Biophysical Chemistry 151 (2010) 105–110

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Modelling of multi-component immunoassay kinetics — A new node-based method for simulation of complex assays

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ARTICLE INFO

Article history:
Received 22 April 2010
Received in revised form 18 May 2010
Accepted 27 May 2010
Available online 2 June 2010

Keywords:
Reaction kinetics
Immunoassay
Simulation
Mechanistic reaction model

ABSTRACT

The behaviour of binding reactions in immunoassays can be predicted and studied by modelling methods. Simple antibody-analyte binding reaction kinetics can be simulated by e.g. a mechanistic assay model based on differential equations. However, the mathematical modelling becomes more complicated if multivalent-structured components are involved and the number of binding complexes increases.

In this paper, a new node-based method to model complex binding reactions is introduced. The principle of this method is to construct a network of the initial components, reaction intermediates and end-products by forming a network of nodes. This network is then solved, node by node, breaking the initial problem into smaller partial problems, still obeying the laws of chemical reaction kinetics and without ignoring any parts of the problem.

This method provides an easy and quick way to study complex binding reactions since simulation networks are simple to construct directly from the reaction scheme. This presented new "NODE"-method is compared with the well known mechanistic assay model.

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1. Introduction

The understanding of the kinetic behaviour of an antibody-analyte binding immunoassay can be important in designing, in setting up the system, and in acquiring a fast and accurate result [1]. The behaviour of an immunoassay reaction can be predicted by simulation models based on antibody-antigen binding reaction kinetics [2–6]. The experimental binding kinetic studies of biomolecules are carried out mainly by optical label-free biosensors [6–9] and label-techniques [2–5]. The mechanistic assay system model, based on antibody-analyte binding reaction kinetics, has proven to be applicable to the prediction of a rapid generic three-component sandwich-type (immunometric) immunoassay [4,5,10]. It is even possible to predict the result of the assay from the kinetics alone at any time during the assay incubation or under varying assay conditions [11].

However, when the structure of an analyte in the binding reaction is multivalent (e.g. C-reactive protein (CRP) or adenovirus), the basic three-component antibody-analyte-labeled antibody binding reaction assay model may fail since the model no longer predicts the results accurately. For example, in the early phase of incubation binding components in the liquid phase may form new intermediates, due to their faster kinetics over solid phase, and end-products that are not accounted for in the basic model, due to the multivalent structure of the analyte. These new intermediates and end-products have their own stability, association and dissociation rates, and they interfere with the basic assay system [12,13]. Thus, the increase in the number of binding partners may have a distinct effect on the immunoassay's kinetic profile, and therefore, the model based on the generic binding complexes will not predict the measured experimental data accurately. The complexity of the model should therefore meet the complexity of the assay system.

Mathematical modelling of reaction kinetics gets more complicated when the number of binding complexes increases. The mechanistic assay model is based on differential equations that describe each of the assay reactions. Extending the model by rewriting the equations becomes exceedingly cumbersome, as the number of binding reaction partners increases. The matrix, representing the ordinary differential equations (ODEs), which has to be solved, will grow and requires care and competence in handling since manual transform of the chemical equations into a matrix is in our experience error-prone.

Advances in biotechnology and computer science have provided an opportunity to develop biochemical reaction networks and toolboxes e.g. Feinberg's Chemical Reaction Network (CRN) theory [14–17]. These chemical reaction networks can be used to describe complex physicochemical systems by utilizing quality and quantitative information [18–24]. The network theories are widely used to describe metabolic pathways and signalling pathways [18,25–28]. Also, the modelling of enzymatic reaction cascades is of interest in drug development mainly to get a better overall view of the biological processes [29–31].

In this paper a new method to model multi-component assays with quantitative results is presented. The method uses a network of

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doi:10.1016/j.bpc.2010.05.012

binding complexes and nodes to calculate and simulate a chemical system. Such a simulation network has not, to our knowledge, been proposed in the area of immunoassays before. The method is a variant of the mechanistic assay model; it obeys the laws of chemical reactions at the node level and calculates the concentrations as a function of time using the same understandable parameters and initial values as the mechanistic model, e.g. concentrations, reaction rates. This NODE-method is used to simulate the behavior of the system as a whole and not to study only one molecule or one type of binding class at a time, typical to stochastic modelling approaches [32,33].

