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Kinetics of the Liposome Adhesion on a Mercury Electrode: Testing of a Mathematical Model

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Liposome adhesion at charged mercury interface is measured by appearance of adhesion signals so-called current transient. The proposed methodology based on new developed mathematical model of consecutive multi-step processes will show how to access kinetic parameters of adhesion process: docking, opening and spreading time constants, respectively. The model was tested using reported current and charge transients of liposome. Model shows agreement with literature value in determination of spreading time constants either by computation from charge or current transients. Contrary, determination of opening time constants shows agreement with computation from current transients only. The fast docking time constant become resolved (at time below the opening time constant), from current transient data and the model of the three-step process. These demonstrate that current and charge transients should be combined in determination of kinetic parameters of adhesion process.

Keywords: amperometry at mercury electrode, current transient, liposome adhesion, kinetic of single adhesion event, mathematical model of multi-step processes

1. INTRODUCTION

The significance of adhesion phenomena in single particle-electrode interaction became apparent since the discovery of adhesion signals of vesicles in seawater samples [1]. Individual soft microparticles such as oil droplets, living cells and liposomes in aqueous media are characterized by their adhesion signals using amperometry at the mercury electrode introduced by Žutić and coworkers [2-8]. Mercury electrode as a substrate for adhesion studies allows controlled variation of own surface properties [9] by changing applied potential. Adhesion signal so-called current transient appears due to the double-layer charge displacement from the inner Helmholtz plane caused by liposome adhesion and spreading at the charged mercury interface [2]. Adhesion signal traces transformation kinetics of a single liposome to a film of a finite surface area in milisecond time scale. Adhesion signals of liposomes were also studied by Scholz and coworkers [10] with the purpose to extracts kinetic parameters of liposome-electrode interaction [11] and characterize effects of a pore-forming polypeptide [12,13]. They reported kinetic model of liposome adhesion on mercury electrode where integrated current transient was modeled with the following empirical equation:

$$Q(t) = Q_0 + Q_1[1 - \exp(-t/\tau_1)] + Q_2[1 - \exp(-t/\tau_2)] \quad (1)$$

$Q(t)$ is the displaced charge at selected time while fitting parameters Q_0, Q_1, Q_2 correspond to docking, opening and spreading components of the liposome adhesion process, respectively. Parameters τ_1 and τ_2 correspond to the opening and spreading time constants, respectively. Opening time constant represents fast component while spreading time constant corresponds to slow component of adhesion process. A docking time constant is understood as being too fast to be resolved in time [11,12]. Validity of kinetic model of liposome adhesion and interpretation of current transients on a molecular level has been debated [6,14].

In this work we tested a new developed mathematical model of consecutive multi-step processes of the first order [15] using reported current and charge transients of DMPC liposome [11]. We will present methodology based on mathematical model in order to obtain kinetic parameters of adhesion process through the analysis of current and charge transients. This methodology is based on the application of semilog plots, where individual exponential functions of time (which are the main parts of equations derived from kinetic models) could be sequentially separated.

2. THEORY

According to our new developed mathematical model, two consecutive processes, irreversible, reversible or mixed, will always produce following type of kinetic equation:

$$Q(t) = Q_2[1 - \exp(-t/\tau_2)] - Q_1[1 - \exp(-t/\tau_1)] \quad (2)$$

Total displaced charge could be derived at the time of adhesion signal completion ($t \to \infty$) in the following way:

$$Q_\infty = Q_2 - Q_1 \quad (3)$$

It is necessary to rearrange the Eqn. 2 in order to obtain only exponential functions of time using difference between the total charge and the displaced charge at selected times. These functions could be sequentially separated if they are presented in the corresponding semilog plot at different time scales:

$$\ln[Q_\infty - Q(t)] = \ln[Q_2 \exp(-t/\tau_2) - Q_1 \exp(-t/\tau_1)] \tag{4}$$

