Characterization of pressure-driven water flows in nanofluidic channels by mass flowmetry

Yutaka Kazoe1 · Sho Kubori2 · Kyojiro Morikawa1 · Kazuma Mawatari1,2 · Takehiko Kitamori1,2

Received: 25 June 2021 / Accepted: 26 July 2021
© The Author(s), under exclusive licence to The Japan Society for Analytical Chemistry 2022

Abstract
With developments in analytical devices promoted by nanofluidics, estimation of the flow rate in a nanochannel has become important to calculate volumes of samples and reagents in chemical processing. However, measurement of the flow rate in nanospaces remains challenging. In the present study, a mass flowmetry system was developed, and the flow rate of water by pressure-driven flow in a fused-silica nanochannel was successfully measured in picoliters per second. We revealed that the water flow rate is dependent on the viscosity significantly increased in a square nanochannel with $10^2$ nm width and depth (3.6 times higher than the bulk viscosity for a representative channel size of 190 nm) and slightly increased in a plate nanochannel with micrometer-scale width and $10^2$ nm depth (1.3 times higher for that of 234 nm), because of dominant surface effects. The developed method and results obtained will greatly contribute to nanofluidics and other related fields.

Keywords Nanofluidics · Nanochannel · Flow rate · Water · Pressure-driven flow

Introduction
Microfluidics has led to the miniaturization of various chemical operations into microchannels and realized integrated analytical devices for biology, medicine, environmental analyses, etc [1]. Recently, the field has downscaled to nanospaces with ultrasmall volumes (aL–fL) to realize novel analytical applications such as high-efficiency chromatography [2, 3], single-molecule detection [4] and sorting [5], and living single-cell protein analyses [6]. Generally, for designing the analytical devices based on micro/nanofluidics, the flow rate in fluidic channels is an important parameter to calculate volumes of samples and reagents in chemical processing. Due to significantly increased fluid resistance of nanochannels compared with that of the microchannels, nanofluidic devices have employed fluidic control by an external pressure or an external voltage [3, 5, 6], rather than that by a syringe pump. Therefore, estimation of the flow rate of liquid in nanochannels is required for the design of nanofluidic analytical devices.

However, in nanospaces, it is difficult to estimate the flow rate from the bulk liquid properties, because various unique properties occur due to dominant surface effects. Previous studies have revealed that surface interactions have a significant effect on the behavior of the nearest molecules to the surface and induce unique phenomena in nanospaces with dimensions of $10^6$ nm, such as the change of water structure in silica nanopores [7, 8]. Even in nanospaces with dimensions of $10^2$ nm, which are 100 times larger than nanotubes and nanopores, we have revealed unique water properties such as 4 times higher viscosity [9, 10], 3 times lower dielectric constant [11] and 8 times higher proton diffusion coefficient [12] than those of bulk water. Based on nuclear magnetic resonance spectroscopy (NMR) data analysis, we revealed that the motion of water molecules is restricted nanochannels, and suggested a hypothesis of a loosely structured water phase within 50 nm of the surface generated by interactions between silanol groups and water molecules [13]. Other research groups have also reported unique properties...
in nanochannels, which have been explained by the influence of a nanoscale layer of ions electrically attracted to the surface, i.e., the electric double layer (EDL) [14, 15].

The appearance of such unique properties has meant that experimental investigation of the flow rate in the nanochannels has become increasingly important. A measurement system for streaming current in nanochannels [16], which is an electric current caused by ions in the EDL flowing in a nanochannel, has been reported; however, it requires calibration between the streaming current and the flow rate. In contrast, mass flowmetry is an efficient approach to the direct measurement of flow rate. Although several studies have reported the measurement of mass flow rate in porous membranes [17], accurate measurements in a nanochannel in the order of less than nanograms per second, even at a pressure of MPa, is still difficult.

In the present study, we developed a system of mass flowmetry for nanochannels with defined dimensions and geometry based on top–down glass nanofabrication and pressure-driven flow control. The flow rate of water confined in a nanochannel in the order of picoliters per second was evaluated. Based on the results, the fluid resistance and the viscosity of water flowing in the nanochannels were determined to reveal effects of channel size and geometry. The results obtained in this study is important for the field of nanofluidics to realize novel analytical devices and also other fields such as physical chemistry and biophysics.

