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MODELLING OF UREA-ENFETS FOR HAEMODIALYSIS APPLICATIONS

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Abstract

The detection properties of enzymatic field effect transistors (EnFETs) based on pH-sensitive chemical field effect transistors (pH-ChemFETs) are modelled by taking into account the enzymatic reaction, the diffusion phenomena of the main chemical species in the electrolyte, the related acid/basic chemical reactions and the pH-ISFET potentiometric response. Thus, the pH distribution near the pH sensitive gate is characterized and the sensor detection properties have been studied as a function of the main analysis parameters. The modelling is developed for haemodialysis applications and more especially for the optimisation of urea-sensitive

EnFETs. However, it is fully compatible with other EnFETs developments based on pH-ChemFET-metry.

Keywords: Modelling, EnFETs, haemodialysis, urea detection

1. Introduction

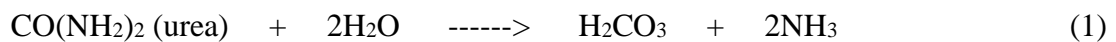
Chronic end-stage kidney failure is a widely spread health disorder and becomes therefore a priority for the development of health-care systems. Thus, dialysis techniques, and haemodialysis in particular, have been in constant development in order to define precisely its efficiency and improve patient life expectancy. In order to do so, two final metabolites of the protein metabolism, urea and creatinine, have to be monitored continuously during haemodialysis treatment. This goal will be achieved by using disposable, low cost and reliable biochemical sensors. Different transduction techniques, based on potentiometry or amperometry, have been developed in parallel but the biochemical detection principle has always been based on the enzymatic production of ammonia due to the hydrolysis of urea and creatinine. Nevertheless, considering only urea detection, two different solutions have been proposed, either by detecting the ammonium ions in solution [1-3], either by monitoring the ammonia influence on the solution pH [4-9]. The first one is a direct measurement method but requires the integration of both urease-based enzymatic and ammonium ionophore-based ionosensitive layers on the same transducers. The second one is an indirect measurement method but is depending of parasitic pH variations and buffer properties of dialysate solutions.

In previous papers, we have reported on the realisation and the characterization of urea-sensitive and creatinine-sensitive enzymatic field effect transistors (EnFETs) based on the

integration of poly vinyl alcohol (PVA) enzymatic layers on pH-sensitive chemical field effect transistors (pH-ChemFETs) [7-8]. Thus, good detection properties have been evidenced on urea or creatinine concentration ranges appropriate to human chronic end-stage kidney failure. However, in order to reach optimisation and reliability in the field of haemodialysis, these EnFETs microsensors have to be modelled. Indeed, this will allow the understanding of their detection and transduction principles as well as the characterisation of the pH-related parasitic influences. Such modelling has been performed for creatinine-EnFETs [10]. It is here improved and extended to urea-EnFETs.

2. Modelling

The studied enzymatic field effect transistor (EnFET) is realised from a SiO₂/Si₃N₄ pH-ISFET adapted to biochemical detection thanks to a poly vinyl alcohol (PVA) enzymatic layer. Indeed, the EnFET sensor operation is based on pH variations related to the enzymatic production of acidic and/or basic chemical products into aqueous solutions. In our case and according to equation (1), the urease enzymatic reaction into the PVA layer is responsible for the urea hydrolysis into aqueous solution, i.e. for the production of ammonia NH₃ and carbonic acid H₂CO₃, and finally for the pH local variation near the pH-ISFET sensitive gate [7].



Thus, the model is based on the solving of mass transport phenomena due to the enzymatic consumption/production phenomena at the sensor surface. By determining the main chemical species concentrations in solution, the spatial and temporal pH distribution is obtained and the sensor response is deduced from the pH value on its sensitive surface [10]. N.F.

Sheppard et al. have already developed such approach for a conductimetric urea biosensor, resolving the mass balance equations within the concentration boundary layer [11]. In our model, by considering the whole analysis channel, it is possible to take into account the flow influence as well as specific phenomena related to the enzymatic substrate exhaustion or to the microfluidic integration.

The EnFET modelling involves the enzymatic reaction, the diffusion phenomena of the main chemical species, i.e. the substrate and the corresponding acidic or basic products in water, the related acid/base reactions in aqueous solution, and finally the SiO₂/Si₃N₄ pH-ChemFET potentiometric response. Starting from the standard description of a haemodialysis fluidic channel where the Si₃N₄/PVA pH-sensitive surface is in direct contact with the electrolyte flow (figure 1), the model has been simplified by considering that the PVA area is infinite in extent. Thus, concentrations vary only according to the x-axis, normal to the sensor surface. Nevertheless, since the influence of the y-axis flow has been taken into account (see hereafter), the whole model has to be considered as pseudo two-dimensional rather than one-dimensional.

A. Modelling of the mass transport phenomena

The modelling is focused on the mass transport phenomena into the channel of the main interfering chemical species, i.e. urea CO(NH₂)₂, ammonia NH₃ and carbonic acid H₂CO₃ (figure 1):

$$\frac{\partial C(x,t)}{\partial t} = D \frac{\partial^2 C(x,t)}{\partial x^2} + \varepsilon g(x,t) + \frac{v(x)}{c} (C_0 - C(x,t)) \quad (2)$$

where C(x,t) is the concentration distribution as a function of the distance normal to the sensor surface x and to the time t.

The equation (2) first term represents the molecule diffusion phenomena where D is the diffusion coefficient into water. Since the PVA enzymatic layer is composed of at least 93% of water [12], this coefficient has been assumed constant whatever the x parameter.

According to Archimede's law, the equivalent volumic mass ρ^* of a "spherical" molecule (equivalent radius r^* , mass m) diluted in a given fluid (volumic mass ρ , viscosity η) is similar to the fluid volumic mass ρ . Then, according to the Einstein's equation, the fluidic diffusion coefficient D of the studied molecules can be estimated as following:

$$D = \frac{kT}{6\pi\eta r^*} = \frac{1}{6\pi\eta} \sqrt[3]{\frac{4\pi\rho^* N_A}{3M}} kT = \frac{1}{6\pi\eta} \sqrt[3]{\frac{4\pi\rho N_A}{3M}} kT = A \frac{kT}{\sqrt[3]{M}} \quad (3)$$

where k is the Boltzman constant, T is the absolute temperature, M is the molar mass of the studied molecule, N_A is the Avogadro's number and A is finally a constant parameter depending only on the fluid characteristics. Finally, the diffusion coefficient into water of the different studied molecules, i.e. urea $\text{CO}(\text{NH}_2)_2$, ammonia NH_3 and carbonic acid H_2CO_3 , have been estimated according to equation (3).

