SUPPLEMENTARY METHODS, TABLES, AND FIGURES

for

DEVELOPMENT OF A DYNAMIC PHYSIOLOGICALLY-BASED MECHANISTIC KIDNEY MODEL TO PREDICT RENAL CLEARANCE

Weize Huang, Nina Isoherranen

Department of Pharmaceutics, School of Pharmacy, University of Washington, Seattle, WA, 98195

Mechanistic kidney model development and governing equations that describe the pharmacokinetic and physiological processes in the model.

The overall dynamic model consists of a simple circulation component and a mechanistic physiologically based kidney model. The simple circulation model was used to connect the renal blood flow out of the kidney with the blood flow into the kidney. The Bowman's capsule was set as the entrance of the blood flow into the mechanistic kidney model. Only unbound drug in the blood was allowed to be filtered into proximal tubule. The kidney model used 35 compartments, based on physiological segmentation of the kidney, to capture the physiology of the human kidney. Longitudinally, the kidney was divided into 4 major segments: proximal tubule, loop of Henle, distal tubule, and collecting duct. These segments were further divided into subsegments as follows: the proximal tubule was divided into 3 subsegments (S1, S2, and S3), loop of Henle into 2 subsegments (descending and ascending), and collecting duct into 5 subsegments (connecting tubule, initial collecting duct, cortical collecting duct, medullary collecting duct, and papillary duct) based on established human kidney physiology. Each subsegment was divided into 3 sections: tubular lumen, cellular compartment, and vascular section. A compartment for bladder was created to serve as a compartment for collecting the eliminated drugs. Glomerular filtration was incorporated into the Bowman's capsule, bidirectional passive diffusion including both passive reabsorption and passive secretion were assumed to occur throughout the entire nephron, and active transporter-mediated secretion, active transporter-mediated reabsorption, and renal intrinsic metabolism were assumed to occur only at proximal segment.

To simulate renal clearance under conditions of distribution equilibrium, drug is infused intravenously to the circulation compartment of the model to initiate the simulation and continue throughout the simulation. The equation describing the change of blood drug concentration in the central compartment (the circulation component) with respect to time is:

$$V_c \frac{d[C_b]}{dt} = Ro\left(1 - e^{-\frac{Q_c}{V_b}t}\right) + Q_{other}C_b(t) + Q_{kidney}C_{CDB,5}(t) - Q_c C_b(t)$$

$$Q_c = Q_{other} + Q_{kidney}$$

where C_b is blood concentration (mg/L), V_b is blood volume (5 L), V_c is central compartment volume (42 L), R_0 is iv infusion rate (mg/hr), Q_c is cardiac output (330 L/hr), Q_{kidney} is blood flow to kidney (60 L/hr), Q_{other} is blood flow to other organs (L/hr), $C_{CDB,5}$ is blood concentration in the vascular section of the fifth subsegment of collecting duct (L/hr)

Once the drug is infused into the system, it may partition into red blood cells and bind to plasma proteins, but only unbound drug in plasma can be filtered into Bowman's capsule from glomerular blood (renal inflow). The equation describing the change of drug concentration in the Bowman's capsule with respect to time is:

$$C_{bowman}(t) = \frac{f_{u,p}}{BP} C_b(t)$$

where $f_{u,p}$ is plasma unbound fraction, BP is blood to plasma concentration ratio, and C_{bowman} is drug concentration in the Bowman's capsule after filtration (mg/L).

After the drug in the blood is filtered into the Bowman's capsule, it enters the mechanistic kidney model divided into the various subsegments and sections as described above. The segments and sections are linked in series as shown in Figure 1.

After entering the tubular lumen of the proximal tubule, drug enters and leaves the tubular lumen by bidirectional passive diffusion across the apical tubular membrane, active secretion from proximal tubule cells, active reabsorption to proximal tubule cells, and tubular fluid inflow and outflow. The equation describing the change of drug concentration in the proximal tubular lumen at each subsegment with respect to time is:

$$V_{PT}\frac{d[C_{PT,i}]}{dt} = Q_{PT,i}C_{PT,i-1}(t) - Q_{PT,i+1}C_{PT,i}(t)$$
$$+ CL_{Api,scr,i}C_{PC,i}(t)f_{u,cell} - CL_{Api,reabs,i}C_{PT,i}(t)$$
$$+ CL_{PD,PT,api,i}(C_{PC,i}(t)f_{u,cell}\cdot\beta - C_{PT,i}(t)\cdot\alpha_i))$$