**Experimental**

**Experimental apparatus**

Figure 1 shows a schematic illustration of the developed measurement system, which consists of a nanofluidic device containing nanochannels, a high-pressure control system for application of megapascal pressures [18], and a microbalance with a resolution of 1 μg (ME36S, Sartorius AG, Göttingen, Germany). The flow rate in a single nanochannel was accurately measured by controlling the size, geometry and number of nanochannels by top-down fabrication. The number of nanochannels was determined to increase the total mass flow rate from nanograms per second of single nanochannel to measurable levels (μg/s). A sample aqueous liquid was injected into the nanochannels by an external pressure, and was flowed through the nanochannels then collected into a vial with an inner diameter of 10.1 mm located on the microbalance to measure the mass of collected liquid. However, there were still large errors for the measurement of mass flow rates at microgram per second levels. It was determined that vibration of the tube causes an error of ±3 mg, which is 1000 times larger than the order of the mass flow rate. Therefore, as shown in Fig. 1, a stainless tube fixed by a rubber and glass cover was used to reduce the error by vibration to a negligibly small amount compared to the resolution of the microbalance. The outer- and inner-diameters of the stainless tube were set to 1.6 mm and 0.8 mm, respectively, to decrease the error by buoyancy acting on the tube to less than 2%. In addition, water that was collected in the vial evaporated at 1.42 μg/s, which also caused a large error. To suppress this error, as shown in Fig. 1, silicone oil was dropped on the collected water to decrease the evaporation rate to $2.8 \times 10^{-2}$ μg/s by an oil film on the water surface, which is negligibly small compared to the mass flow rate. Accordingly, accurate measurement of the mass flow rate in nanochannels was realized in the order of micrograms per second.

**Preparation of nanofluidic device**

Nanofluidic devices containing nanochannels with defined dimensions and geometry were fabricated, as reported previously [10]. Figure 2a shows a schematic diagram of the nanochannels, which were interfaced with microchannels for injection and ejection of sample liquid. Nanochannels were fabricated on a fused-silica glass substrate by electron beam lithography and dry etching. The width $W$, and depth $D$, of the nanochannels were measured from scanning electron microscopy (SEM) and atomic force microscopy (AFM) observations, respectively. For nanochannels deeper than 800 nm, the depth was measured with a nanoscale optical profiler due to difficulties in the application of AFM. The length of nanochannels $L$, was 100 μm, which is much longer than the representative size of nanochannels, so that the
Characterization of pressure-driven water flows in nanofluidic channels by mass flowmetry

1.3
entrance effects on fluid flow were negligible. SEM observation confirmed that approximately 99% of the nanochannels were successfully fabricated without breaking. Microchannels that were 500 μm wide and 40 μm deep were fabricated on another fused-silica glass substrate by photolithography and wet etching. Two substrates were then bonded by thermal fusion at 1080 °C to construct a nanofluidic device.

Figure 2b shows the two types of nanochannels prepared: square nanochannels that are 10^2 nm wide and 10^2 nm deep (W/D ~ 1), and plate nanochannels that are a micrometer wide and 10^2 nm deep (W/D > 20), to examine the effects of channel size and geometry. The width, depth and length of the nanochannels are listed in Table 1. When the pressure ΔP is applied to a nanochannel, the volumetric flow rate Q is given by:

\[ \Delta P = C Q, \]  

where \( C \) is the fluid resistance. For all conditions, the Reynolds number was expected to be \( Re = \rho UD_r/\mu \leq 10^4 \) (\( \rho \): density, \( U \): representative velocity, \( D_r \): representative size, \( \mu \): viscosity); therefore, the fluid flow in the nanochannels was laminar flow. For the square nanochannels, \( W = D \) is substituted into an analytical solution of pressure-driven laminar flow \([10, 19]\), so that the fluid resistance is approximately given by:

\[ C = \frac{128 \mu L}{4.5 D_h^4}, \]  

where \( D_h \) is the hydraulic diameter, which is given by \( D_h = 2WD/(W + D) \). For plate nanochannels, the fluid resistance is given by \([19]\)

\[ C = \frac{12 \mu L}{WD^3}. \]  