Further, current transient equation can be obtained by differentiation of the Eqn. 2:

$$dQ(t)/dt = (Q_2/\tau_2)\exp(-t/\tau_2) - (Q_1/\tau_1)\exp(-t/\tau_1) \tag{5}$$

Now, Eqn. 5 can be presented in the corresponding semilog plot:

$$\ln(dQ/dt) = \ln[(Q_2/\tau_2)\exp(-t/\tau_2) - (Q_1/\tau_1)\exp(-t/\tau_1)] \tag{6}$$

3. RESULTS AND DISCUSSION

Reinterpretation of charge-time data of DMPC liposome [11] based on Eqn. 4 is shown on Fig. 1. It is evident that at $t>2\tau_1$, the exponential function of time is controlled mainly by τ_2. Consequently, logarithm of time function has the form of a straight-line (a) where τ_2 value determined from the slope is 0.55 ms. In addition, intercept of the extrapolated straight-line (a) with ordinate produces the corresponding extrapolated charge value (Q_2=4.95×10^{-10} C). Subtracted data shown with symbol (o) correspond to difference between extrapolated straight-line (a) and displaced charge values (thick line) at $t<2\tau_1$. Intercept of the extrapolated straight-line (b) with ordinate produces the corresponding extrapolated charge value (Q_1=2.01×10^{-10} C). τ_1 value obtained from the slope of straight-line (b) is 0.23 ms. Extrapolated charge values Q_1 and Q_2 satisfy the Eqn. 3 and τ_1 value is in agreement with the continuity relation of the model of a simple two-step process:

$$Q_1/\tau_1 = Q_2/\tau_2 = Q_\infty/(\tau_2 - \tau_1) \tag{7}$$

It is interesting to note, that τ_1 value depends on charge or current-time data. τ_1 value determined from the slope of straight-line (c) equals 0.17 ms (Fig.1).

We interpreted current-time data of DMPC liposome [11] using Eqn. 6, as shown on Fig. 2. According to the same methodology, at $t>2\tau_1$ the exponential function of time is controlled mainly by τ_2 which is the same as determined from charge transient (Fig.1). In addition, the intercept of the corresponding extrapolated straight-line (a) with ordinate produces the corresponding extrapolated current value (i_2=9.09×10^{-7} A). Values of extrapolated current, i_2 and charge, Q_2 controlled by τ_2 satisfy the Eqn.:

$$i_2 = (dQ/dt)_2 = Q_2/\tau_2 \tag{8}$$

Subtracted data shown with symbol (o) correspond to difference between extrapolated straight line (a) and experimental current-time values (thick curve) at $t<2\tau_1$. Intercept of the extrapolated straight-line (b) with ordinate produces the corresponding extrapolated current value (i_1=12.90×10^{-7}

A). τ_1 value obtained from the slope of straight-line (b) is 0.17 ms. Values of extrapolated current, i_1 and charge, Q_1 controlled by τ_1 satisfy the Eqn.:

$$i_1 = (dQ/dt)_1 = Q_1/\tau_1 \qquad (9)$$

Subtracted data deviates at t<0.2 ms from the extrapolated straight-line (b) with a tendency to approach to the i_2 value. This is expected since extrapolated current values i_1 and i_2 are not the same and initial value of the experimental current is zero. Therefore, τ_1 value does not satisfy the Eqn. 7.