where i=1, 2, and 3, represent the three different proximal tubule subsegments, $C_{PT,i}$ is the drug concentration in the tubular lumen of i^{th} subsegment of proximal tubule (mg/L), $C_{PT,0}= C_{bowman}$, V_{PT} is the volume of each subsegment of proximal tubular lumen (L) as listed in Supplementary Table S1, $Q_{PT,i}$ is the tubular flow rate into the tubular lumen of i^{th} subsegment of proximal tubule (L/hr), $Q_{PT,i+1}$ is the tubular flow rate out of tubular lumen of i^{th} subsegment of proximal tubule (L/hr). The flow rates for each subsegment are listed in Table 1. Of note, $Q_{PT,1}= Q_{GFR}$ (120mL/min). The tubular flow rate out of any subsegment is equal to the tubular flow rate into the next subsegment. $CL_{Api,scr,i}$ is the active transporter-mediated secretion clearance on the apical side of cell compartment of i^{th} subsegment of proximal tubule (L/hr). $CL_{Api,reabs,i}$ is the active transporter-mediated reabsorption clearance on the apical side of cell compartment of i^{th} subsegment of proximal tubule (L/hr), $f_{u,cell}$ is the intracellular unbound fraction in the renal cell, $CL_{PD,PT,api,i}$ is the intrinsic passive diffusion clearance on the apical side of cell compartment of i^{th} subsegment of proximal tubule (L/hr), α_i is the uncharged fraction inside the tubular lumen of i^{th} subsegment of proximal tubule, and β is the uncharged fraction inside cell. As the pH inside tubular cells is 7.2 through the kidney, β is a constant for a specific drug and its value is determined by the pKa of the drug. The α and β are calculated from the known pKa and acid/base characteristics of the drug.

Once the drug flows out of the proximal tubule, it flows into the loop of Henle (descending and ascending), distal tubule, and collecting duct (connecting tubule, initial collecting duct, cortical collecting duct, medullary collecting duct, and papillary duct). No active secretion or active reabsorption was assumed to occur in the loop of Henle, distal tubule, and collecting duct. Only bidirectional passive diffusion, tubular inflow, and tubular outflow were incorporated. The equation describing the change of tubular drug concentration in the loop of Henle, distal tubule, and collecting duct with respect to time is:

$$V_{Tj,i}\frac{d[C_{Tj,i}]}{dt} = Q_{Tj,i}C_{Tj,i-1}(t) - Q_{Tj,i+1}C_{Tj,i}(t)$$
$$+ CL_{PD,j,i}(C_{Cj,i}(t)f_{u,cell}\cdot\beta - C_{Tj,i}(t)\cdot\alpha_{j,i}))$$

where i=1,2 for loop of Henle, i=1 for distal tubule, i=1,2,3,4,5 for collecting duct, j=loop of Henle, distal tubule, or collecting duct. $C_{Tj,i}$ is the drug concentration of the tubular lumen of i^{th} subsegment of j segment (mg/L), $V_{Tj,i}$ is the volume of the tubular lumen of i^{th} subsegment of j segment (L), $Q_{Tj,i}$ is the renal tubule inflow of the tubular lumen of i^{th} subsegment of proximal tubule of j segment (L/hr), $Q_{PT,i+1}$ is the renal tubule outflow of the tubular lumen of i^{th} subsegment of proximal tubule of j segment (L/hr), $CL_{PD,j,i}$ is the intrinsic passive diffusion clearance of both apical and basolateral sides of cell compartment of i^{th} subsegment of j segment (L/hr), $\alpha_{j,i}$ is the uncharged fraction inside the tubular lumen of i^{th} subsegment of segment j.