The $\varepsilon g(x,t)$ term represents the enzymatic consumption/production phenomena per time unit. The integer parameter ε is representative of the number of molecules consumed ($\varepsilon < 0$) or produced ($\varepsilon > 0$) according to the studied enzymatic reaction. Furthermore, since this reaction only occurs in the PVA layer (thickness e_{PVA}), the $g(x,t)$ parameter is assumed null elsewhere (figure 1). Finally, according to the Michaelis-Menten equation, $g(x,t)$ is given by:

$$g(x,t) = a_M n_{\text{enz}} \frac{[S](x,t)}{[S](x,t) + K_M} \quad (0 \leq x \leq e_{\text{PVA}}) \quad (4)$$

where a_M is the maximal activity ($a_M = 16.67 \times 10^{-9}$ mol/s for one enzymatic unit), n_{enz} is the enzymatic units number per PVA volume unit, $[S](x,t)$ is the substrate concentration distribution in solution and K_M is the enzyme Michaelis constant.

Last, the equation 2 third term represents the contribution of the electrolyte flow to the concentration variations with time. This phenomenon has been estimated through a pseudo two-dimensional model (figure 2). It is representative of the global dilution phenomena estimated on the characteristic length c of the PVA pattern. Thus, this term has been related to the solution velocity profile $v(x)$ in the channel as well as to the initial and the current concentration of the studied chemical species C_0 and $C(x,t)$. In our case, since the electrolyte (water) is a Newtonian liquid and considering laminar flow conditions into the fluidic channel, the electrolyte velocity profile $v(x)$ has been estimated according to the Poiseuille's law:

$$v(x) = \frac{32f}{\pi d^4} x(d - x) \quad (5)$$

where f is the electrolyte flow in steady-state regime and d is the channel diameter.

In order to solve the mass transport equation using finite element model, the following initial and boundaries conditions have been chosen:

$$(C(x, t))_{t=0} = C_0 \quad (6)$$

$$\left(\frac{\partial C(x,t)}{\partial x}\right)_{x=0} = \left(\frac{\partial C(x,t)}{\partial x}\right)_{x=d} = 0 \quad (7)$$

Thus, initial conditions assume spatially uniform concentrations of all chemical species and boundary conditions assume that no flux goes through the physical barrier related to the sensor surface ($x = 0$) or the channel upper wall ($x = d$).

Finally, by solving the mass transport equations (equation 2) system related to each interfering chemical species, their different concentration distribution in solution $C(x,t)$ have been defined. In the case of the urea-EnFET based on the urease enzymatic reaction (equation 1), this modelling has been applied thoroughly for urea ($\varepsilon = -1$), ammonia ($\varepsilon = +2$) and carbonic acid ($\varepsilon = +1$) in order to determine the following concentration distributions into the channel: $[\text{urea}]_p(x,t)$, $[\text{NH}_3]_p(x,t)$ and $[\text{H}_2\text{CO}_3]_p(x,t)$

B. Modelling of the acid/basic chemical reactions in the electrolyte

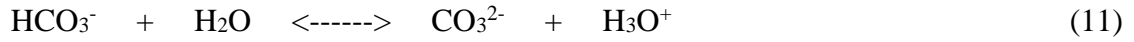
Starting from the different concentration distributions $C(x,t)$ of acidic and basic chemical species consumed or produced by the enzymatic reaction, the electrolyte chemical equilibrium can be studied according to the conservation law, the solution electroneutrality as well as the different acid/base equilibria in aqueous phase:

$$K_a = \frac{[\text{base}](x,t) \cdot [\text{H}_3\text{O}^+](x,t)}{[\text{acid}](x,t)} = \frac{[\text{base}](x,t) \cdot 10^{-\text{pH}(x,t)}}{[\text{acid}](x,t)} \quad (8)$$

where K_a is the acidity constant of the studied acid/base couple.

Since water molecules are obviously in excess compared to the others chemical species ($[\text{H}_2\text{O}] \approx 55,55 \text{ mol.L}$), the water-based chemical system will be assumed to be continuously in equilibrium. Thus, the different acidic/basic phenomena (equations 8) will be considered as instantaneous compared to the mass transport ones (equations 2). Thanks to such assumption, the $[\text{H}_3\text{O}^+](x,t)$ concentration distribution, i.e. the $\text{pH}(x,t)$ function, can be finally determined from the different acid/base concentration distributions $C(x,t)$ by solving the related chemical reactions system.

In the case of the urease hydrolysis (equation 1), the acid/base chemical reactions related to ammonia NH_3 and carbonic acid H_2CO_3 should be taken into account:



Thus, whatever the x and t spatial and temporal parameters, the $[\text{H}_3\text{O}^+]$ ion concentration, i.e. the pH value, can be calculated by studying the standard chemical equations related to the $\text{NH}_4^+/\text{NH}_3$ and the $\text{H}_2\text{CO}_3/\text{HCO}_3^-/\text{CO}_3^{2-}$ acid/base couples in aqueous solutions:

$$K_1 = \frac{[\text{NH}_3][\text{H}_3\text{O}^+]}{[\text{NH}_4^+]} \quad (12)$$

$$K_2 = \frac{[\text{HCO}_3^-][\text{H}_3\text{O}^+]}{[\text{H}_2\text{CO}_3]} \quad (13)$$

$$K_3 = \frac{[\text{CO}_3^{2-}][\text{H}_3\text{O}^+]}{[\text{HCO}_3^-]} \quad (14)$$

$$K_e = [\text{H}_3\text{O}^+][\text{OH}^-] \quad (15)$$

$$[\text{NH}_3] + [\text{NH}_4^+] = [\text{NH}_3]_0 + [\text{NH}_3]_p(x,t) \quad (16)$$

$$[\text{H}_2\text{CO}_3] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}] = [\text{H}_2\text{CO}_3]_0 + [\text{H}_2\text{CO}_3]_p(x,t) \quad (17)$$

$$[\text{H}_3\text{O}^+] + [\text{NH}_4^+] = [\text{OH}^-] + [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + C_t \quad (18)$$

where K_1 , K_2 and K_3 are the acidity constants of the $\text{NH}_4^+/\text{NH}_3$, the $\text{H}_2\text{CO}_3/\text{HCO}_3^-$ and the $\text{HCO}_3^-/\text{CO}_3^{2-}$ acid/base couples respectively ($K_1 = 5,62 \cdot 10^{-10}$, $K_2 = 4,30 \cdot 10^{-7}$, $K_3 = 5,61 \cdot 10^{-11}$), K_e is the water ionic product ($K_e = 10^{-14}$), $[\text{NH}_3]_0$ and $[\text{H}_2\text{CO}_3]_0$ are the initial concentrations of chemical species deviated from ammonia and carbonic acid respectively, $[\text{NH}_3]_p(x,t)$ and $[\text{H}_2\text{CO}_3]_p(x,t)$ are the ammonia and carbonic acid concentration distribution due to the urease

enzymatic reaction (defined previously), and C_t is a constant parameter describing the initial acidic/basic properties of the analysed solution.