In characterization of the pressure-driven flow in the nanochannels, \( D_s \) and \( D \) were used as the representative size \( D_r \) of the square- and plate nanochannels, respectively. The microchannels were designed to make the pressure resistance

| Table 1 List of nanochannels used for experiments and measured values of fluid resistance and viscosity |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Channel type    | Representative size, \( D_r \) (nm) | Width, \( W \) (nm) | Depth, \( D \) (nm) | Number of channel (–) | Fluid resistance (Pa s/m³) | Viscosity (Pa s) |
| Square (pure water) | 190 ± 8 | 163 ± 12 | 230 ± 2 | 11,100 | 6.92 ± 0.21 × 10^{21} | 3.21 ± 0.54 × 10^{-3} |
| | 394 ± 6 | 410 ± 12 | 380 ± 5 | 5500 | 2.93 ± 0.12 × 10^{20} | 2.50 ± 0.19 × 10^{-3} |
| | 760 ± 11 | 777 ± 19 | 745 ± 12 | 814 | 9.16 ± 0.51 × 10^{18} | 1.08 ± 0.09 × 10^{-3} |
| | 1691 ± 10 | 1572 ± 14 | 1830 ± 14 | 44 | 3.52 ± 0.01 × 10^{17} | 1.01 ± 0.02 × 10^{-3} |
| Square (KCl 10 mM) | 190 ± 8 | 163 ± 12 | 230 ± 2 | 11,100 | 6.62 ± 0.10 × 10^{21} | 3.07 ± 0.51 × 10^{-3} |
| | 234 ± 3 | 10,000 ± 15 | 234 ± 3 | 1022 | 1.10 ± 0.04 × 10^{19} | 1.17 ± 0.06 × 10^{-3} |
| | 417 ± 4 | 10,360 ± 15 | 417 ± 4 | 110 | 1.28 ± 0.14 × 10^{18} | 0.80 ± 0.09 × 10^{-3} |
| | 745 ± 12 | 15,400 ± 15 | 745 ± 12 | 22 | 1.84 ± 0.12 × 10^{17} | 0.98 ± 0.08 × 10^{-3} |

Note that due to the difficulty of direct evaluation, the standard deviations of plate nanochannel width, which are caused by electron beam lithography, were obtained by averaging the standard deviations of square nanochannel widths determined from SEM observations.
negligibly smaller than that of the nanochannels; therefore, the external pressure applied to the nanofluidic device was regarded as the pressure $\Delta P$ applied to the nanochannels.

**Results and discussion**

**Flow rate in nanochannel**

The measurement system was used to evaluate the flow rate of the pressure-driven flow of pure water in the nanochannels. Figure 3 shows the results obtained for nanochannels of $D_r = 190$ nm. When the pressure was applied, the mass of collected water, $\Delta M$, in the vial increased linearly. As the pressure increased, the mass increase rate of water flowed through the nanochannels became higher. The volumetric flow rate in a single nanochannel was estimated from the results using a water density of 997 kg/m$^3$ and the number of nanochannels (Table 1). Figure 4 shows the volumetric flow rates as a function of the applied pressure. The standard deviation of the flow rate was determined from three repeated measurements. For entire nanochannels, a linear relationship between the flow rate and the pressure was obtained at $R^2 > 0.9993$. This linear relationship at $\Delta P \leq 2.0$ MPa suggested that water confined in the nanochannel can be regarded as an incompressible Newtonian fluid with the viscosity independent of the shear rate. The obtained flow rates were compared with the theoretical flow rates, which were calculated from Eqs. (1) to (3) with the viscosity of bulk water indicated according to the literature [20] and the representative channel size including the uncertainty at a confidence level of 95% based on the coverage factor of 3.18. For the square nanochannels, we found that the obtained flow rate in the channels smaller than 760 nm was significantly lower than the theoretical flow rate, while that in the channels larger than 760 nm was almost similar to the theoretical value. In contrast, for the plate nanochannels, the obtained flow rate corresponded to the theoretical flow rate, except the nanochannel with $D_r = 234$ nm indicating a slightly lower flow rate. Accordingly, we succeeded in the measurement of the flow rate of water confined in the fused-silica nanochannel within the order of pL/s to nL/s.

We found that the water flow rate in the nanochannel is significantly decreased compared to that predicted from the theory with the bulk water properties, and the difference between the experimental and theoretical values depends on the size and geometry of the nanochannel. Based on the obtained flow rate as a function of the applied pressure, the fluid resistance was calculated from the slope of the fitting line, as listed in Table 1. For entire nanochannels, the fluid resistance was obtained with the coefficient of variation ranging from 0.2 to 11%. Therefore, it is concluded that the developed mass flowmetry system has a sufficient degree of accuracy for characterization of pressure-driven flow in nanochannels with the applied pressure in the order of $10^{-1}–10^0$ MPa.