In terms of the renal cellular compartment, at the proximal region, the drug enters and leaves the renal cell compartment by bidirectional passive diffusion, active secretion, active reabsorption, and renal metabolism. The equation describing the change of drug concentration in the proximal renal cell with respect to time is:

$$V_{PC}\frac{d[C_{PC,i}]}{dt} = CL_{bsl,scr,i}C_{PB,i}(t)\frac{f_{u,p}}{BP} - CL_{Api,scr,i}C_{PC,i}(t)f_{u,cell}$$
$$+ CL_{Api,reabs,i}C_{PT,i}(t) - CL_{bsl,reabs,i}C_{PC,i}(t)f_{u,cell}$$
$$+ CL_{PD,PT,bsl,i}(C_{PB,i}(t)\cdot\frac{f_{u,p}}{BP}\cdot\gamma - C_{PC,i}(t)f_{u,cell}\cdot\beta)$$
$$+ CL_{PD,PT,api,i}(C_{PT,i}(t)\cdot\alpha_i - C_{PC,i}(t)f_{u,cell}\cdot\beta)$$
$$- CL_{\text{int},i}(C_{PC,i}(t)f_{u,cell})$$

where i=1, 2, and 3, represent the three different proximal tubule subsegments, $C_{PC,i}$ is the drug concentration of the cell compartment of i^{th} subsegment of proximal tubule (mg/L), V_{PC} is the volume of kidney proximal cell compartment of each subsegment (L), $CL_{PD,PT,bsl,i}$ is the intrinsic passive diffusion clearance on the basolateral side of the cell compartment of i^{th} subsegment of proximal tubule (L/hr), $CL_{int,i}$ is the intrinsic metabolic clearance due to enzymes residing in the cell compartment of i^{th} subsegment of proximal tubule (L/hr), γ is uncharged fraction in the plasma. Since plasma pH is always 7.4, γ is a constant for a specific drug and its value is determined by the pKa of the drug.

At the other regions (except proximal region), the drug enters and leaves the renal cell compartment by bidirectional passive diffusion, with no active secretion, active reabsorption, and metabolism. The equation describing the change of drug concentration in the proximal renal cell with respect to time is:

$$V_{Cj,i}\frac{d[C_{Cj,i}]}{dt} = CL_{PD,j,i}(C_{Bj,i}(t)\cdot\frac{f_{u,p}}{BP}\cdot\gamma + C_{Tj,i}(t)\cdot\alpha_{j,i}$$
$$- 2\times C_{Cj,i}(t)f_{u,cell}\cdot\beta)$$

where i=1,2 for loop of Henle, i=1 for distal tubule, i=1,2,3,4,5 for collecting duct, j=loop of Henle, distal tubule, or collecting duct, $C_{Cj,i}$ is the drug concentration of the cell compartment of i^{th} subsegment of j segment (mg/L), $V_{Cj,i}$ is the volume of the cell compartment of i^{th} subsegment of j segment (L)

In terms of the vascular section, right after glomerular filtration, the equation describing the change of drug concentration in the blood with respect to time is:

$$C_{PB,0}(t) = (1 - \frac{\frac{f_{u,p}}{BP} Q_{GFR}}{Q_{kidney}}) C_b(t)$$

where $C_{PB,0}$ is the drug concentration in the blood right after glomerular filtration.

At the proximal region, the drug enters and leaves the vascular section by bidirectional passive diffusion, active secretion, active reabsorption, and inflow and outflow processes. The equation describing the change of drug concentration in the blood with respect to time is:

$$V_{PB} \frac{d[C_{PB,i}]}{dt} = Q_{Kidney}(C_{PB,i-1}(t) - C_{PB,i}(t))$$
$$- CL_{bsl,scr,i} C_{PB,i}(t) \frac{f_{u,p}}{BP} + CL_{bsl,reabs,i} C_{PC,i}(t) f_{u,cell}$$
$$+ CL_{PD,PX,bsl,i}(C_{PC,i}(t) f_{u,cell} \cdot \beta - C_{PB}(t) \cdot \frac{f_{u,p}}{BP} \cdot \gamma)$$

where i=1, 2, or 3, represent three different proximal subsegments, $C_{PB,i}$ is the drug concentration of the vascular section of i^{th} proximal tubule subsegment (mg/L), V_{PB} is the volume of vascular section of each proximal tubule subsegment (L), $CL_{PD,PX,bsl,i}$ is the intrinsic passive diffusion clearance on the basolateral side of the renal cell of the i^{th} proximal tubule subsegment (L/hr)