This last parameter C_t is representative of all the non-specifically studied ions initially present in solution and interfering with its electroneutrality (equation 18). It can be determined by solving the system of equations (12) to (18) while taking into account specific initial chemical conditions ($t = 0$). For instance, for a water-based solution characterised by a given pH_0 value, i.e. by an initial concentration $[H_3O^+]_0$, and by an initial concentration of chemical species deviated from carbonic acid $[H_2CO_3]_0$, C_t is given by:

$$C_t = [H_3O^+]_0 - \frac{K_e}{[H_3O^+]_0} - [H_2CO_3]_0 \left(\frac{K_2[H_3O^+]_0 + 2K_2K_3}{[H_3O^+]_0^2 + K_2[H_3O^+]_0 + K_2K_3} \right) \quad (19)$$

Thus, in the following and thanks to equation (19), it will be possible to determine the influences of the pH_0 and $[H_2CO_3]_0$ values in standard water-based solutions. Nevertheless, in real haemodialysis case, the dialysate will have to be known in order to define the C_t parameter according to its chemical composition. This will allow to take into account any pH-related phenomenon: pH-related chemical equilibrium, buffer effect,...

C. Modelling of the pH-ISFET response

Finally, the EnFET threshold voltage V_T is related to the pH at the silicon nitride Si_3N_4 surface according to the simplified site-binding model [13,14]:

$$V_T = V_{T0} + s_0[pH(0,+\infty) - pH_{pzc}] \quad (20)$$

where V_{T0} is the threshold voltage of the corresponding field effect transistor, s_0 is the pH-ISFET sensitivity (given theoretically by the Nernst law or estimated experimentally), $pH(0,+\infty)$ is the pH at the silicon nitride gate surface when the diffusion phenomena "steady state" is reached, and pH_{pzc} is the point of zero charge (pH_{pzc} has been estimated around 4 for Si_3N_4 [14,15]).

In the following, since the V_{T0} value is only related to the pH-ChemFET technological fabrication [16], it is of no influence concerning the C-EnFET detection properties and it will not be taken into account, i.e. it will be chosen equal to zero.

3. Results and discussion

In the following, the EnFET model has been studied by considering standard values for the different parameters according to previous experimental developments [7, 10, 12] and to haemodialysis application:

- $T = 300$ Kelvins
- $\epsilon_{PVA} = 1$ micrometer
- $d = 2$ millimetres
- $c = 1$ millimetre
- $s_0 = 59.6$ mV/pH

A. Study of the main chemical species concentrations

Figures 3, 4 and 5 represent respectively typical variations of the urea, ammonia and carbonic acid concentrations $[S](x,t)$, $[NH_3](x,t)$ and $[H_2CO_3](x,t)$ in the analysis channel.

They clearly show the urea enzymatic consumption and diffusion phenomena towards the sensor surface ($x = 0$) as well as the ammonia and carbonic acid enzymatic production and diffusion phenomena from the sensor surface. Nevertheless, the most representative result of the enzymatic reaction is given by the pH variations $\text{pH}(x,t)$ in proximity to the pH-ChemFET sensitive surface (figure 6). Starting from a 7.5 initial pH value, the production of ammonia NH_3 and carbonic acid H_2CO_3 due to the urea hydrolysis (equation 1) is found to be responsible for a global pH increase [7]. The pH wave effect with time should be related to the opposite chemical properties, i.e. basic against acidic, of ammonia and carbonic acid in aqueous solution, and to the difference between their diffusion coefficients D_{NH_3} and $D_{\text{H}_2\text{CO}_3}$.

Then, since the pH is modified on millimetric distances from the sensor surface, the PVA enzymatic layer thickness is insignificant compared to the typical distances related to the pH increase. Therefore, the enzymatic reaction is really a surfacic phenomenon occurring at the sensor sensitive gate ($x \approx 0$).

Finally, a steady-state is reached in less than 1 second, evidencing a pH limit value (figure 7). Thanks to this $\text{pH}(0,+\infty)$ value, the urea-EnFET detection properties can be thoroughly studied (equation 20).

B. Study of the most influential parameters

According to theoretical equations, the most influential parameters are the urease Michaelis constant K_M , the number of urease enzymatic units per volume unit in the PVA layer n_{enz} , the solution initial pH value pH_0 , the solution initial concentration of chemical species deviated from carbonic acid $[\text{H}_2\text{CO}_3]_0$ and the solution flow f . Their influences on the urea-EnFET sensor detection properties have therefore been studied according to the following ranges (main values are given in bold characters):

- [urea] (mol/L): [$10^{-4} - 10^2$] (haemodialysis range [$10^{-3} - 10^{-1}$])
- K_M (mol/L): [$10^{-3} - 10^3$] (**0,1**)
- n_{enz} (unit/cm³): [$10^5 - 10^9$] (**10⁷**)
- pH₀: [5.5 - 10] (**7.5**, standard value in most dialysate solutions...)
- [H₂CO₃]₀(mol/L): [0 - 10^{-1}] (**32 10⁻³**, standard value in most dialysate solutions...)
- f (mL/s): [0 - 10^4] (**0**, standard value in dialysis: 10)

Since the urea-EnFET detection/transduction principle is based on pH-metry, it is necessary to first study the influence of the solution initial pH (figure 8). Whatever the pH₀ value, the urea-EnFET response tends towards a constant value for the highest urea concentrations. This phenomenon should be related to the well-known pH limit (equal to 9.17) characteristic of the urea hydrolysis in aqueous solution [7]. Compared to the creatinine hydrolysis in aqueous solution [10], this is a real drawback since no or low detection properties are obtained for pH₀ values around 9 and non linear variations are evidenced in the other cases. Nevertheless, for other pH values and therefore for neutral initial solutions ($7 \leq \text{pH}_0 \leq 8$) generally used for haemodialysis applications, the urea-EnFET response shows variations on a wide urea concentration range. In the following, the presented results have been obtained for the 7.5 pH₀ value, characteristic of most dialysate solutions.