The benefits of this method are in usability, when the number of binding complexes increases. In the NODE-method, the reactions are calculated as parts like in a puzzle. The calculation is carried out by using calculation nodes; each node concentrates only on one association-dissociation reaction process and only the full network of nodes describe the whole assay system. Each node is calculated one at a time, and once at each time step of the simulation. Thus, the principle of this NODE-method is to model the kinetic curve by constructing a network of nodes connecting the initial components, reaction intermediates and end-products that react with each other. The network is then solved, node by node, by breaking down the initial problem into smaller partial problems, while still obeying the laws of chemical reaction kinetics.

In this paper we show how the binding reaction network with a time coordinate can be used to study the kinetics of immunoassay binding reactions and return a quantitative result. The new NODE-method is used to model the behaviour of human thyroid stimulating hormone assay (hTSH) kinetics. The initial values used in this NODE-method are the same as in our earlier publication, in which the same kinetics were modelled by using mechanistic assay modelling method [4]. We conclude by comparing the results of both modelling methods.

1.1. Mechanistic assay system kinetic model

Different modelling approaches have been used to study the kinetic behaviour of assay systems [2–4,34]. Pure mathematical models, which are not based on the laws of chemical reaction kinetics, may produce several solutions for one set of input parameters. A good model is valid for any occasion with varying input parameters and should provide an unambiguous answer, with a single set of input parameters. A mechanistic assay model considers the antigen-antibody reaction, laws of chemical reaction kinetics and it offers identifiable, physical and chemical parameters such as time, concentrations and association/dissociation rates [3,4]. It can be used, e.g. to study rapid immunoassays, which rely on interrupted incubation [4,10]. The reaction scheme for a three component immunometric assay reactions can be written as Eqs. (1)–(4):

$$Ag + Ab \underset{k2}{\overset{k1}{\rightleftarrows}} AgAb \tag{1}$$

$$Ag + Ab' \underset{k4}{\overset{k3}{\rightleftarrows}} AgAb' \tag{2}$$

$$Ab + AgAb' \underset{k6}{\overset{k5}{\rightleftarrows}} AbAgAb' \tag{3}$$

$$Ab' + AgAb \underset{k8}{\overset{k7}{\rightleftarrows}} AbAgAb' \tag{4}$$

where k_{2n-1} stands for association and k_{2n} for dissociation rate constants. Ab is denoting the primary binding reagent, Ag the analyte (sample) and Ab′ the secondary binding reagent (the labeled antibody). The assay reaction scheme of a three component binding reaction can be seen in Fig. 1.

The respective ordinary differential equations (ODEs) of the mechanistic model approach for three component immunometric assay reactions are shown in Eq. (5). If the association and dissociation constants are known, the status of the immunoassay can be calculated at any point in time by solving these equations numerically for that particular time point — starting from the time point ($t = 0$) with initial concentrations of the reaction components and ending at a defined time point. The mechanistic assay reaction model has been shown to predict the behaviour of the assay kinetics over a wide range of analyte concentrations [3–5]. In practice, in the mechanistic model the differential equations are written for each component as a function of time and from these equations a Jacobian matrix can be constructed and solved. In this study the mechanistic assay

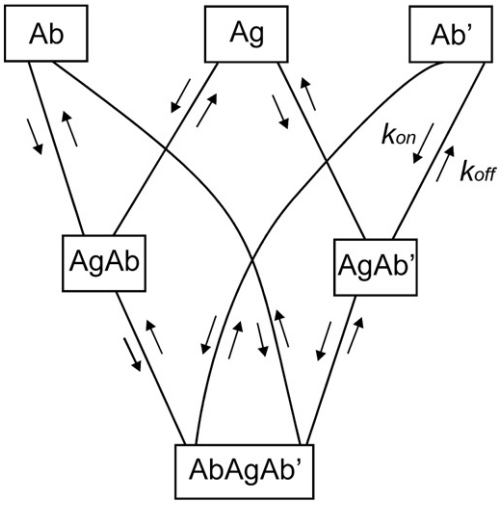

Fig. 1. The reaction scheme of a three component binding assay.