At the other regions (except proximal region), the drug enters and leaves the vascular section by bidirectional passive diffusion, inflow, and outflow processes. The equation describing the change of drug concentration in the blood with respect to time is:

$$V_{Bj,i} \frac{d[C_{Bj,i}]}{dt} = Q_{Kidney}(C_{Bj,i-1}(t) - C_{Bj,i}(t))$$
$$+ CL_{PD,j,i}(C_{Cj,i}(t) f_{u,cell} \cdot \beta - C_{Bj,i}(t) \cdot \frac{f_{u,p}}{BP} \cdot \gamma)$$

where i=1,2 for loop of Henle, i=1 for distal tubule, i=1,2,3,4,5 for collecting duct, j=loop of Henle, distal tubule, or collecting duct, $C_{Bj,i}$ is the drug concentration of the vascular section of i^{th} subsegment of j segment (mg/L), $V_{Bj,i}$ is the volume of vascular section of i^{th} subsegment of j segment (L), $CL_{PD,j,i}$ is the intrinsic passive diffusion clearance of both apical and basolaterial sides of i^{th} subsegment of j segment (L/hr)

Renal clearance simulation with consideration of pH and microvilli effect on both in vitro experimental system and in vivo

The percent unionized of the drug of interest in the pH of the renal tubule (α), renal cell (β), and blood (γ) was calculated using equations 1-3.

$$unionized\ \%\ of\ acid = \frac{1}{1+10^{pH-pKa}} \quad (1)$$

$$unionized\ \%\ of\ base = \frac{1}{1+10^{pKa-pH}} \quad (2)$$

$$unionized\ \%\ of\ zwitterion = unionized\ \%\ of\ acid \times unionized\ \%\ of\ base \quad (3)$$

The intrinsic permeability for the test compounds was calculated from the reported apparent permeability measured at the pH (6.5 or 7.4) of apical side of transwell system using equation 4,

$$P_{int} = \frac{P_{app}}{unionized\%\ at\ apical\ experimental\ pH} \quad (4)$$

where P_{int} is intrinsic permeability, P_{app} is reported Caco2 or MDCK in vitro permeability and experimental pH is either 6.5 or 7.4 as reported. Active transporter-mediated secretion, active transporter-mediated reabsorption, and renal intrinsic metabolism were assumed to be zero unless otherwise stated. Effective passive diffusion (CL_{PD}) at each subsegment was calculated using equation 5,

$$CL_{PD,i} = P_{int} \times TSA_i \times unionized\ \%_i \quad (5)$$

where $CL_{PD,i}$ is passive diffusion between either tubule and cell or cell and blood of each subsegment, TSA_i is the relevant tubular surface area of each subsegment and unionized % is the percent unionized at subsegment i with a given pH. CL_{PD} is the same for apical and basolateral sides except for the proximal tubule where apical side has 30 fold higher TSA than basolateral side due to presence of microvilli.

Renal clearances were simulated at distribution equilibrium to avoid confounding effects of distribution processes. An infusion of the test drug was administered to the circulation compartment of the model to initiate each simulation and continued throughout the simulation. The renal clearance was calculated as the steady state urinary excretion rate (amount of drug excreted into the bladder in a unit time) divided by plasma (circulation compartment) concentration at steady state. For test compounds with more than one published in vitro permeability value, the renal clearance was predicted separately using each of the reported values and the mean predicted renal clearance was calculated as a mean of all individual predictions.

As the expression of microvilli in cell culture systems is highly variable and not accurately defined, with poor knowledge of the role of the unstirred water layer on the cell surface area, a scaling factor of the surface area of the in vitro systems representing the impact of microvilli expression in the in vitro systems was optimized by testing scaling factors of 1.25, 1.5, and 2 with neutral drugs, to define the real intrinsic permeability of the drugs from the in vitro systems. The optimized scaling factor was subsequently used for all renal clearance predictions for neutrals, acids, bases and zwitterions.

Table S1. Physiological parameters used in the model. Tubular radius and length for each tubule are values for single nephron. Volume and surface area of the tubule are reported for total of two kidneys assuming 0.9 million nephrons per kidney. The method to calculate the surface area of the collecting duct was adapted from Scotcher et al 2016. Microvilli adjustment for the apical surface area is 30 of proximal tubule and 1 for all other tubule according to Brown et al 2010. All physiological parameters were calculated from literature values as described in Methods section.