Figure 9 represents the typical urea-EnFET responses for different urease Michaelis constant K_M . It appears that the urea-EnFET is characterised by no-detection properties for the lowest urea concentrations, by a linear variation on two urea concentration decades (sensitivity around 80 mV/pUrea), and finally by a saturation phenomenon for the highest concentrations. In fact, the urease Michaelis constant K_M is found to have only little influence on the urea-EnFET detection properties. Its increase is only responsible for the shift of the linear detection

range towards higher urea concentrations. In the same way, the enzymatic units number per volume unit in the PVA layer n_{enz} has similar influences (figure 10). Its decrease is responsible for the shift of the linear detection range towards higher urea concentrations, and finally (and logically) for the detection sensitivity decrease. Thus, these two parameters K_M and n_{enz} will be crucial for defining precisely the urea-EnFET detection range according to the haemodialysis application.

Since the carbonic acid is a direct product of the urea hydrolysis (equation 1), it is very interesting to study the influence of the solution initial concentration of chemical species deviated from carbonic acid $[H_2CO_3]_0$ on the urea-EnFET response (figure 11). The $[H_2CO_3]_0$ increase is found to be responsible for the shift of the linear detection range towards higher urea concentrations. Nevertheless, it is very interesting to study the variations of the urea detection sensitivity (estimated on the linear detection range) with the $[H_2CO_3]_0$ parameter. Starting from a 35 mV/pUrea for the lowest values, the urea detection sensitivity increases to reach a maximal sensitivity value (80 mV/pUrea, see below) on a wide range of intermediary concentrations. Such phenomenon is related to specific acid/base equilibriums of the NH_4^+/NH_3 and $H_2CO_3/HCO_3^-/CO_3^{2-}$ chemical system. Thus, low urea concentration variations are amplified, leading to high pH variations near the sensor surface and finally to high urea detection sensitivities (in agreement with the Nernst law). Finally, for the highest $[H_2CO_3]_0$ values, the acidic properties of carbonic acid tend to limit the global basic influences of the urea hydrolysis and the urea detection sensitivity decreases towards zero mV/pUrea.

Lastly, the influence of the solution flow on the urea-EnFET detection properties has been studied (figure 12). The flow increase is found to be responsible for a global decrease of the urea detection sensitivity. Since this decrease is not obtained when the initial concentration of chemical species deviated from carbonic acid $[H_2CO_3]_0$ is equal to zero, it is related to the variations of the urea detection sensitivity previously evidenced (see below). Indeed, for

intermediary $[\text{H}_2\text{CO}_3]_0$ concentrations, the flow increase is responsible for an increasing contribution of carbonic acid deviated chemical species at the sensor surface, and therefore for the urea detection sensitivity decrease towards zero mV/pUrea.

4. Conclusion

The urea-EnFET detection principles based on pH-ChemFET-metry has been modelled by taking into account the enzymatic reaction, the diffusion phenomena of the main chemical species in the electrolyte, the related acid/basic chemical reactions into aqueous solution, and finally the detection properties on the pH-ISFET sensitive gate. Thus, it has been possible to characterize the concentration variations of the main interfering chemical species near the sensor surface, and to define the most influential parameters on the urea-EnFET detection properties.

The modelling results demonstrate that pH-based urea-EnFETs can be used in the field of haemodialysis. Indeed, for neutral ($\text{pH}_0 \approx 7.5$) dialysate solutions, detection ranges are evidenced on two urea concentration decades. Obviously, the detection properties depend on many physical, chemical, biochemical and technological parameters: the urease Michaelis constant K_M , the urease units number per volume unit in the PVA layer n_{enz} and/or the solution flow f . Nevertheless, they can be finally defined and controlled in order to be compatible with the [5 – 50 mmol/L] urea concentration ranges appropriate to human chronic end-stage kidney failure.

The modelling has still to be improved by taking into account the buffer properties of the analysed solutions thanks to the C_t parameter. For haemodialysis applications, this requires a complete knowledge of the dialysate chemical composition used. According to the modelling results related to the influence of the initial concentration of chemical species deviated from

carbonic acid $[H_2CO_3]_0$, such study will not reconsider the use of urea-EnFETs but will require to take into account the interferences of specific acid/base equilibriums. Nevertheless, the model enables a real understanding of the EnFETs detection principle based on pH-ChemFET-metry. It opens solutions for improving EnFETs sensor in terms of performances by fitting the detection range specifically with the application, of reliability by taking into account biochemical aspects related to the enzyme processing, storage or ageing, and of cost by decreasing the necessary amount of often expensive enzymes to reach the best detection properties. It has been developed for the detection of urea in the field of haemodialysis, but is also fully compatible with other pH-related enzymatic transducers.

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Biographies

Pierre Temple-Boyer was born on October 25, 1966. He received his Engineer Master's Degree in electronic engineering from the Ecole Supérieure d'Electricité (Paris – France) in 1990 and his Master's Degree in microelectronics from the Université Paul Sabatier de Toulouse (France) in 1992. He joined the Laboratoire d'Architecture et d'Analyse des Systèmes of the French Centre National de la Recherche Scientifique (LAAS-CNRS) in 1992 and received the PhD degree from the Institut National des Sciences Appliquées de Toulouse (France) in 1995. Since then, as a senior researcher, he has been working on the development of micro- and nanotechnologies.

Ahmed Benyahia was born on November 3, 1980. He received her Master's Degree in microelectronics from the Université Paul Sabatier de Toulouse (France) in 2006. He joined the Laboratoire d'Architecture et d'Analyse des Systèmes of the French Centre National de la Recherche Scientifique (LAAS-CNRS) in 2006. He is working on the development of ChemFETs micro sensors for chemical and biochemical detection.

