Atypical Diffusion Monitored by Flow Cytometry: Slow Diffusion of Small Molecules in Polyelectrolyte Multilayers

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1) Derivation of the relative fluorescence: the case of classical diffusion.

In the classical case, the system of differential equations that approximately describes the quenching process of dithionite with labels in polyelectrolyte film will be written in the following form:

$$\frac{\partial c_Q(x,t)}{\partial t} = D\frac{\partial^2 c_Q(x,t)}{\partial x^2}$$	(1)
$$\frac{\partial c_L(x,t)}{\partial t} = -kc_L(x,t)c_Q(x,t)$$	(2)

Where D is diffusion coefficient of quenchers in polyelectrolyte film, k is the binding rate constant of quenchers with labels. In order to solve the system of equations (1) - (2), we need to set the initial and boundary conditions. Since at the initial moment of time there were no quenchers in the film and the concentration of labels has its maximal value c_{L0}, then the initial conditions will be as follows:

$c_Q(x,0) = 0$	(3)
$c_L(x,0) = c_{L0}$	(4)

At the interface with the bulk solution, the concentration of quenchers is constant and equals to c_Q^{Eq}, and the substrate is impermeable for quenchers. Therefore, boundary conditions can be written as follows:

$$\left(\frac{\partial c_Q(x,t)}{\partial x}\right)_{x=l} = 0 \qquad c_Q(0,t) = c_Q^{Eq} \tag{5}$$

The equation (1) can be solved using variables separation method and the change of the quenchers' concentration with time will be as follows:

$$c_Q(x,t) = c_Q^{Eq}\left(1 - \frac{4}{\pi}\sum_{n=0}^{\infty}\frac{\sin\left(\frac{(2n+1)\pi x}{2l}\right)}{(2n+1)}\exp\left(-\frac{(2n+1)^2\pi^2 D}{4l^2}t\right)\right) \tag{6}$$

The solutions of equation (2) will be as follows:

$$c_L(x,t) = c_{L0}\exp\left(-\int_0^t kc_Q(x,s)ds\right) \tag{7}$$

If k do not depend on time, then by substituting (6) in (7) we get the final expression for concentration of quenchers:

$$c_L(x,t) = c_{L0}\exp\left(-kc_Q^{Eq}\left(t - \frac{l^2}{D}\frac{16}{\pi^3}\sum_{n=0}^{\infty}\frac{\sin\left(\frac{(2n+1)\pi x}{2l}\right)}{(2n+1)^3}\left(1-\exp\left(-\frac{(2n+1)^2\pi^2 D}{4l^2}t\right)\right)\right)\right) \tag{7a}$$

The kinetics of quenching of the label is determined by the following formula

$$\frac{F(t)}{F(0)} = \frac{\int_0^l c_L(x,t)\,\mathrm{dx}}{\int_0^l c_L(x,0)\,\mathrm{dx}} \tag{8}$$

Substituting the expression for concentration of the quencher (7a) in (8) we obtain the following final expression for the kinetics of quenching:

$$\frac{F(\tau)}{F(0)}=\int_0^1 \exp\left(-\tilde{k}\left(\tau-\frac{16}{\pi^3}\sum_{n=0}^{\infty}\frac{\sin\left(\frac{(2n+1)\pi}{2}\xi\right)}{(2n+1)^3}\left(1-\exp\left(-\frac{(2n+1)^2\pi^2}{4}\tau\right)\right)\right)\right)\mathrm{d}\xi$$

$$\tilde{k}=4\pi l^2 R_c c_Q^{Eq} \qquad \tau=\frac{D}{l^2}t, \qquad \xi=\frac{x}{l} \qquad (9)$$

where l is polyelectrolyte film thickness, R_c is effective reaction radius of labels.

For the numerical calculation of $\tilde{k}$ it is necessary to multiple the right part of the expression with Avogadro's number and divide to 1000, since during derivation of the formula, we considered collision of species in 1 cm^3 volume.

For the analysis of experimental data, we rewrite (9) in the following form:

$$\frac{F(\mathrm{t})}{F(0)}=$$

$$\int_0^1 \exp\left(-\tilde{k}\left(\frac{t}{t_1}-\frac{16}{\pi^3}\sum_{n=0}^{\infty}\frac{\sin\left(\frac{(2n+1)\pi\xi}{2}\right)}{(2n+1)^3}\left(1-\exp\left(-\frac{(2n+1)^2\pi^2}{4}\frac{t}{t_1}\right)\right)\right)\right)d\xi$$

$$\tilde{k}=4\pi l^2 R_c c_Q^{Eq}, \qquad t_1=l^2/D, \qquad \xi=\frac{x}{l} \qquad (10)$$

