Direct Measurement of In-Vitro Transport of Water and Peritoneal Dialysis Solution

Phonethipsavanh Nouanthong[1, 3], Panadda Dechadilok[2], Talerngsak Kanjanabuch[3]

[1]Faculty of Graduate School, Chulalongkorn University, Bangkok, Thailand

[2]Department of Physics, Faculty of Science, Chulalongkorn University, Bangkok, Thailand

[3]Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Abstract: ***Icodextrin is clinically employed as an osmotic agent in continuous ambulatory peritoneal dialysis (CAPD). However, the mechanism of water transport induced by this glucose polymer solution is yet to be completely elucidated. In this study, we performed a simple experiment to shade light on properties of high molecular weight icodextrin and low molecular weight dextrose as osmotic agents. The materials and methods used include 10 ml cellophane bags containing a peritoneal dialysis solution were placed in a 200 ml container full of either water or blood plasma. The experiments are performed using cellophane bags with 3.5 kDa and 10kDa molecular weight cut-off. 24-hour monitoring of water transport induced by 7.5% icodextrin solution, and that induced by 1.5% and 4.25% dextrose solutions are compared. It is observed in the study that in a beaker containing water, mass of the cellophane bags containing 1.5% and 4.5% dextrose solutions increase up to 6.6% and 10.7% respectively within 24 hours. In blood plasma containers, the cellophane bags containing 1.5% and 4.25% dextrose solution exhibited mass loss, indicating an overall fluid flux flowing from the cellophane bag. In contrast, masses of cellophane bags containing 7.5% icodextrin solution increase up to 30%. Moreover, the constant slope of the averaged mass changes after the dwell time of 8 hours also indicated a constant incoming fluid flux. We may conclude that in plasma beaker, cellophane bags containing dextrose solution exhibit mass decrease and volume reduction. On the other hand, cellophane bags containing icodextrin solution exhibits mass increases regardless of whether the cellophane bags were suspended in water or blood plasma despite the similar osmolarity of blood plasma and icodextrin solution.***

Keywords: peritoneal dialysis, Icodextrin, dextrose, water transport

1. Introduction

During the standard care for patients with end-stage renal disease (ESRD), glucose is commonly employed as a conventional osmotic agent for peritoneal dialysis. However, it had been documented that the high concentration, particularly 4.25% Dextrose, has direct deleterious effects on peritoneal structure and hence its function [1]. In addition, the glucose molecule, with its molecular weight being 180 Da, is relative small and can be absorbed through peritoneum rapidly, causing a decrease in osmotic pressure gradient and, therefore, a fast reduction of permeated fluid flow. To avoid the complications caused by a glucose-based dialysis solution and to sustain an osmotic-driven ultrafiltration, a starch-derived glucose polymer, icodextrin, with its molecular weight being between 1.6 – 45 kDa has been used as an osmotic agent during peritoneal dialysis. Comparing to conventional glucose-based dialysis solution, because of its larger molecular size, the peritoneum absorption rate of icodextrin is smaller, allowing it to induce sustained ultrafiltration flow [2-5].

In-vivo peritoneal dialysis involves transport of water and solutes through multiple barriers that constituted the peritoneal membrane with the driving mechanisms involved being heavily intertwined. Solute concentration profile is governed by convection, and diffusion and fluid flow across the peritoneal membrane is driven by the crystalloid, and colloid osmotic pressure difference, as well as the hydrodynamic pressure gradient. The indirect methods were used to evaluate the water transport and ultrafiltration[6-9]. However, lymphatic reabsorption and enzymatic digestion of glucose polymer also influenced the solute concentration and flow profiles. In our experiments, we attempted to gain a better understanding of glucose and icodextrin as osmotic agents by simplifying the situation and having an osmotic-driven flow being transported through a single-layered semipermeable membrane. The objective of the present work was to explore in a simple Vitro Experiment using ability of isodextrin and dextrose as osmotic agents in water osmosis transport. The study will be simple useful for other osmotic agent development

2. Materials and Methods

Dialysis solutions: Glucose based peritoneal dialysis having concentration of 1.5% and 4.25% dextrose with osmolarity 346 and 485 mOsm/L from (Baxter, Health Care, Phillippines INC) was used as a low molecular weight osmotic agent. 100 ml solution contains 1.5 g or 4.25 g dextrose hydrous USP, 538 mg sodium chloride USP, 448 mg sodium lactate, 25.7 mg calcium chloride USP, 5.08 mg magnesium chloride USP, pH 5.2. While 7.5% Icodextrin with osmolarity 278 mOsm/L from (Baxter Health Care, Singapore Branch) was used as a high molecular weight as osmotic agent. The electrolyte composition and pH were similar to dextrose but contained a high molecular weight glucose polymer 7.5 g icodextrin instead of dextrose. Normal blood donated aphaeresis' plasma was obtained from Thai Red Cross. The experiments were performed using the same batch of SnakeSkin® Pleated Dialysis Tubing molecular weight cutoff (MWCO) 3.5-kDa and 10-kDa purchased from Thermo Scientific (www.piercenet.com).