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FIGURE CAPTIONS

Figure 1: description of the EnFET-based detection structure

Figure 2: pseudo-2D description of the flow influence in the channel

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Figure 9: urea-EnFET response with urease Michaelis constant K_M

Figure 10: urea-EnFET responses with number of enzymatic units per volume unit n_{enz}

Figure 11: urea-EnFET responses with initial concentration $[\text{H}_2\text{CO}_3]_0$

Figure 12: urea-EnFET responses with solution flow f

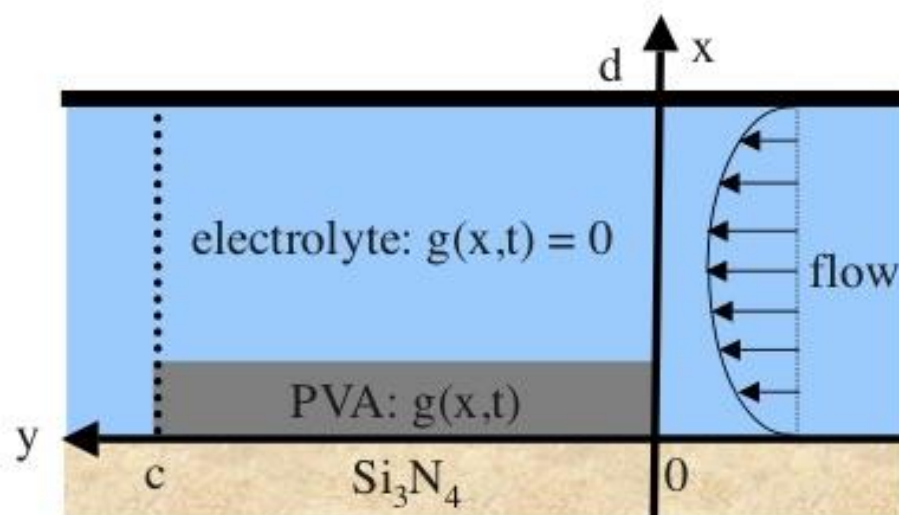


Figure 1: description of the EnFET-based detection structure

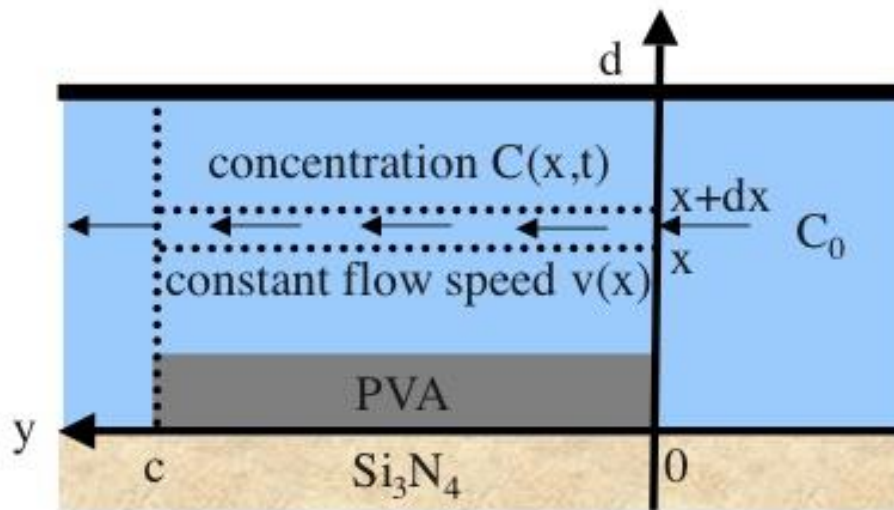


Figure 2: pseudo-2D description of the flow influence in the channel

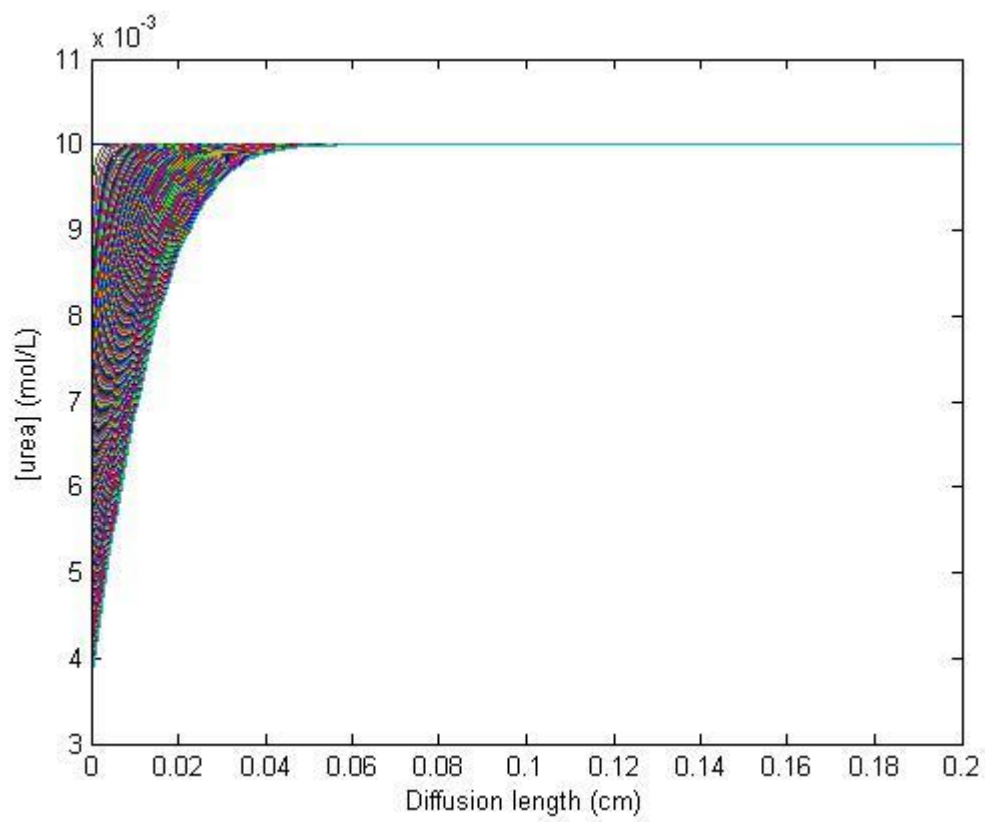


Figure 3: urea concentration [S](x,t) into the analysis channel

