Research Article

Computer Simulation for Studying Complexation between a Model Drug and a Model Protein

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Abstract

Computer simulation is one of effective tools for instructors to illustrate complexation or binding interaction between a model drug and a model serum protein in its entire intricacy since the students can be economically exposed to a large variety of results of laboratory design within a relative short period of time. The program of computer simulation was created with Microsoft Access^{TM}. In this simulation program, theoretical parameters such as stoichiometric ratio and binding constants were assigned. After users defined initial concentrations for drug and protein, the program would calculate free drug after complexation and adding noise with zero mean and standard deviation according to the user-defined relative standard deviation. The noise added would make the dataset to be more realistic. Users could use this obtained data to further create a Scatchard plot. The fourth-year pharmaceutical care students of the Faculty of Pharmaceutical Sciences, Prince of Songkla University used this program in studying "complexation" topic. Satisfaction of the students on the instruction using this computer simulation program was determined using a five-choice questionnaire. The results indicated that this learning method was useful and satisfactory. Most responses on the satisfaction with the study via this simulation program were averagely rated above 3 from 5.

Key Words: Computer simulation; Complexation; Protein binding

Introduction

Formation of complexes or coordination compounds is occurred via donor-acceptor mechanism or Lewis acid-base reaction between two or more different chemical constituents. Protein binding with drug is a good example of most commonly found pharmaceutical complexes. The binding of a drug to plasma proteins, e.g., albumin and α_1-acid glycoprotein, can influence the inactivation or retardation of the activity of the entrapped drug. Moreover, complexation may increase the drug activity due to competitive binding of two drugs or more when administered together (Martin, 1993). Therefore, it is necessary for pharmaceutical care students to clearly understand about complexation. In order to study binding constant of complexation, useful data analyzed from this tool includes Scatchard plot and stoichiometric ratio of drug-to-protein. However, due to the delicacy of analytical methods, the experiment consumes both time and resources, and the specific assay techniques are required.

Practicing in data analysis may be practically cost-prohibitive.

In order to set-up a laboratory session for data analysis of binding constant in complexation, computer simulation is an interesting tool of choice since it can reduce time and resources. Students can fully concentrate on the data analysis methodology instead of performing very lengthy experiments in order to obtain a few datasets that may not be suitable to data analysis methodology at all. Moreover, the data analysis resulted from simulation could be validated against the theoretical values used in the software. This should enable the learning process to be performed easily within the resource-constrained teaching environment. Many reports have shown that computer simulations were used as study tools successfully in various topics such as in pharmaceutical industry management (Nelson and Gagnon, 1975), pharmacokinetics (Hayton and Collins, 1991), pharmacotherapy (Chiholm et al., 1996), pharmaceutical calculation (Ramanathan et al., 1997), and pharmaceutical technology (Mezei et al., 1990; Wongpoowarak and Boonme, 2005; Wongpoowarak et al., 2008).

A computer simulation program for studying complexation between a model drug and a model protein is demonstrated here. The software was constructed in Microsoft Access^{TM} using automated tools for generating user-interfaces and embedded the queries. There are limitations in the reliability of simulation methodology due to the unrealistic assumptions, i.e., all binding sites are equivalent thermodynamically, relative probability of vacancy in binding process would be independent from the concentrations of both proteins and drug molecules and the average binding site could be used for representing the actual distribution of binding sites. With these limitations, the simulation should be used for a simple scenario that is not affected by the aforementioned assumptions. Hence, this program was expected to be used as a studying tool for skill improvement in data analysis of the students in a very short period. The fourth-year pharmaceutical care students of the Faculty of Pharmaceutical Sciences, Prince of Songkla University were assigned to study the complexation between a model drug and a model protein via the simulation program from "581-401 Pharmaceutical Preparation III" course. The satisfaction of the students with the program was also determined using five-choice questionnaires.

The aim of this study was to evaluate the satisfaction of the generated computer simulation program on the students' learning of the subject "complexation".

Materials and Methods

Theoretical aspects

Theoretical aspects of complexation are presented as follows (Martin, 1993). The interaction between a drug molecule *D* and a free receptor *P* in a protein can be written as Eq. 1.

$$P + D \rightleftharpoons PD \quad \text{(Eq. 1)}$$

The binding or association constant, assuming the equivalent between activities and concentrations, can be calculated by Eq. 2.

$$K = \frac{[PD]}{[P][D_f]} \qquad \text{(Eq. 2)}$$

where *K* is the binding constant, [*P*] is the concentration of the protein in the term of free binding sites, [D_f] is the free drug, and [*PD*] is the concentration of the drug-protein complex.

If the total protein concentration is appointed as [P_t], [*P*] can be replaced with Eq. 3 and Eq. 2 can be rewritten as Eq. 4.

$$[P] = [P_t] - [PD] \qquad \text{(Eq. 3)}$$

$$\frac{[PD]}{[P_t]} = \frac{K[D_f]}{1 + K[D_f]} \qquad \text{(Eq. 4)}$$

The stoichiometric ratio, *r,* or number of moles of drug bound [*PD*] per mole of total protein [P_t] can be found with Eq. 5.

$$r = \frac{[PD]}{[P_t]} = \frac{K[D_f]}{1+K[D_f]} \quad \text{(Eq. 5)}$$

If independent binding sites, υ, referred to as *nSite* here, are accessible, *r* can be performed as Eq. 6 and then rearranged as exhibited in Eq. 7 providing a graph called a Scatchard plot.

$$r = \upsilon\frac{K[D_f]}{1+K[D_f]} \quad \text{(Eq. 6)}$$

$$\frac{r}{[D_f]} = \upsilon K - rK \quad \text{(Eq. 7)}$$

Simulation methodology for average binding site calculation

In order to simulate the behavior of Eq.1 – Eq.7, we had to start from guessing the complex formation first and compute all related values from the complex formation. If all related values could provide exact value of binding constants, it is equivalent to using binding constants to calculate all species involved.