	Proximal Tubule	Loop of Henle	Distal Tubule	Collecting Duct
Tubule radius (mm)	0.03	0.009	0.025	0.1
Tubule length (mm)	18	12	5.5	21
Volume of tubule (L)	0.092	0.0055	0.019	1.19
Volume of each subsegment in the model (L)	0.0305	0.0027	0.0194	0.237
Surface area of tubule (dm^2)	611	122	156	33
Microvilli adjustment (dm^2)	18321	122	156	33
Each subsegment in the model (dm^2)	6107	61	156	6.7

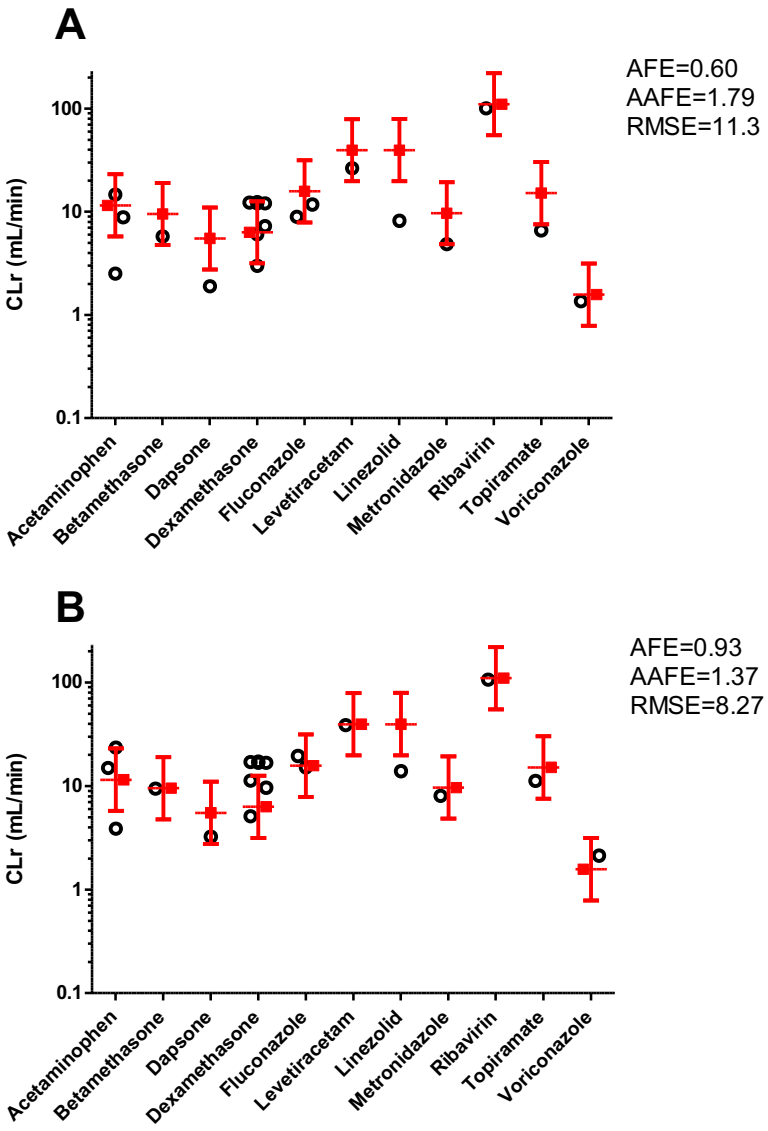

Figure S1. Simulation of renal clearance of 11 neutral test compounds before and after adjustment for microvilli expression level in the in vitro experimental system used to determine permeability values. Red symbols represent the observed renal clearances of the 11 test compounds with 2-fold error range. Black symbols represent the simulated renal clearances using the available different in vitro permeability data. Panel (a) shows the comparison between simulated and observed renal clearance and the overall AFE, AAFE, and RMSE using the intrinsic permeability calculated under the assumption that microvilli expression is completely absent in the experimental system used to determine permeability values. Panel (b) shows the comparison of simulated and observed renal clearance and the overall AFE, AAFE, and RMSE for the same 11 neutral test compounds using the intrinsic permeability calculated under the assumption that microvilli expression in the in vitro system accounts for 1.5 fold higher measured permeability than would be expected from intrinsic permeability in the absence of microvilli.

Table S3. Prediction of in vivo PAH basolateral and apical secretion clearances based on in vitro transporter data (Hotchkiss et al. 2015, Uchino et al 2000, and Smeets 2004) assuming the transporter expression level per mg of in vitro system is equal to the transporter expression level per mg of human kidney and 300 grams of kidney per person.