Assay methods: A simple defined experiment was performed. Shortly, the dialysis dialysis tubing length of 12

cm was used in all experiments. Cellophane bags containing the dialysis solution were placed in a beaker full of either water or blood plasma in container size 250 ml, and the weight of each cellophane bags was measured to determine the mass change at 30, 60, 120, 240, 480, 720 and 1440 minutes. At the first stage of this study, where the cellophane bags were placed in a beaker containing water, the hydrodynamic pressure gradient was also minimized, allowing a direct comparison between fluid flow driven by crystalloid osmotic agent, dextrose, and that driven by colloid osmotic agent, icodextrin. In beakers containing water, the dialysis bags with MWCO 10-kDa containing of 10 ml dialysis solutions 1.5%, 4.25% dextrose and 7.5% icodextrin and water as control were placed in an individual container size 250 ml full of water. All beakers were placed on shaker with a slow movement (70 rpm). For experiments done in the beaker containing blood plasma, we used two different molecular weight cutoff MWCO 3.5 and MWCO 10-kDa containing of 10 ml dialysis solutions 1.5%, 4.25% dextrose and 7.5% icodextrin and water. The second stage of the study was devoted to an examination of icodextrin and dextrose as osmotic agents where the bulk solution was blood plasma. The percentage of the mass change of the cellophane bag as a function of time is also reported. Experiments were performed at room temperature (25-28^{o}C). Calculation and Statistical Analysis: Direct examination of mass changes were determined by measuring the difference between the baseline weight of each cellophane bag after being placed in a beaker for 5 minutes (w_0) and its weight after each dwelling times interval (w_i). The percentage of mass increases was calculated as shown in equation (1).

$$\%\ \text{mass change} = \frac{w_0 - w_i}{w_0} \quad (1)$$

Mean values and standard deviation of the percentage of mass changes are assessed.

3. Results

Mean values and standard deviation of the percentages of mass changes of the cellophane bags at the dwell time of 30, 60, 120, 240, 360, 480, 720 and 1440 minutes are reported in Table 1&2.

Table 1: Beaker containing water

Dwell Time (Min)	*Percent mass increase (Mean ± SD) MWCO 10-kDa*		
	7.5% Icodextrin	*4.25% Dextrose*	*1.5% Dextrose*
30	4.21 ± 0.41	6.80 ± 0.59	4.28 ± 0.97
60	7.04 ± 0.35	9.88 ± 0.09	5.35 ± 0.83
120	9.05 ± 0.67	12.22 ± 0.90	7.44 ± 1.31
240	14.17 ± 1.97	14.32 ± 1.58	8.28 ± 1.50
360	17.02 ± 2.52	13.76 ± 1.44	8.27 ± 2.10
480	19.90 ± 1.68	13.53 ± 1.75	8.05 ± 2.16
720	23.66 ± 0.46	12.92 ± 1.46	7.19 ± 2.60
1440	27.15 ± 0.31	10.74 ± 1.30	6.56 ± 2.30

Table 2: Beaker containing plasma

Dwell Time (Min)	*Percent mass increase (Mean ± SD)*			
	MWCO 3.5-kDa		*MWCO 10-kDa*	
	7.5% Icodextrin	*4.25% Dextrose*	*7.5% Icodextrin*	*4.25% Dextrose*
30	-0.02 ± 0.61	2.33 ± 0.62	0.44 ± 0.13	2.48 ± 0.13
60	0.45 ± 0.04	2.83 ± 0.44	1.19 ± 0.36	3.39 ± 0.55
120	0.38 ± 0.14	2.75 ± 0.57	2.75 ± 0.21	3.52 ± 0.33
240	1.41 ± 0.30	2.08 ± 0.44	5.77 ± 0.72	2.80 ± 0.56
360	3.06 ± 0.58	1.60 ± 0.40	9.52 ± 0.39	1.47 ± 0.30
480	7.05 ± 0.33	-0.09 ± 0.46	15.91 ± 0.24	0.59 ± 0.37
720	8.85 ± 1.41	-1.08 ± 0.13	19.30 ± 0.48	-0.59 ± 0.44
1440	19.52 ± 1.33	-5.27 ± 0.11	-	-4.39 ± 0.27