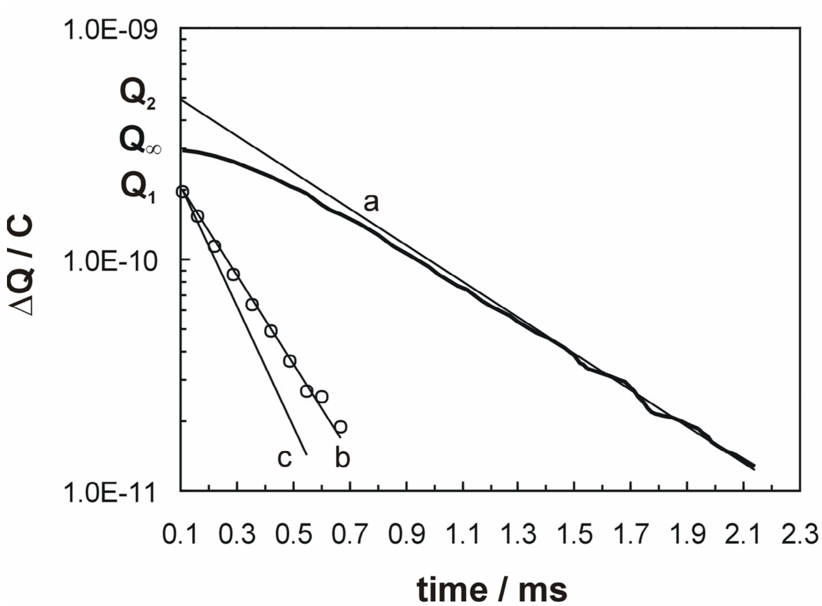

Figure 1. Time dependence of charge difference, $\Delta Q = Q_\infty - Q(t)$. Thick curve is obtained from charge-time data of DMPC liposome (taken from Figure 11b in [11]); extrapolated straight-line (a) is associated with spreading step; symbol (o) presents discrete values as a difference between the extrapolated straight-line (a) and charge-time data; extrapolated straight-line (b) is associated with opening step; straight-line (c) is associated with opening step based on current-time data calculation. Q_∞ is difference between extrapolated charge values Q_2 and Q_1 (Eqn. 3).

In order to interpret the observed deviation, we presented corresponding difference (Δ) between extrapolated straight-line (b) and current data (o) in Fig. 2. According to developed methodology, τ_0 value determined from the slope of straight-line (c) is 0.05 ms. Intercept of the extrapolated straight-line (c) with ordinate produces the corresponding extrapolated current value ($i_0 = 3.81 \times 10^{-7}$ A).

We suggest the use of both charge-time and current-time data in interpretation of adhesion kinetics of organic particles with electrode interfaces. The charge transient data at t>2τ_1 very slowly converge to its maximum value. This requires appropriate adjustment in selection of this maximum charge value, so that charge transient at t>2τ_1 produce a straight-line in the corresponding semilog plot (Fig. 1). However, integration procedure of current transient might cause decrease of precision in determination of component which occur at $t<2\tau_1$. Contrary, experimental current transient at $t>2\tau_1$ becomes less precise due to the relatively large data dispersion. However, experimental current transient at $t<2\tau_1$ seems to provide detailed information of faster component of adhesion process.

