \] \tag{4}

in which \( a, b, \) and \( c \) are the function parameters and \( z \) is the variable. Such an infinite-series representation in (4), as often referred to as the hypergeometric series, is convergent for arbitrary \( a, b, \) and \( c, \) provided that \( c \) is neither a negative integer nor zero and that \( a \) or \( b \) is not a negative integer for real \(-1 < z < 1 \) (or \(|z| < 1\)), and for \( z = \pm 1 \) if \( c > a + b \). The GHFs used in the solutions of the gradually-varied-flow profiles herein have the specified property of that \( a, b, \) and \( c \) are fixed with specified relations, \( a = 1 \) and \( c = b + 1 \). Considering that the first argument is always unity, the second and third arguments differ in one unity, and the fourth argument is a variable only, we use a simpler expression as shown in (5) to reduce the related equations to shorter expressions for facilitating the reading of the manuscript.

\[ g(b, z) = _2F_1(1, b; b+1; z) = b \sum_{s=0}^{\infty} \frac{z^s}{b+s} \] \tag{5}

There is a recurrence formulas for GHF as shown below

\[ g(b, z) = 1 + \frac{bz}{b+1} g(b+1, z) \] \tag{6}

4. GHF-based analytical solutions of GVF equation

Since the solutions of (2), if expressed in terms of GHF, are subject to the convergence criterion of GHF, i.e., \(|u| < 1\). To derive the alternative form of (2) in order for its GHF-based solutions to be valid for \(|u| > 1\), (2) on substitution of \( u = w^{-1} \) and \( du = -w^{-2} dw \) yields

\[ \frac{dx_\ast}{dw} = \frac{-w^{-2} + \lambda^M w^{M-2}}{1 - w^N} \] \tag{7}
Obviously, the analytical solutions of (7), if expressed in terms of GHF, are convergent for \(|w| < 1\), which on substitution of \(w = u^{-1}\) yields \(|u| > 1\). The complete GHF-based solutions of the dimensionless GVF equation are obtained in the following by executing the integration of the two indefinite integrals in (2) for \(|u| < 1\) as well as those derived from (7) for \(|w| < 1\) (or \(|u| > 1\) by substitution of \(w = u^{-1}\) into its solution).

For \(|u| < 1\), using the commercial software of the Mathematica [Wolfram, 1996], we can find the solution of the indefinite integral in terms of GHF as shown below.

\[
\int \frac{u^\phi}{1-u^N} \, du = \frac{u^{\phi+1}}{\phi+1} g\left(\frac{\phi+1}{N}, u^N\right) + \text{Const}
\]  

(8)

Replacing the two indefinite integral terms in (3) using (8), we can express (3) in terms of GHF as

\[
x_* = u \left[1 - g\left(\frac{1}{N}, u^N\right)\right] + \lambda^M \frac{u^{N-M+1}}{N-M+1} g\left(\frac{N-M+1}{N}, u^N\right) + \text{Const}
\]  

(9)

which is valid only for \(|u| < 1\).

For \(|u| > 1\) (or \(|w| < 1\) by substitution of \(u = w^{-1}\) into \(|u| > 1\)), by the same token, we can similarly express the analytical solution of (7) in terms of GHF as

\[
x_* = \frac{1}{w} \left[1 - g\left(\frac{1}{N}, w^N\right)\right] + \lambda^M \frac{w^{N-M+1}}{N-M+1} g\left(\frac{N-M+1}{N}, w^N\right) + \text{Const}
\]  

(10)

which is valid for \(|w| < 1\). To express (10) in terms of \(u\), substituting \(w = u^{-1}\) into (10) yields

\[
x_* = u \left[1 - g\left(\frac{1}{N}, u^{-N}\right)\right] + \lambda^M \frac{u^{N-M+1}}{M-N+1} g\left(\frac{M-N+1}{N}, u^{-N}\right) + \text{Const}
\]  

(11)

which is valid for \(|u| > 1\). Thus, except for \(x_* = \pm \infty\) at \(u = 1\), the complete solutions of (2) should consist of the combination of (9) in the domain of \(0 \leq u < 1\) with (11) in the domain of \(u > 1\).

5. Conclusions

The 1-D approach has been traditionally used by hydraulicians to solve the GVF problem for free-surface water flow in channels with gradually changes in its water surface elevation. Using the direct-integration method, many investigators have attempted to analytically solve the GVF equation. Despite their efforts to overcome the drawback of the conventional direct-integration method in the evaluation of the two indefinite integrals appearing in (3), our prospect to develop a viable technique to counter it without recourse to the table of the varied-flow function (VFF) has not been bright for decades until recently there has been the extensive and growing use of the mathematics software, such as the Mathematica. The availability of such powerful software has helped integrate successfully the two indefinite integrals using the Gaussian hypergeometric function (GHF) without recourse to the VFF table as shown in this paper. This merits attention because the GHF embraced in the GHF-based solution can henceforth replace the VFF. The GHF-based solutions can henceforth play the role of the VFF table in the interpolation of the VFF-values. So, after decades’ long efforts made by hydraulicians to solve the GVF equation using exclusively the VFF to compute GVF profiles in channels with cross-sectional shape other than wide rectangle, we have finally come up with a novel approach, in which the VFF is no longer needed in the integration of the two indefinite integrals. In addition, the mathematical capability of \(M\) and \(N\) in the GHF-based solutions to accept real numbers will provide a potential to expand the GHF-based solutions for GVF profiles in wide channels to those in channels with regular cross-sectional shape, such as rectangle, triangle, trapezoid, and circle, in which the values of \(M\) and \(N\) vary with the flow depth, \(h\). Such superiority of the GHF-based solutions will exclusively enable one to apply the GHF-based solutions to the numerical computation of GVF profiles in channels with cross-sectional shape other than wide rectangle.

References


Enhancing butanol production with Clostridium pasteurianum CH4 using sequential glucose-glycerol addition and simultaneous dual-substrate cultivation strategies

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Adding butyrate significantly enhanced butanol production from glycerol with Clostridium pasteurianum CH4, which predominantly produces butyrate (instead of butanol) when grown on glucose. Hence, the butyrate produced from assimilating glucose can be used to stimulate butanol production from glycerol under dual-substrate cultivation with glucose and glycerol. This proposed butanol production process was conducted by employing sequential or simultaneous addition of the two substrates. The latter approach exhibited better carbon source utilization and butanol production efficiencies. Under the optimal glucose to glycerol ratio (20 g L\textsuperscript{-1} to 60 g L\textsuperscript{-1}), the simultaneous dual-substrate strategy obtained maximum butanol titer, productivity and yield of 13.3 g L\textsuperscript{-1}, 0.28 g L\textsuperscript{-1} h\textsuperscript{-1}, and 0.38 mol butanol/mol glycerol, respectively. Moreover, bagasse and crude glycerol as dual-substrates were also converted into butanol efficiently with a maximum butanol concentration, productivity and yield of 11.8 g L\textsuperscript{-1}, 0.14 g L\textsuperscript{-1} h\textsuperscript{-1}, and 0.33 mol butanol/mol glycerol, respectively.