Figure 1 shows the percentage of mass changes induced by glucose and glucose polymer based solutions through dialysis tubing MWCO 10-kDa in a water container as a function of dwell time. Error bars are one standard deviation. Within experimental error, the weight of cellophane bags containing water did not changed, demonstrating that there was not a water flux exchange between the volume inside the cellophane bag and the bulk fluid in the beaker. This confirms our assumption that the effect of hydrodynamic pressure difference across the cellulose membrane was indeed minimized. The mass of the cellophane bags containing 1.5% and 4.25% dextrose solution exhibited a sharp increase during the first four hours reaching a peak at 8.28% and 14.28%, respectively, before starting to show a slight mass loss. This indicated that the osmotic pressure differences induced by 1.5% and 4.25% dextrose solution dissipated after approximately 4 hours. After 24 hours, the total mass increases induced by 1.5% and 4.25% dextrose were 6.6% and 10.7% respectively. In contrast, the mass of the cellophane bags containing 7.5% icodextrin continued to increase up to 24 (%) in 12 hours and reached 27% after 24 hours, with the slope of the curve being non-zero showing that there was still a water flux flowing from the outside bulk fluid into the cellophane bags.

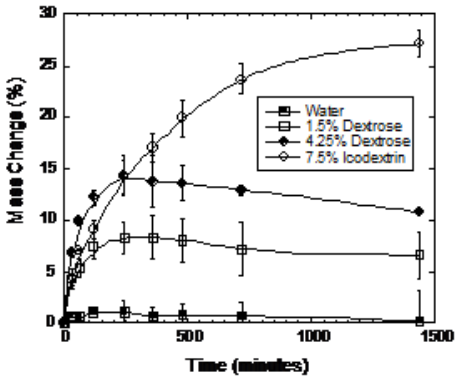

Figure 1: Percentage of mass changes of water and dialysis solutions inside cellophane bags as a function of dwell time. The membrane molecular weight cut-off was 10 kDa. Results are shown for solutions being water, 1.5% and 4.25% dextrose solution, and 7.5 % icodextrin solution. The cellophane bags were placed in a 200 mL beaker containing water. Error bars are one standard deviation.

Figure 2 shows the percentage of mass changes due to water and solute exchanges through dialysis tubing with MWCO 10-kDa in a beaker containing blood plasma as a function of dwell time. The cellophane bags containing water exhibited

a mass loss down to approximately 21% after 24 hours. The very same trend was also observed in the case of cellophane bags containing 1.5% dextrose solution during the first 8 hours, with the mass decrease happening more slowly, before reaching a plateau (within experimental error) at the dwell time of 8 hours. Its averaged total mass loss, after 24 hours, was approximately 14%. In contrast, the mass of cellophane bags containing 4.25% dextrose solution increased slightly during the first 2 hours up to the total mass increase of approximately 4%. Then a slow mass decrease started to become apparent, resulting in the overall mass loss of 5% within 24 hours. A very different trend was observed for cellophane bags containing 7.5% icodextrin solution of which mass increased slowly up to 3% within the first 2 hours, and then the mass increase started to happen more rapidly, with the total mass increase being 34% after 24 hours. The slope of its curve shown in Figure 1 is approximately constant from the dwell time of 8 hours to the dwell time of 24 hours, indicating an approximately constant incoming fluid flux from the outside bulk solution into the cellophane bag during that time interval.

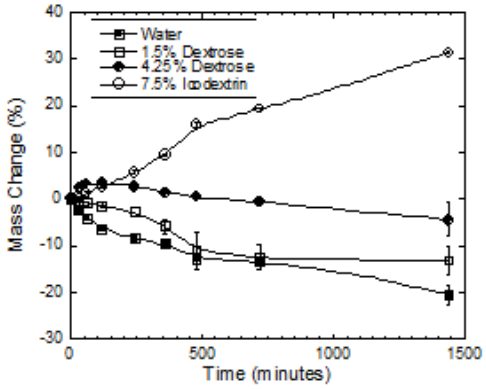

Figure 2: Percentage of mass changes of water and dialysis solutions inside cellophane bags as a function of dwell time. The membrane molecular weight cut-off was 10 kDa. Results are shown for solutions being water, 1.5% and 4.25% dextrose solution, and 7.5 % icodextrin solution. The cellophane bags were placed in a 200 mL beaker containing blood plasma. Error bars are one standard deviation.