**Effects of channel size and geometry on water viscosity**

To clarify the effects of channel size and geometry, as listed in Table 1, the water viscosity was determined from the fluid resistance and the channel size using Eqs. (2) and (3). Figure 5 summarizes the estimated viscosity of pure water normalized with respect to the bulk viscosity $\mu_0$, as a function of the representative channel size. Error bars were calculated from Eqs. (2) and (3) for the square- and plate nanochannels, respectively, based on the error propagation law with consideration of the standard deviations of fluid resistance and channel size. For channel sizes larger than 800 nm, in which unique liquid properties do not occur according to previous studies [10, 12, 13], the viscosity in the nanochannel was almost similar to the bulk viscosity. For channel sizes smaller than 700 nm, the viscosity increased with a decrease in the channel size, and became 3.6 times higher at $D_r = 190$ nm in the case of the square nanochannels. In the case of the plate nanochannels, the viscosity also increased with a decrease in the channel size, but became only 1.3 times higher at $D_r = 234$ nm.

The results obtained in the present study agrees well with the viscosity of water confined in nanochannels measured by a different method. We have reported measurements of liquid viscosity in nanochannels by capillary filling, which is the spontaneous injection of a liquid into nanochannels by the surface tension at the gas–liquid
The results also suggested that the viscosity of water in fused-silica nanochannels, which is determined from the speed of capillary filling, significantly increases for square nanochannels rather than plate nanochannels. This agreement validated the proposed mass flowmetry method developed for nanochannels, and suggested that the flow rate in the nanochannel is significantly affected by unique properties of confined water.

To elucidate a dominant factor for unique properties in nanospaces, both experimental and numerical studies have been reported. Most of the previous studies have dealt with plate nanochannels made of glass, where a 1.1–1.5 times
higher water viscosity at a channel depth of 200 nm was interpreted as due to electroviscous effects caused by the electric body force generated from the EDL near the surface [14, 21, 22]. The 1.3 times higher water viscosity in the plate nanochannel with \( D_r = 234 \) nm obtained in the present study (Fig. 5) is in agreement with the results of previous studies; therefore, this increased viscosity is considered to be due to electroviscous effects. In contrast, few studies on the viscosity of liquid confined in square nanochannels have been reported. To examine the electroviscous effects, we measured the flow rate of 10 mM potassium chloride (KCl) in the square nanochannel with \( D_r = 190 \) nm. While the Debye length (the EDL thickness) of pure water in the nanochannel was around 100 nm based on our previous study [23], that of 10 mM KCl is theoretically calculated to be 3 nm, [24] which is much smaller than the channel size. Therefore, the electroviscous effects with 10 mM KCl are expected to be much smaller than those with pure water. However, the estimated viscosity of 10 mM KCl determined from the fluid resistance was \( 3.08 \times 10^{-3} \) Pa s and still 3.4 times higher than the bulk viscosity, which is almost the same as that of pure water \( (3.21 \times 10^{-3} \) Pa s), as listed in Table 1. Therefore, the electroviscous effect is not a dominant factor in the increased viscosity observed in the square nanochannels. Alternatively, the effect of loosely structured water near the surface on the viscosity of water confined in fused-silica nanochannel was suggested. The mass flowmetry developed in this work is available for the characterization of fluid flow in other top-down fabricated nanospaces such as graphene nanochannels [25, 26] and lipid bilayer-modified nanochannels [27], which have been developed for fundamental studies on energy harvesting and biophysics, respectively. The knowledge obtained will accelerate understanding of the properties of fluids confined in nanospaces, which is important in the field of nanofluidics and other fields such as physical chemistry and biophysics.

**Conclusions**

We have reported the development of a mass flowmetry measurement for fused-silica nanochannels fabricated in a nanofluidic device. We succeeded in the measurement of the flow rate in a nanochannel within the order of pL/s to nL/s. Based on the results, we revealed the effects of size and geometry of nanochannels on the flow rate depending on the water viscosity, which increases with decreasing the representative channel size especially in the square nanochannels smaller than 800 nm, rather than the plate nanochannels. It was also confirmed that the electroviscous effect is not a dominant factor in the increased viscosity observed in the square nanochannels. Alternatively, the effect of loosely structured water near the surface on the viscosity of water confined in fused-silica nanochannel was suggested. The mass flowmetry developed in this work is available for the characterization of fluid flow in other top-down fabricated nanospaces such as graphene nanochannels [25, 26] and lipid bilayer-modified nanochannels [27], which have been developed for fundamental studies on energy harvesting and biophysics, respectively. The knowledge obtained will accelerate understanding of the properties of fluids confined in nanospaces, which is important in the field of nanofluidics and other fields such as physical chemistry and biophysics.