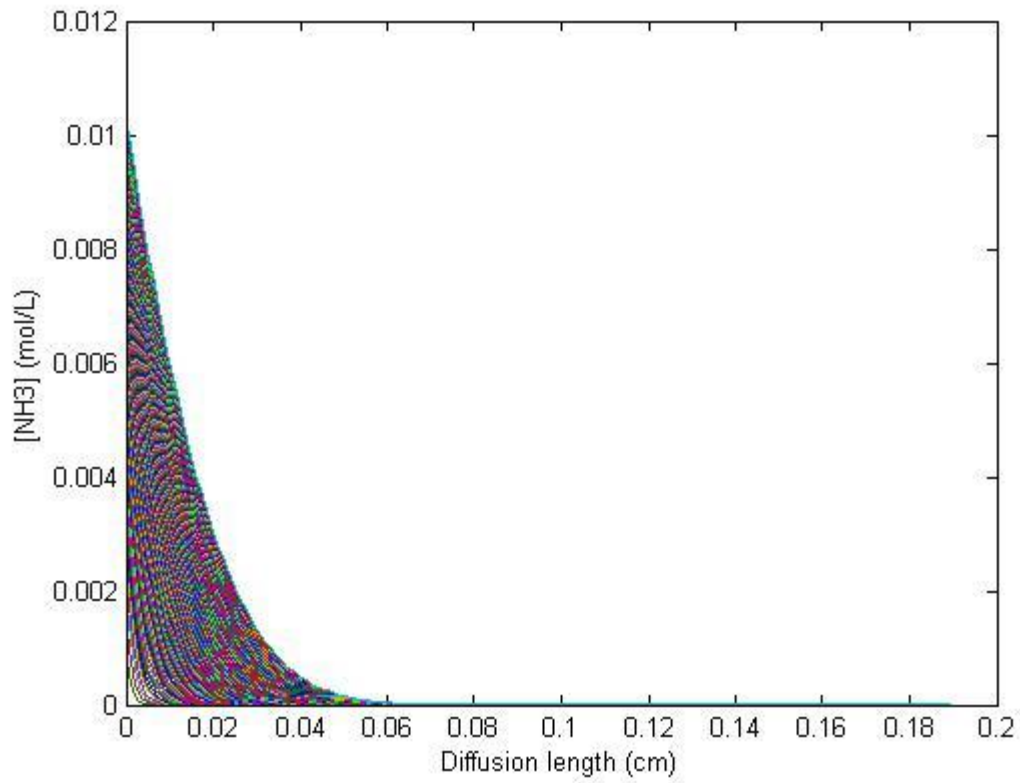


Figure 4: ammonia concentration $[NH_3](x,t)$ into the analysis channel

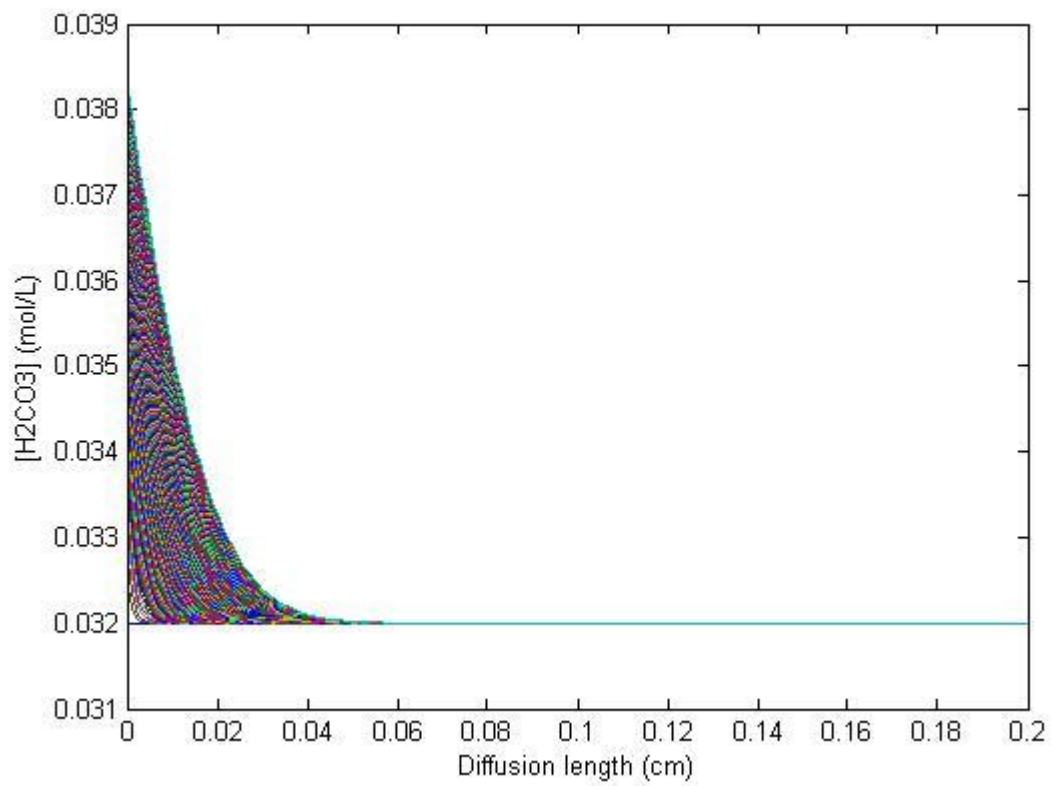


Figure 5: carbonic acid concentration $[H_2CO_3](x,t)$ into the analysis channel

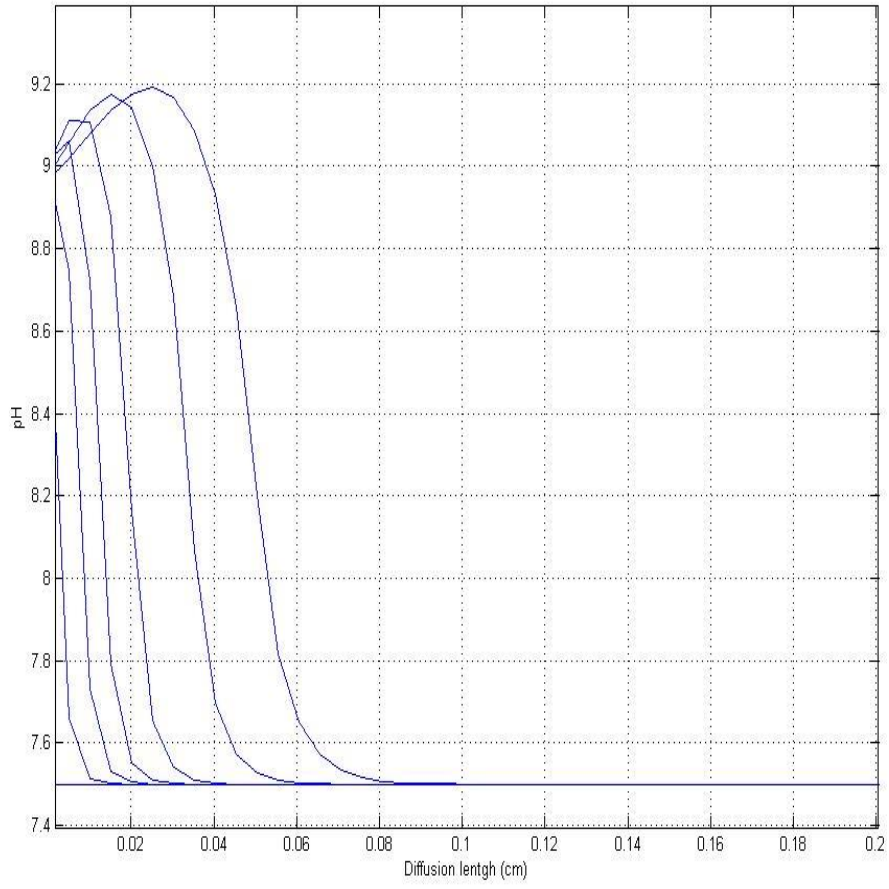


Figure 6: pH(x,t) function into the analysis channel

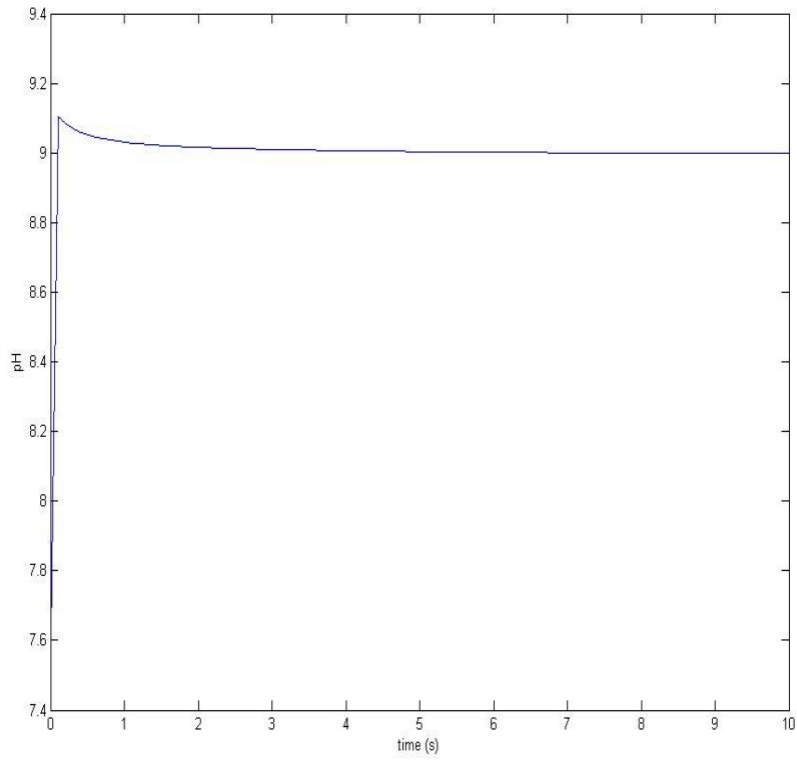


Figure 7: pH temporal variations at the pH-ISFET sensitive surface ($x = 0$)