The very same experiment was repeated but with the cellophane bags having the molecular weight cut-off being 3.5 kDa. Shown in Figure 3 is the relationship between the dwell time and the percentage of mass change of cellophane bags containing water, 1.5% and 4.25% dextrose solution, and 7.5% icodextrin solution, placed in a container full of blood plasma. Generally, the percentage of the mass changes observed exhibited a similar trend previously seen in the case of the cellophane bag having the molecular weight cut-off being 10kDa. The mass of cellophane bags containing water also steadily decreased, although the averaged total mass loss, after 24 hours, was smaller at 14%. Cellophane bags containing 4.25% dextrose solution exhibited a slight mass increase to 3% during the first hour and then a slow mass decrease amounting to an averaged mass loss, within 24 hours, being 5.6%. The difference induced by the different molecular weight cut-off of the dialysis tubing is shown in the percentage of the mass changes of cellophane bags containing 1.5% dextrose solution of which trend became similar to that of cellophane bags containing 4.25% dextrose. During the first hour, the averaged mass increased slightly up to 0.4%, and then a mass decrease was observed. After 24-hour monitoring, the overall averaged mass loss was 6.7%. In contrast, the cellophane bags containing 7.5% icodextrin solution demonstrated a different trend from those of cellophane bags containing 1.5% and 4.25% dextrose solution. The averaged mass of the cellophane bags containing icodextrin remained approximately constant during the first 30 minutes, then starting to increase slowly until the dwell time of 3 hours. After that, the averaged percentage of mass change increased more rapidly, with the total mass increase being approximately 21% after 24 hours.

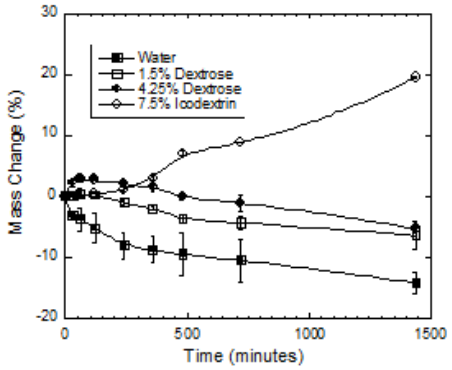

Figure 3: Percentage of mass changes of water and dialysis solutions inside cellophane bags as a function of dwell time. The membrane molecular weight cut-off was 3.5 kDa. Results are shown for solutions being water, 1.5% and 4.25% dextrose solution, and 7.5 % icodextrin solution. The cellophane bags were placed in a 200 mL beaker containing blood plasma. Error bars are one standard deviation. The slope of its curve shown in Figure 3 became approximately constant after 8 hours, indicating a constant incoming flux similar to that previously seen in the case of the cellophane bag MWCO being 10 kDa.

4. Discussion

A simple in-vitro experiment is conducted to investigate properties of high molecular weight icodextrin and low molecular weight dextrose as osmotic agents. By eliminating several factors such as the lymphatic solute reabsorption and the enzymatic glucose digestion, we focused mainly on the osmotic gradient being the driving factor of the fluid flow across a single semipermeable membrane. The molecular weight cut-off of 10 kDa was chosen in order to mimic the endothelium with its small pores having the sizes of approximately 4-6 nm. An experiment in a beaker full of blood plasma was also repeated with cellophane membranes with molecular weight cut-off being 3.5 kDa to confirm the previous experiment and our hypothesis.

A relationship between a volume flux of a fluid flow (J_v), an actual pressure difference (ΔP), and osmotic pressure difference across a semipermeable membrane is given by the well-known Sterling equation

$$J_V = L_p\left(\Delta P - \sum_i \sigma_{oi}\Delta\Pi_i\right). \quad (2)$$

L_p is the membrane hydraulic permeability. $\Delta\Pi_i$ is an osmotic pressure difference caused by unequal concentration of solute *i* at both sides of the membrane. For a dilute, ideal

solution, according to van't Hoff's law, the osmotic pressure due to a presence of solute *i* can be written as

$$\Pi_i = kTC_i \quad (3)$$

where k is Boltzmann's constant, and C_i is solute concentration in the unit of numbers of molecules per volume. In equation (2), if J_v is defined as the volume flux from the left to the right, then ΔP and $\Delta\Pi_i$ are the pressures in the left-hand solution minus that of the right-hand solution. It can be deduced from equation (3) that the osmotic pressure is higher in the region with higher solute concentration (or solute osmolarity), the direction of the osmotic driven flow is towards the region with higher solute concentration.