**Acknowledgements** This work was supported by a Kakenhi Grant-in-Aid (No. JP21000007) from the Japan Society for the Promotion of Science (JSPS). Fabrication facilities were provided in part by the Academic Consortium for Nano and Micro Fabrication from four universities (The University of Tokyo, Tokyo Institute of Technology, Keio University and Waseda University, Japan).

**References**

1. X. Ou, P. Chen, B.-F. Liu, Anal. Sci. 35, 609 (2016)
2. L.F. Giraldo, B.L. López, L. Pérez, S. Urrego, L. Sierra, M. Mesa, Macromol. Symp. 258, 129 (2007)
3. H. Shimizu, K. Toyoda, K. Mawatari, S. Terabe, T. Kitamori, Anal. Chem. 91, 3009 (2019)
4. N. Varongchayakul, J. Song, A. Meller, M.W. Grinstaff, Chem. Soc. Rev. 47, 8512 (2018)
Characterization of pressure-driven water flows in nanofluidic channels by mass flowmetry

5. B.R. Cipriany, P.J. Murphy, J.A. Hagarman, A. Cerf, D. Latulippe, S.L. Levy, J.J. Benítez, C.P. Tan, J. Topolancik, P.D. Soloway, H.G. Craighead, Proc. Natl. Acad. Sci. USA 109, 8477 (2012)
6. T. Nakao, Y. Kazoe, E. Mori, K. Morikawa, T. Fukasawa, A. Yoshizaki, T. Kitamori, Analyst 144, 7200 (2019)
7. G.K. Lockwood, S.H. Garofalini, J. Phys. Chem. C 118, 29750 (2014)
8. R. Zhou, C. Sun, B. Bai, J. Chem. Phys. 154, 074709 (2021)
9. A. Hibara, T. Saito, H.B. Kim, M. Tokeshi, T. Ooi, M. Nakao, T. Kitamori, Anal. Chem. 74, 6170 (2002)
10. L. Li, Y. Kazoe, K. Mawatari, Y. Sugii, T. Kitamori, J. Phys. Chem. Lett. 3, 2447 (2012)
11. K. Morikawa, Y. Kazoe, K. Mawatari, T. Tsukahara, T. Kitamori, Anal. Chem. 87, 1475 (2015)
12. H. Chinen, K. Mawatari, Y. Pihosh, K. Morikawa, Y. Kazoe, T. Kitamori, Angew. Chem. Int. Ed. 51, 3573 (2012)
13. T. Tsukahara, A. Hibara, Y. Ikeda, T. Kitamori, Angew. Chem. Int. Ed. 46, 1180 (2007)
14. N.R. Tas, J. Haneveld, H.V. Jansen, M. Elwenspoek, A. van den Berg, Appl. Phys. Lett. 85, 3274 (2004)
15. S. Liu, Q. Pu, L. Gao, C. Korzeniewski, C. Matzke, Nano Lett. 5, 1389 (2005)
16. K. Morikawa, K. Mawatari, M. Kato, T. Tsukahara, T. Kitamori, Lab. Chip 10, 871 (2010)
17. M. Whitby, L. Cagnon, M. Thanou, N. Quirke, Nano Lett. 8, 2632 (2008)
18. R. Ishibashi, K. Mawatari, K. Takahashi, T. Kitamori, J. Chromatogr. A 1228, 51 (2012)
19. J.P. Brody, P. Yager, R.E. Goldstein, R.H. Austin, Biophys. J. 71, 3430 (1996)
20. J. Kestin, M. Sokolov, W.A. Wakeham, J. Phys. Chem. Ref. Data 7, 941 (1978)
21. M. Wang, C.-C. Chang, R.-Y. Yang, J. Chem. Phys. 132, 024701 (2010)
22. S.I. Kim, S.J. Kim, Microfluid. Nanofluid. 22, 12 (2018)
23. Y. Kazoe, K. Mawatari, Y. Sugii, T. Kitamori, Anal. Chem. 83, 8152 (2011)
24. R.F. Probstein, Physicochemical Hydrodynamics (Wiley, New York, 1994)
25. Q. Xie, F. Xin, H.G. Park, C. Duan, Nanoscale 8, 19527 (2016)
26. Q. Xie, M.A. Alibakhshi, S. Jiao, Z. Xu, M. Hempel, J. Kong, H.G. Park, C. Duan, Nat. Nanotech. 13, 238 (2018)
27. Y. Kazoe, K. Mawatari, L. Li, H. Emon, N. Miyawaki, H. Chinen, K. Morikawa, A. Yoshizaki, P.S. Dittrich, T. Kitamori, J. Phys. Chem. Lett. 11, 5776 (2020)