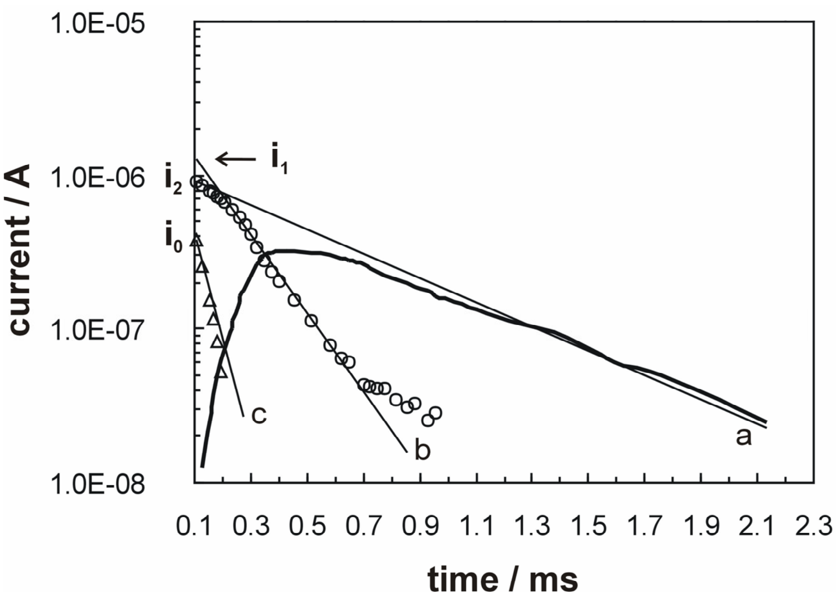

Figure 2. Current-time data of DMPC liposome (taken from Figure 11a in [11]) presented in semilog plot. Thick curve corresponds to reported experimental values; extrapolated straight-line (a) is associated with spreading step; symbol (o) presents the difference between the extrapolated straight-line (a) and experimental data; extrapolated straight-line (b) is associated with opening step; symbol (Δ) is a difference between the extrapolated straight-line (b) and current data denoted as (o); extrapolated straight-line (c) is associated with docking step. Extrapolated current values, i_2, i_1 and i_0 are determined from intercept of extrapolated straight-lines (a), (b) and (c) with ordinate, respectively.

The simple model of two-step process cannot be used for the interpretation of complete set of experimental data. There are several evidences to support this statement: (i) $i_1 \neq i_2$; (ii) different τ_1 values obtained from charge and current-time data and (iii) deviation of subtracted current data (o) from extrapolated straight-line (b). These evidences might suggest existence of an additional component of adhesion process known as docking which did not extinct yet at the t<0.2 ms. Accordingly, mathematical model of consecutive three-step process is described by:

$$Q(t) = Q_2[1 - \exp(-t/\tau_2)] - Q_1[1 - \exp(-t/\tau_1)] + Q_0[1 - \exp(-t/\tau_0)] \tag{10}$$

and

$$dQ(t)/dt = (Q_2/\tau_2)\exp(-t/\tau_2) - (Q_1/\tau_1)\exp(-t/\tau_1) + (Q_0/\tau_0)\exp(-t/\tau_0) \tag{11}$$

Synthetic charge and current-time data were generated for two and three-step processes using similar time constants according to Eqns. 10 and 11 from our concurrently published model [15]. We confirmed existence of the third, very fast docking component according to the following evidences: (i) $i_1 \neq i_2$; (ii) τ_1 value does not satisfy continuity Eqn. 7 and (iii) deviation of subtracted current data from corresponding extrapolated straight-line at t<0.2 ms.

Kinetic parameters of adhesion determined from charge and current-time data sets and calculated values according to mathematical model of multi-step processes are summarized in Table 1. Determined values of τ_2 and Q_2 are in good agreement with reported data [11]. Determined values of Q_1 and i_2 agree well with calculated data using Eqns. 7 and 8. Determined value of τ_1 obtained from charge-time data is in good agreement with the calculated one, according to Eqn. 7. Determined value of τ_1 obtained from current-time data agrees well with reported one [11].

Table 1. Comparison of determined and calculated values of kinetic parameters of liposome adhesion (Q / 10^{-10} C, i / 10^{-7} A, τ / ms). Calculated values were obtained according Eqns. 7, 8, 9 and 11 derived from the mathematical model of consecutive multi-step adhesion process. Total displaced charge, Q_∞ is 2.94×10^{-10} C.

Parameters	Determined values		Calculated values
	Charge-time	Current-time	
Q_2	4.95	-	-
Q_1	2.01	-	2.01
Q_0	-	-	0.19
τ_2	0.55	0.55	-
τ_1	0.23	0.17	0.22
τ_0	-	0.05	-
i_2	-	9.09	9.00
i_1	-	12.90	11.82
i_0	-	3.81	-

4. CONCLUSIONS

Mathematical model of consecutive multi-step processes of the first order was tested on the case study. Presented methodology based on mathematical model shows how to access kinetic parameters of adhesion process through analysis of well-defined and time resolved current transients. It was illustrated need to use both current and charge transients in order to increase precision of data analysis. The main advantage of our methodology is application of semilog plots in order to sequentially separate individual exponential functions of time. Following findings are major: (i) the model of two-step process cannot be used for the interpretation of complete set of experimental data and (ii) the fast docking time constant became resolved at t<0.2 ms through analysis of current transient and a model of three-step process.

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