The ability of solute to induce an osmotic flow is characterized by its osmotic reflection coefficient (σ_{oi}). The theoretical calculations of reflection coefficient for neutral solutes transported through porous membranes [10, 11] and fibrous media [12] indicate that the value of σ_{oi} increases with the increasing ratio between the solute size, and the spaces of the membrane. In the case of an ideal semipermeable membrane (where the solute size is larger or equal to that of the membrane spaces), σ_{oi} = 1, meaning that the osmotic driven flow is maximized. In contrast, for a completely permeable membrane, where the spaces are large enough that the membrane does not distinguish the solute from the solvent (and the ratio between solute size and the membrane spaces is miniscule), σ_{oi} = 0, and there is no osmotic-driven flow. In absence of the actual pressure difference, it is the product of the reflection coefficient and the osmotic pressure difference that determines the fluid volume fluxes caused by different solutes across the same semipermeable membrane.

For experiments conducted in the water container, we assumed zero solute concentration outside the cellophane bag initially. Therefore, for cellophane bags containing water, there was no osmotic pressure difference across the membrane. As mentioned previously, the approximately constant mass of the cellophane bags indicates that the hydrodynamic pressure difference is negligible, and the only factor driving the fluid volume flux is the osmotic pressure difference. In the cases of cellophanes containing 1.5% and 4.25% dextrose solution, with their osmolarities being 346 mOsm/L and 485 mOsm/L respectively, the osmotic pressure in the cellophane bag was higher than that in the outside bulk solution. Therefore, the fluid volume flux was flowing from the outside bulk solution into the cellophane bag, resulting in mass increases. The mass of cellophane bags containing 4.25% dextrose solution increases faster due to the higher solute osmolarity, resulting in a higher osmotic pressure difference across the membrane. At the same time, the glucose molecules were also diffusing from the regions inside the cellophane bags towards the outside bulk fluid. Competing against solute diffusion is solute convection (because the osmotic-driven flow direction is from the outside fluid towards the region inside the cellophane bags). The experimental results showed that for dextrose molecules transported through cellulose membrane with molecular weight cut-off being 10 kDa (with the maximum membrane spaces being 4-6 nm), diffusion was a more dominant factor. Eventually, the combination of incoming fluid flux and solute diffusion caused the solute concentration on both sides of the membrane to be in equilibrium, and the osmotic flow to cease within a time scale of 4 hours.

An interesting comparison would be to compare the fluid volume flux in the case of cellophane bags containing 7.5% icodextrin solution with that of cellophanes containing dextrose solution. The dextrose solutions had higher solute osmolarities, with the icodextrin osmolarity being 278 mOsm/L. Therefore, according to van't Hoff's Law, the dextrose solutions had higher osmotic pressure difference, which might explain why, initially, the flow rate induced by the dextrose solution was higher. However, because of its larger molecular size, the osmotic reflection coefficient of icodextrin was higher than that of dextrose. In addition, because of their sizes being comparable or larger than that of the membrane spaces, a number of smaller icodextrin molecules would be allowed to diffuse through the membrane through restricted diffusion, while a larger number of icodextrin molecules were completely retained. This would explain why icodextrin solution was able to maintain osmotic pressure difference for a longer period of time, resulting in an averaged mass increase that was almost three times higher than those of dextrose solutions after 24 hours.

For experiments done in a beaker containing blood plasma, the osmolarity of the outside bulk solution was assumed to be that of the blood plasma (in the range of 275-299 mOsm/L). The mass of the cellophane bags containing water was in decline, indicating that the fluid flow direction was towards the outside bulk solution. This was to be expected since the outside blood plasma had higher osmolarity, and thus higher osmotic pressure. The mass decrease happened more rapidly in the cases of dialysis tubing with higher molecular weight cut-off because of its higher hydraulic permeability (L_p) due to larger membrane spaces, resulting in higher volume fluxes. In the case of cellophanes containing 4.5 % dextrose solutions, because of the higher osmolarity of the dextrose solution, there was a fluid flow coming into the cellophane bags initially. However, there was also an exchange of dextrose molecules, and solute molecules in the blood plasma through diffusion. The electrolytes as well as very small solutes in blood plasma could diffuse through the membrane freely while the diffusion of macromolecules and proteins was restricted. According to the experimental results, because of this solute exchange and the initial fluid flow direction, after the dwell time of 2-3 hours, the osmotic pressure difference of the blood plasma became higher than that of the dextrose solution, and the flow direction was reversed. It could be concluded that, in absence of lymphatic reabsorption and enzymatic glucose digestion, the osmotic pressure difference across a fibrous membrane with maximum space size of 4-6 nm induced by 4.25% dextrose solution dissipated within 2-3 hours.