$$\begin{aligned}
\frac{d[Ag]}{dt} &= k2\cdot[AgAb] - k1\cdot[Ag][Ab] + k4\cdot[AgAb'] - k3\cdot[Ag][Ab'] \\
\frac{d[Ab]}{dt} &= k2\cdot[AgAb] - k1\cdot[Ag][Ab] + k6\cdot[AbAgAb'] - k5\cdot[Ab][AgAb'] \\
\frac{d[Ab']}{dt} &= k4\cdot[AgAb'] - k3\cdot[Ag][Ab'] + k8\cdot[AbAgAb'] - k7\cdot[Ab'][AgAb] \\
\frac{d[AgAb]}{dt} &= k1\cdot[Ag][Ab] - k2\cdot[AgAb] + k8\cdot[AbAgAb'] - k7\cdot[Ab'][AgAb] \\
\frac{d[AgAb']}{dt} &= k3\cdot[Ag][Ab'] - k4\cdot[AgAb'] + k6\cdot[AbAgAb'] - k5\cdot[AgAb'][Ab] \\
\frac{d[AbAgAb']}{dt} &= k7\cdot[Ab'][AgAb] - k8\cdot[AbAgAb'] + k5\cdot[AgAb'][Ab] - k6\cdot[AbAgAb']
\end{aligned} \tag{5}$$

model simulations are carried out by open source R language and packages, which are collections of R subroutines and function collections for solving specific tasks. In this work the R odesolve-package was used as a solver for ODEs [35,36].

2. Methods

2.1. NODE-method kinetic model

The NODE-method was implemented with National Instrument's LabVIEW™ program — mainly because programs can be constructed as networks of functions, similarly to the main principle of the NODE-method.

The NODE-method is a variant of the mechanistic assay model. It also obeys the laws of chemical reactions and it calculates the concentrations of all reaction components as a function of time using the same understandable parameters: concentrations, reaction rates (in concentration-time units) and incubation time. Thus, the NODE-method performs the same task with the same initial values as the

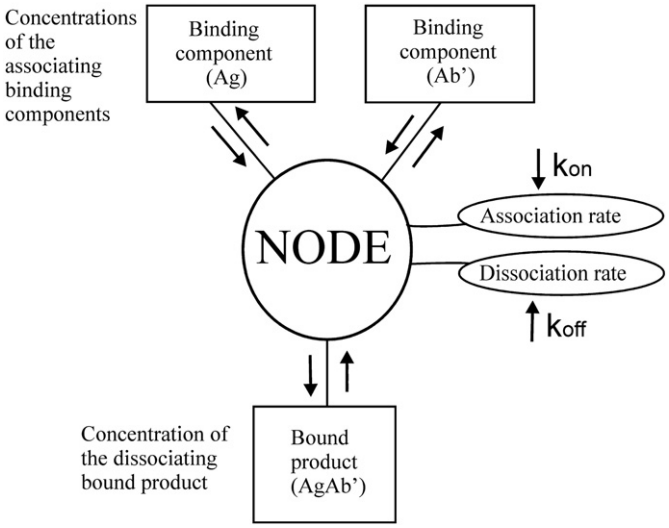

Fig. 2. An example of a node. Each node has specific association and dissociation rates. The node is linked to its respective binding components and to the bound product.

mechanistic assay model, but in a different way. Instead of solving a single matrix, the NODE-method will calculate the constructed network (of any shape) by decomposing the 'problem' into separate partial problems in small time steps by using nodes. Each node has specific association and dissociation rates and corresponds to a single binding reaction of two components, which form the bound product (end-product or intermediate), see Fig. 2.