	Jmax (pmol/min/mg protein)	K_m (μM)	CL (μl/min/mg protein)	CL (L/hr)	Sum (L/hr)
OAT1	1095	22.3	49.1	884	884
NPT1	1880	2660	0.71	12.7	22.3
MRP2	160	5000	0.032	0.58	
MRP4	80	160	0.5	9	

Table S4. Prediction of in vivo cimetidine basolateral and apical secretion clearances based on in vitro transporter data (Burt et al. 2016) using scaling factor of 60 million proximal tubule cells per gram of kidney (Neuhoff et al 2013) and 300 grams of kidney per person. PTC, proximal tubule cells, Sum refers to the combined contribution of OCT2 and OAT3 to the basolateral secretion and the combined contribution of the two MATEs to the apical secretion.

	J_{max} (pmol/min/ million PTC)	K_m (μM)	CL (μl/min/ million PTC)	CL (μl/min/mg kidney)	CL (L/hr)	Sum (L/hr)
OCT2	2170	72.6	29.9	1793	32.28	
OAT3	1232	161.5	7.6	458	8.24	40.5
MATE1	135.5	7.7	17.6	1056	19.01	
MATE2-K	216	18.2	11.9	712	12.82	31.8

Table S5. Setup of the pH gradient for the different subsegments of the model to simulate effects of altered urine pH on renal clearance of memantine. The pH assumed for each subsegment is reported for the four different urine pH values.

Segment	Uncontrolled urine pH	Urine pH 5.1	Urine pH 7.9	Urine pH 8.1
Proximal $tubule_1$	7.2	7.2	7.4	7.4
Proximal $tubule_2$	7.1	7	7.4	7.4
Proximal $tubule_3$	7	6.8	7.4	7.4
Loop of $henle_D$	7	6.8	7.4	7.4
Loop of $henle_A$	7	6.8	7.4	7.4
Distal tubule	6.9	6.4	7.5	7.5
Collecting $duct_1$	6.8	6	7.6	7.6
Collecting $duct_2$	6.7	5.8	7.7	7.7
Collecting $duct_3$	6.6	5.6	7.8	7.8
Collecting $duct_4$	6.5	5.4	7.9	7.9
Collecting $duct_5$	6.5	5.1	7.9	8.1

Table S6. Setup of the pH gradient for the different subsegments of the model to simulate effects of altered urine pH on renal clearance of salicylic acid. The pH assumed for each subsegment is reported for the four different urine pH values.

Segment	Urine pH 5	Urine pH 6	Urine pH 7	Urine pH 8
Proximal $tubule_1$	7.2	7.2	7.2	7.4
Proximal $tubule_2$	7	7.1	7.1	7.4
Proximal $tubule_3$	6.8	6.9	7	7.4
Loop of $henle_D$	6.8	6.9	7	7.4
Loop of $henle_A$	6.8	6.9	7	7.4
Distal tubule	6.4	6.8	7	7.5
Collecting $duct_1$	6	6.7	7	7.6
Collecting $duct_2$	5.8	6.6	7	7.7
Collecting $duct_3$	5.6	6.4	7	7.8
Collecting $duct_4$	5.4	6.2	7	7.9
Collecting $duct_5$	5	6	7	8

Table S7. Simulation of urine pH-dependent renal clearance of memantine. The observed data is shown for five different conditions (uncontrolled urine pH and flow; urine pH of 5.1, urine flow of 0.99 mL/min; urine pH of 5.1, urine flow of 2.72 mL/min; urine pH of 8.1, urine flow of 1.15 mL/min; urine pH of 7.9, urine flow of 2.6 mL/min). The pH dependent renal clearance of memantine was simulated under the same five conditions according to the process described in the Methods sections using in vitro permeability values reported in MDCK cells and Caco-2 cells, and the average permeability value from the two studies.

	Observed CL_r (mL/min)		Simulated CL_r (mL/min)		
Study group	Median (25%-75%)	99% Confidence Interval	MDCK	Caco-2	Average
Uncontrolled	149 (139-183)	64.8 - 233	155	121	136
pH=5.1 Qu=0.99mL/min	210 (183-220)	139 - 281	220	197	208
pH=5.1 Qu=2.72mL/min	219 (194-233)	144 - 294	220	197	208
pH=8.1 Qu=1.15mL/min	19.4 (17.2-24.4)	5.63 - 33.2	12.6	4.88	7.64
pH=7.9 Qu=2.60mL/min	30.5 (26.6-34.4)	15.6 - 45.4	23.2	10.1	15.0