For the cellophane containing 1.5% dextrose solution, initially the osmolarity of the dextrose solution was slightly higher than that of the blood plasma. A trend similar to that of the cellophane containing 4.25% dextrose solution was observed when the membrane molecular weight cut-off was 3.5 kDa. When the molecular weight cut-off was 10 kDa, the larger membrane spaces implied a higher hydraulic permeability (L_p), and an easier solute exchange. Therefore, the early increase of the mass of the cellophane bags was not observed.

In the case of cellophane bags containing 7.5 % icodextrin solution placed in a container full of blood plasma, the osmolarities of the icodextrin solution and blood plasma was intitially equal, resulting in the fact that there was either no fluid flux or only slight fluid fluxes in the beginning. Very small solutes suspended in the blood plasma could freely diffuse into the cellophane bags, while the diffusion of the macromolecules such as proteins in blood plasma, and small-sized icodextrin was restricted. The diffusion of very small solutes from the outside blood plasma into the cellophane bag caused the osmotic pressure in the cellophane bags to become higher than that of the bulk solution, causing the outside fluid to flow into the cellophane bags. This fluid flow resulted in a convection of very small solutes into the cellophane bags, which, along with diffusion, kept building up the osmotic pressure inside the cellophane bags and caused more fluid to flow in. This resulted in a seemingly steady fluid flow into the cellophane bags after the dwell time of 8 hours. The flow movement was more rapid when the molecular weight cut-off of the membrane was higher, which meant that the membrane higher hydraulic permeability and that diffusion and convection were less restricted.

5. Conclusion

After a 24-hour monitoring, the cellophane bags containing 1.5% and 4.25% dextrose solution placed in blood plasma containers exhibited mass loss, indicating an overall fluid flux flowing from the cellophane bag. In contrast, masses of cellophane bags containing 7.5% icodextrin solution increase up to 30%. Moreover, the constant slope of the averaged mass changes after the dwell time of 8 hours also indicated a constant incoming fluid flux. It is commonly believed that icodextrin is a better osmotic agent due to its smaller absorption rate. Our experimental result indicates that another factor which might enable incodextrin solution to sustain ultrafiltration can also be the restricted diffusion of icodextrin molecules along with a faster diffusion of small solutes in blood plasma. These two factors caused a building up of osmotic pressure difference, convection of small solutes and fluid flow, despite the similar osmolarity of icodextrin solution and blood plasma. This model is simple measurement method to evaluate the osmotic agent on water transportation.

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Author Profiles

Phonethipsavanh Nouanthong received the B.S. degree in Medical Technology at Khone Kean University, Thailand (1998), M.S. degree at Chiang Mai University, Thailand (2005), and completed a post graduate course in Biostatistics in Public Health I & II and Epidemiology of HIV/AIDS, Johns Hopkins Bloomberg School of Public Health, the JHU, Maryland, United State. She is currently a Ph.D candidate in Biomedical Science, Graduate School, Chulalongkorn University, Bangkok, Thailand.

Panadda Dechadilok received B.A. Physics and Applied Mathematics, 2002, Northwestern University, Evanston, IL, then S.M. Applied Mathematics, 2008, Harvard University, Cambridge, MA and Ph.D. Applied Mathematics, 2009, Harvard University,

Cambridge, MA. She is currently a lecturer at Department of Physics, Faculty of Science Chulalongkorn University, Bangkok, Thailand.

Talerngsak Kanjanabuch received M.D. (first class honor) Songkhlanagarind University, Thailand, Thai Board of Internal Medicine, The Royal College of Physicians of Thailand, Thai Board of Nephrology, The Royal College of Physicians of Thailand, M.S. - Medical Science and Clinical Fellow, Division of Nephrology, Department of Medicine, Chulalongkorn University Chulalongkorn University. He also became a Clinical Fellow, Renal Pathology, Vanderbilt University Medical School, Nashville, TN, USA Postdoctoral Fellow, Vanderbilt University Medical School, Nashville, TN, USA.