In Fig. 2, the binding components labelled antibody [Ab′] and analyte [Ag], and the bound product [AgAb′] are connected to the node. When the nodes are added in the binding junctions of the assay reaction scheme (Fig. 1), the NODE-method based network will appear as the net in solid lines, in Fig. 3. Each node (1–4) in Fig. 3 corresponds to the Eqs. (1)–(4) presented in mechanistic assay system kinetic model: NODE 1 corresponds to Eq.(2), NODE 2 to Eq.(1), NODE 3 to Eq.(3) and NODE 4 to Eq.(4).

When the number of nodes is increased due to increased number of possible intermediates and endproducts, the NODE-method based network could appear as seen in Fig. 3, including the dashed lines.

The network of nodes is calculated in a loop each loop representing an indefinitely small incubation time step, Δt. After every loop all the calculated concentrations for the studied end-product (or any other component) are saved for the next loop. The loop is repeated until the desired total incubation time is reached. In the end, the NODE-method will return the simulated curve of any component's (intermediate's or end-product's) concentration as a function of time. More information about constructing a NODE-method based modelling program can be found in the supplementary information.

The conservation of mass is considered in the NODE-method model. After each loop the concentrations of the mass components are calculated separately in order to confirm the conservation of masses.

The most undisputed feature of this method is the ease of modifying the binding process model. Adding a new complex in the model does not require determination of the differential equations of the whole system all over again. Thus, a fast growing matrix (as in the full mechanistic assay model) is not needed. The problem is kept simple for the programmer and the calculation engine by insertion or removal of linkages between nodes and binding complexes in the net. This makes it easy to the user to study the effect of existence and strength of the possibly appearing complexes. For instance, in the early phase of the incubation short term fractions may appear. These short term fractions may be formed by liquid phase components, e.g. when free analyte (Ag) binds to a labeled antibody (Ab′), blocking all available epitopes and forming a fraction such as (Ab′AgAb′) or the