2) **Derivation of the relative fluorescence: the case of atypical diffusion.**

In the case of atypical equation the diffusion equation will be written in the following form:

$$\frac{\partial c_Q(x,t)}{\partial t}=D(t)\frac{\partial^2 c_Q(x,t)}{\partial x^2} \qquad (1)$$

To solve the equation (1) the following change of variable t will be carried out:

$$\theta=\int_0^t D(s)ds \qquad (2)$$

With considering of (2), the equation (1) will be rewritten in the following form:

$$\frac{\partial c_Q(x,\theta)}{\partial \theta} = \frac{\partial^2 c_Q(x,\theta)}{\partial x^2} \qquad (3)$$

The change of variable (2) do not lead to a change of initial and boundary conditions and they stay the same as for system (3) – (4) as in a) . The solution of this case is similar to the classical case and it equals:

$$c_Q(\xi,\tau) = c_Q^{Eq}\left(1 - \frac{4}{\pi}\sum_{n=0}^{\infty}\frac{\sin\left(\frac{(2n+1)\pi\xi}{2}\right)}{(2n+1)}\exp\left(-\frac{(2n+1)^2\pi^2}{4}\tau\right)\right)$$
$$\xi = \frac{x}{l}, \qquad \tau = \frac{\theta}{l^2} \qquad (4)$$

Most often, the dependence of the diffusion coefficient on a time is given in the following form:

$$D(t) = \frac{D_0}{t^{1-\alpha}} \qquad (5)$$

This leads to the following expression for τ

$$\tau = \frac{\theta}{l^2} = \frac{1}{l^2}\int_0^t \frac{D_0}{s^{1-\alpha}}ds = \frac{D_0}{l^2}\frac{t^\alpha}{\alpha} \qquad (6)$$

Let us note that in the formula (5) and (6) dimension of D_0 does not coincide with common dimension cm²/s. By substituting (6) in (4) we get the following expression for the quenchers' concentrations in the dimension form:

$$c_Q(x,t) = c_Q^{Eq}\left(1 - \frac{4}{\pi}\sum_{n=0}^{\infty}\frac{\sin\left(\frac{(2n+1)\pi x}{2l}\right)}{(2n+1)}\exp\left(-\frac{(2n+1)^2\pi^2 D_0}{4l^2\alpha}t^\alpha\right)\right) \qquad (7)$$

The change of concentration of labels will be described by the same formula as in classical case in a) - (2) and it's solution will be given by the expression Appendix1 - (7). By substituting (16) in Appendix1 - (7), we get dependence of quenchers' concentration on time:

$$c_L(x,t) = c_{L0}\exp\left(-c_Q^{Eq}\int_0^t k\left(1 - \frac{4}{\pi}\sum_{n=0}^{\infty}\frac{\sin\left(\frac{(2n+1)\pi x}{2l}\right)}{(2n+1)}\exp\left(-\frac{(2n+1)^2\pi^2 D_0 s^\alpha}{4l^2\alpha}\right)ds\right)\right) \qquad (8)$$

Then, substituting in a) - (8) the expression for quenchers' concentration from (8) we get the following final expression for quenching kinetics:

$$\frac{F(\tau)}{F(0)}=\int_0^1 \exp\left(-\tilde{k}\left(\tau^\alpha-\frac{16}{\pi^3}\sum_{n=0}^{\infty}\frac{\sin\left(\frac{(2n+1)\pi\xi}{2}\right)}{(2n+1)^3}\left(1-\exp\left(-\frac{(2n+1)^2\pi^2}{4}\tau^\alpha\right)\right)\right)\right)d\xi$$

$$\tilde{k}=4\pi l^2 R_c c_Q^{Eq},\qquad \tau=t/t_1,\qquad t_1=\left(l^2\alpha/D_0\right)^{\frac{1}{\alpha}}. \tag{9}$$

For the analysis of experimental data, we rewrite (9) in the following form:

$$\frac{F(\mathrm{t})}{F(0)}=\int_0^1 \exp\left(-\tilde{k}\left(\left(\frac{t}{t_1}\right)^\alpha-\frac{16}{\pi^3}\sum_{n=0}^{\infty}\frac{\sin\left(\frac{(2n+1)\pi\xi}{2}\right)}{(2n+1)^3}\left(1-\exp\left(-\frac{(2n+1)^2\pi^2}{4}\left(\frac{t}{t_1}\right)^\alpha\right)\right)\right)\right)d\xi$$

$$\tilde{k}=4\pi l^2 R_c c_Q^{Eq},\qquad t_1=\left(l^2\alpha/D_0\right)^{\frac{1}{\alpha}}. \tag{10}$$

From (5) and (10) follows that in case of α = 1, as it was expected, the formula (10) identically transforms to analogue formula (10) for classical diffusion.