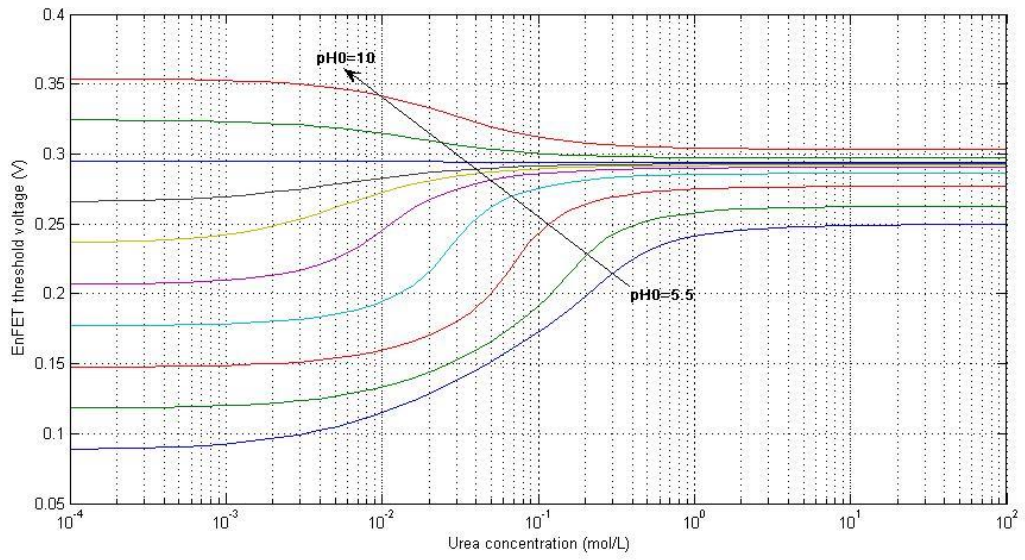


Figure 8: urea-EnFET responses with solution initial pH value pH_0

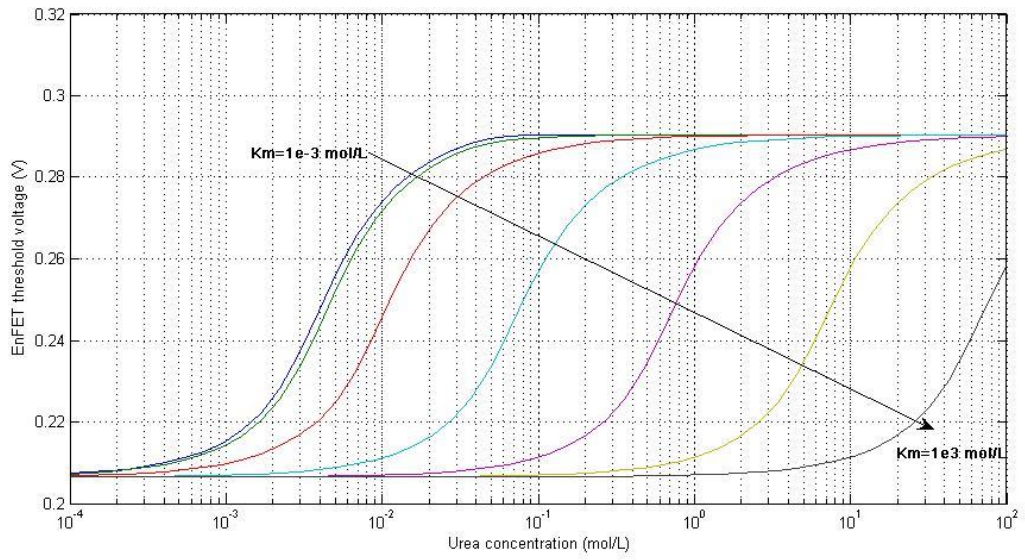


Figure 9: urea-EnFET response with urease Michaelis constant K_M

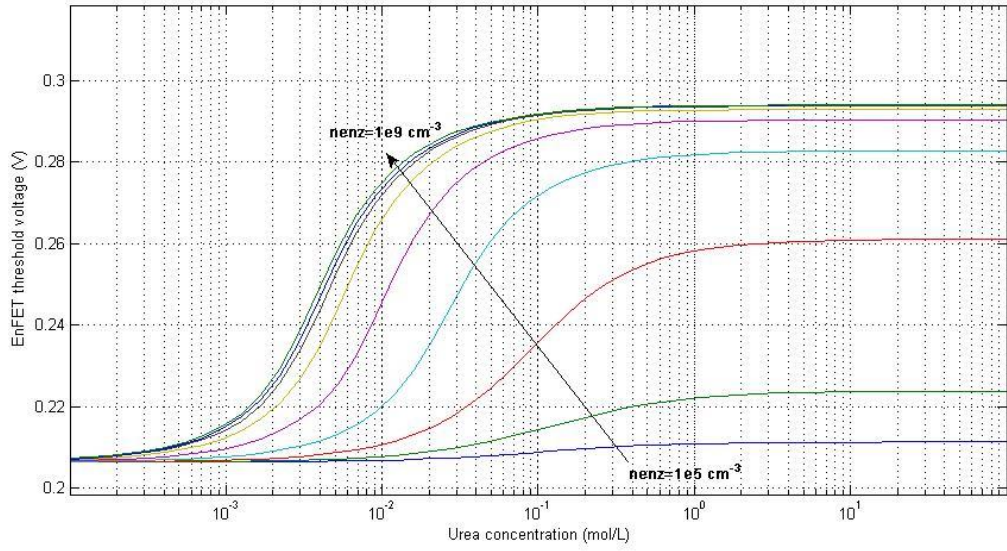


Figure 10: urea-EnFET responses with number of enzymatic units per volume unit n_{enz}

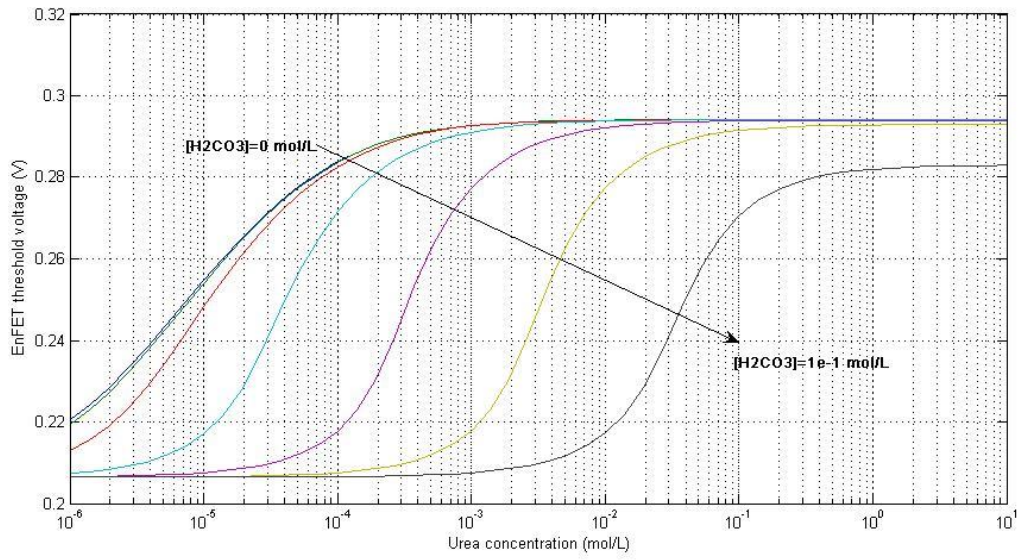


Figure 11: urea-EnFET responses with initial concentration $[H_2CO_3]_0$

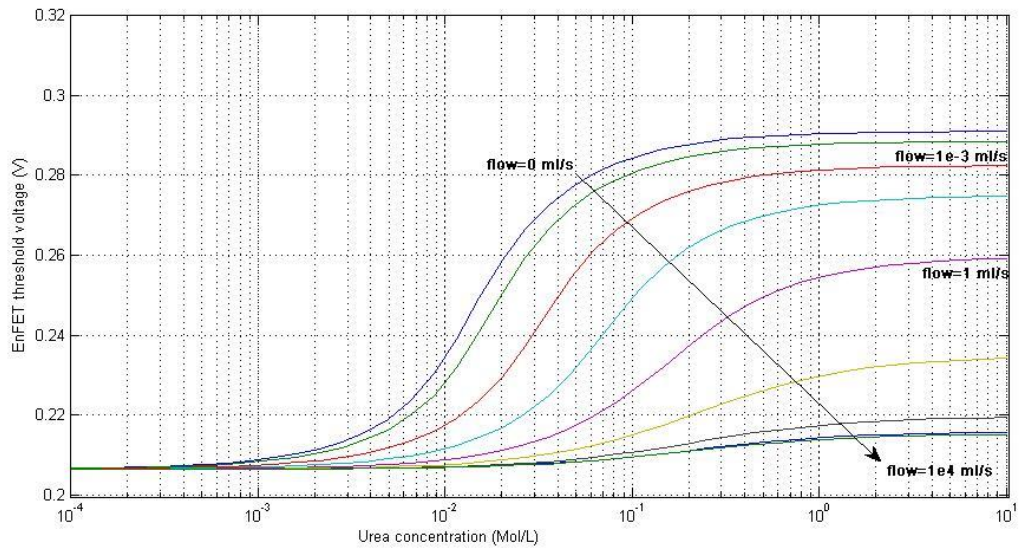


Figure 12: urea-EnFET responses with solution flow f