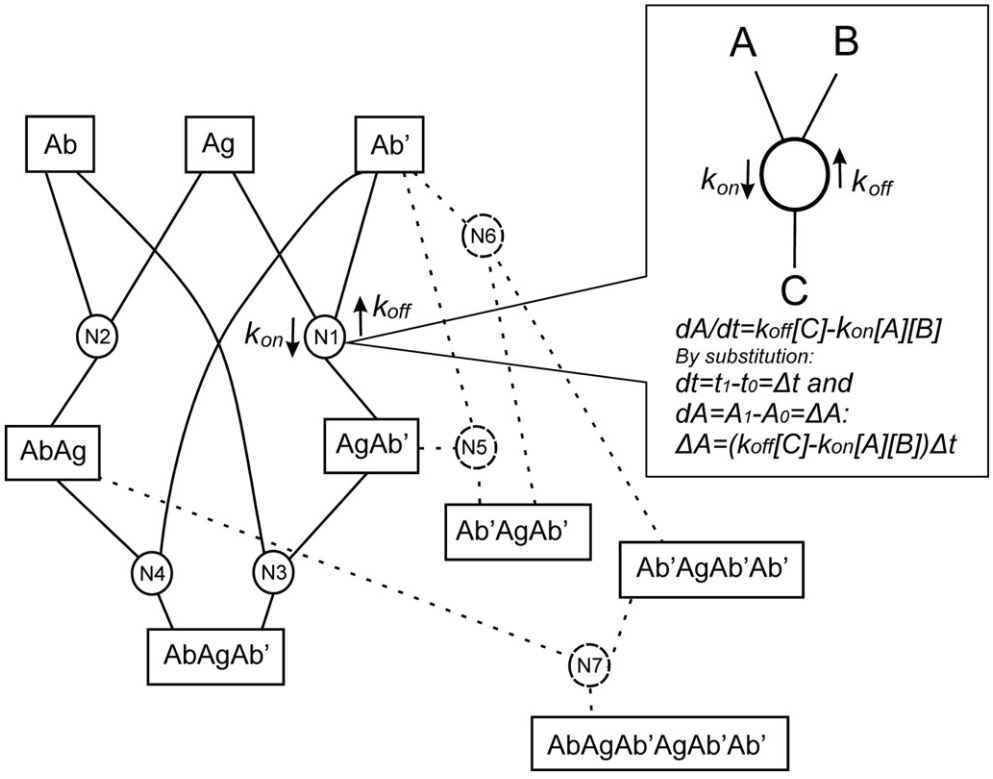

Fig. 3. The reaction scheme of an immunoassay reaction based on the NODE-method. Nodes (circles with N, from 1 to 4) and components (rectangles with solid lines) present a generic three-component assay. Nodes and components connected by dashed lines illustrate the additions of possible binding reactions in the model. The calculation of a generic node in one component's point of view (A representing component Ag) during one time step is presented in top-right corner.

more complex (Ab′AgAb′Ab′), which will shortly break in to parts. These fractions cause errors due to the consumption of the analyte and prevent signal producing complexes (i.e. labeled complexes bound to the solid antibody) from appearing. Such short term (and invisible) fractions may play a role in immunoassays, when the results are predicted even before the reaction reaches its plateau.

2.2. The NODE-method versus the mechanistic assay model simulation and error estimation — standard sandwich-type assay model

Parameters obtained for sandwich-type (immunometric) human thyroid stimulating hormone (hTSH) assay were used in the simulations. The data used was determined in our earlier work [4].

2.2.1. Concentrations and kinetic rates

The concentration range for analyte [Ag] was chosen to cover the dynamic range of the assay: 1.4 mIU/l—700.0 mIU/l (approximately 10^{-11} M–$5*10^{-9}$ M). Kinetic curves were simulated for the analyte concentrations: 4 mIU/l, 50 mIU/l, 150 mIU/l, and 1200 mIU/l.

A concentration of 1200 mIU/l was introduced as an example of analyte concentration exceeding the solid phase antibody capacity. The parameters are equivalent to those determined in our earlier work [4], Eq. (6)

$$\begin{aligned} k_1 &= k_5 = k_7 = 9*10^5 M^{-1}s^{-1} \\ k_2 &= k_6 = k_8 = 4.5*10^{-5}s^{-1}, (K_a = 2*10^{10}M^{-1}) \\ k_3 &= 8*10^6 M^{-1}s^{-1} \\ k_4 &= 1.33*10^{-3}s^{-1}, (K_a = 6*10^9 M^{-1}) \end{aligned} \quad (6)$$

2.2.2. Time-step size (Δt)

The time-step size (Δt) defines the time passed in an immunoassay during one computing loop. Optimally this parameter will be set to infinitesimal. However, in practice, to a value sufficiently small for a reliable result with a reasonable calculation time. It may also be allowed to vary depending on the rate of changes in the system that is described by the network. We have opted to use a fixed step size for simplicity. Generally, there are several methods for how the time-step size is used in ordinary differential ODE solvers. These sophisticated solvers can be adaptive, and therefore the time-step size can be dynamic and automatically adjusted in computing processes. Since the calculations in NODE-method are fast and simple by modern day standards, small Δt values are preferred — however the tolerance of the system to larger step sizes is also descriptive for the model accuracy, thus we varied the step size to verify the NODE-method. The incubation time (total time) is an integer multiple of a time step.

In the traditional mechanistic assay modelling we used an open-source "R" language and its ODE-solve packages to calculate the results for the model. [35,36] In these simulations, the time step size was determined by this ODE solver. In brief, this solver overshoots its targets (the time points), by a predefined rate by the user, and it interpolates the ordinate values for these time points. The solver uses an optimized value for the step size, which can vary between the minimum and maximum, again given by the user, and the error tolerances of the result defined by the user in the beginning.

For simplicity, this newly presented NODE-method is based on the simple Euler's approach of fixed time steps. This is also the easiest and simplest way to keep the computation of the binding reactions synchronized throughout the network. Since, the assay curve is continuous and the step size can be kept small, the results should not deviate too much from the "truth" obtained by more sophisticated approaches. Technically, the next step could be an implementation of a separate ODE solver in each node, but then the issue of synchronization must be carefully considered.

The influence of the step size (Δt) is studied for 1 (one), 0.1 and 0.01 s. The error estimation is done by comparing the NODE-method with the mechanistic assay model for the previously mentioned analyte concentrations.

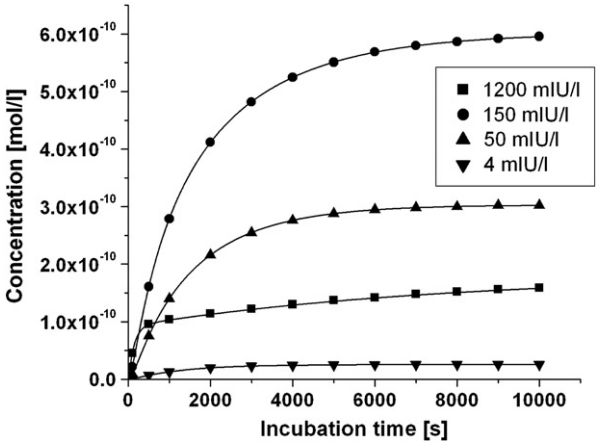

Fig. 4. Kinetic curves for analyte concentrations of 4 mIU/l (up-turned triangle), 50 mIU/l (triangle), 150 mIU/l (circle) and 1200 mIU/l (square). The NODE-method model (solid lines) is compared to the mechanistic assay model (symbols), respectively. The last concentration is an example of analyte concentration exceeding the antibody concentration. This phenomenon called "hook effect" saturates the signal curve to a lower level and will result a false quantity in end-point analysis.

3. Results

A NODE-method network was constructed and compared with the mechanistic assay model by using different step sizes in the simulations by the NODE-method. The conservation of mass was ascertained in all simulations.

3.1. The NODE-method versus the mechanistic assay model

The immunoassay kinetic curves were simulated for analyte concentrations of 4 mIU, 50 mIU, 150 mIU, and 1200 mIU (hTSH) using the NODE-method and the mechanistic model. The highest analyte concentration is used as an example of antigen concentration exceeding reagent capacity. This leads to the well known high analyte "hook-effect" that could result in false quantity with end-point analysis [34]. The simulations carried out by these two methods resulted in congruent binding reaction curves, as seen in Fig. 4. This simulation was done with a time-step size of one second.

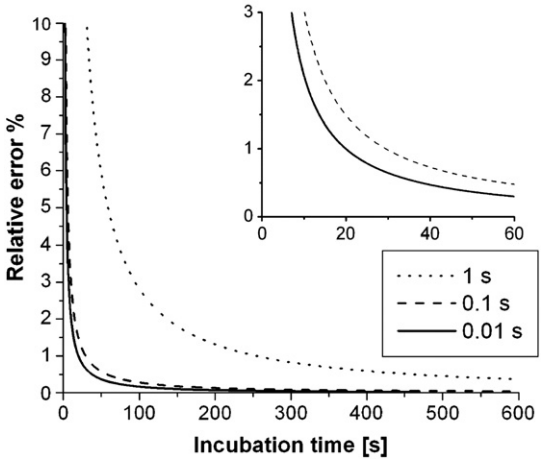

Fig. 5. The effect of the time-step size in the NODE-method compared to the mechanistic assay model method for step sizes of on 1, 0.1 and 0.01 s. The relative error % curves as a function of incubation time are presented for the analyte concentration of 150 mIU/l. The insert illustrates the decrease of the relative error % for time-step sizes of 0.1 and 0.01 s.

3.2. The influence of the time-step size (Δt) in the NODE-method

The effect of the time-step size used in the NODE-method was studied by simulating kinetic curves with one (1) second, (0.1) and (0.01) of a second as a step size. It is understandable that the simulations deviate at some level, since they are both approximations. This deviation between the NODE-method and the mechanistic assay model method is calculated as a relative error at each time point (t), Eq.(7).

$$R_{relative\_error} = |Signal_{NODE\_method}(t) - Signal_{Mechanistic}(t)| \,/\, Signal_{Mechanistic}(t) \quad (7)$$

The relative error percentage was calculated for the analyte concentrations: 4 mIU/l, 50 mIU/l, 150 mIU/l and 1200 mIU/l. The relative error caused by the step size for all analyte concentrations was at its highest in the early phase of the incubation, but decreased over time when the incubation (time) proceeded. The deviation profiles (amount of error and the curve shape) were equivalent for different analyte concentrations for the same time-step size. As expected, the relative error was diminished for a reduced time-step size. The simulation of kinetic curves resulted in a relative error of about 0.01% in the equilibrium for the longest time-step size of one (1) second. The effect of a different time-step size for the analyte concentration of 150 mIU/l can be seen in Fig. 5. This figure illustrates how the deviation, explained by the relative error %, between these two methods reaches 1% in each case:

- step size (1 s): 250 s
- step size (0.1 s): 30 s
- step size (0.01 s): 20 s

The use of the reduced time-step size $\Delta t = 0.01$ s is obviously computationally more intensive, but minimizes the error to 1%, only ten seconds faster when compared to the step size (Δt) of 0.1 s.

4. Conclusions and discussion

Modelling network methods, to our knowledge, have not been implemented in binding reaction kinetics studies in immunoassays. The newly presented NODE-based network method was compared to the mechanistic assay model which is based on solving differential equations. The kinetic simulations created by these two methods were congruent for the same initial value setup. The methods were compared by calculating deviation between the simulation time points. The simulation kinetic curves resulted in a relative error of about 0.01% in the equilibrium for the step size of one (1) second. This difference is insignificant compared to the other error factors caused by the usual experimental practice.

The conservation of mass was taken care of and was calculated with each simulated concentration point as a function of time. This is considered an adequate procedure at this point of the study. In the future, the conservation relations in chemical reaction systems could be studied further [37].

Considering the reliability of the non-equilibrium state rapid immunoassays, the early phase of the incubation must be examined more critically. The effect of the reduced time-step size in the NODE-method was studied in order to determine how much the time-step size affected in the quality of the NODE-method. The use of the shortest time-step size (0.01 s) minimized the relative error to 1% in only 20 s from the beginning, compared to the time-step size 0.1 s in 30 s. The error caused by the use of a step size of 0.1 s is insignificant compared to the usual experimental practice in non-equilibrium rapid immunoassays, such as an inaccuracy in incubation timing [10]. It should also be noted that the simulation carried out by the mechanistic assay model method is an approximation.

This study points out that the NODE-method presented has significant benefits in usability over mechanistic assay modelling methods by providing an easy and practical way to study binding reaction kinetics without having to construct and solve a large set of differential equations. The extension or the shrinkage of the network can be done quickly and in an error-safe way. A large matrix of differential equations is not needed in the NODE-method, compared to mechanistic model, and therefore competence in software programming is not required from the user. The method is applicable for both basic simulation studies of immunoassays, when kinetic curves need to be predicted, and also in studying the effect of a separate component in binding reaction processes. The current study indicates that the method is reliable and can be expected to perform well in more complex situations as the used sandwich assay model — verification of this, however, requires practical data and was considered to be beyond the scope of this work.

This NODE-method may also provide possibilities in studying and modeling metabolic pathways with quantitative results. Likewise in immunoassay kinetics equations, the enzyme kinetics equations represent the rates of consumption and production of compounds, while some nodes can be set to model inhibition of competition by other enzymes. Applications of artificial neural networks in enzyme kinetics have been studied by Bas et. al. [38,39].

The presented NODE-method has the ability to solve both kinetics and end-results of multi-component reactions or pathways of reactions with high accuracy.

Acknowledgements

This work was supported financially by the National Graduate School for Informational and Structural Biology, Turku Finland.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bpc.2010.05.012.

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