Suppose that a molecule of *P* could bind to a maximum of *n* molecules of *D*. Under an assumption that all binding sites are all equivalent thermodynamically, occupation of *P* by *D* would distribute with equal probability for all binding sites.

Considering a molecule of protein *P*, the relative probability of vacancy is supposed to be 1. With *n* sites of binding, the relative chance that 1 molecule of *D* would bind to this site will be $1/n$. The relative chance that 2 molecules of *D* bind to *P* simultaneously would be $1/n$ multiplied with $1/n$, i.e., $1/n^2$. The chance that 3 molecules of *D* bind to *P* simultaneously would be $1/n^3$, and so on. For full occupation, the occupancy chance will be the sum for all binding situation. The relative chance that a molecule of *P* will be in bound state is thus equal to $1/n + 1/n^2 + 1/n^3 + \ldots + 1/n^n$, while the relative chance of staying in the vacancy state equal to 1. The average occupied binding site should thus be equal to such relative chance multiplied with total binding site, *n*. This provides an average binding site (*nSite*) formula as presented in Eq. 8.

$$nSite = \sum_{i=1}^{n}\frac{1}{n^{i-1}} \quad \text{(Eq. 8)}$$

where *n* is the maximum binding site.

If one molecule of drug could bind to several proteins, the value of average binding site will be reversed from the Eq. 8.

Simulation methodology for equilibrium concentration

Due to unbalance concentration of drug and protein, the maximum amount of complex formation will be determined from the minimum value between {drug amount/number of drug molecules involved in the complex formation} and {protein amount/number of protein molecules involved in the complex formation}. Moreover, the actual complex will be formed only partially in relative to this maximum amount of complex formation. We could express the actual complex formed by using formation efficiency (*f*), which always between 0 and 1, multiply with this maximum amount of complex formation. For each value of *f*, all chemical species involved could be calculated explicitly, i.e., by assigning *QD* for stoichiometric ratio for drug and *QP* for stoichiometric ratio for protein as illustrated in Eq. 9 – Eq. 12.

$$[PD] = (f)(Maximum\ complex\ formation) \quad \text{(Eq. 9)}$$

$$[D_f] = [D] - (QD)[PD] \quad \text{(Eq. 10)}$$

$$[P] = [P_t] - (QP)[PD]/nSite \quad \text{(Eq. 11)}$$

$$K = \frac{[PD]}{[D_f]^{QD}[P]^{QP}} \quad \text{(Eq. 12)}$$

One could clearly see that *K* is monotonically depends on *f*. At very low value of *f*, *K* would be very small. At very high value of *f*, *K* would be very high. If we use trial-and-error method to find the condition that the computed *K* (as a function of *f*) is exactly equal to the assigned value of *K*, we will know all species involved with such *K*. By using binary search for *f* that could provide the desired *K*, we could limit the trial-and-error test to within 60 iterations in order to obtain 16-significant digits of accuracy ($2^{60} \approx 1.2*10^{18}$, this mean that by using 60 trials, we would obtain accuracy to within a fraction of 10^{-18}) and all species could be computed directly.

In brief, by varying *f* between 0 and 1 (using binary-search), all species related to that *f* could be known and *K* could be computed. The best value of *f* providing the desired *K* could give all related chemical species of interest. This algorithm could be implemented as a user-defined function in Visual Basic Code with maximum of 60 iterations to provide the result that is reliable to 16 significant digits.

Description of computer simulation program

The program of computer simulation for studying complexation between a model drug and a model protein was created with Microsoft Access^{TM}. The source code for implementing in the software was exhibited in the Appendix. The Microsoft Access^{TM} was preferable since it has been easier to make the computation process invisible to the user's attention, and the user can control recalculation to be made manually only when needed. In addition, it was proved to be suitable program for simulation tools for teaching (Wongpoowarak and Boonme, 2005; Wongpoowarak et al., 2008). In this study, the 2003 version of Microsoft Access^{TM} was used, and the program could be upgraded to other versions since it was created with minimal user-interfaces and involved a few queries.

The simulation intends to mimic equilibrium dialysis data. According to the equilibrium dialysis method, a model protein was placed in a number of dialyzing-membrane vesicles. The vesicles were tied firmly and suspended in beakers containing the drug in various concentrations. Finally, the concentrations of the drug in free form were determined by analytical assay. This program simulates instead of actual chemical assay, by allowing users to define their own experimental conditions at will.

By assigning theoretical parameters such as stoichiometric ratio (*QD/QP*) and binding constants (*K*), users could define initial concentrations for drug ([*D*]) and protein ([*P*]) for the software to generate simulated data according to the protocol according to the theoretical concept. The generated noise, using log-and-trig formula (Daykin et al., 1994), normally distributing around zero mean with an assigned relative standard deviation (RSD) was added to the generated data. This will make the dataset to be more realistic. The students could use these simulated data to practice data analysis process. They could also learn that different research designs would also provide different quality of the obtained parameters from a Scatchard plot.

Laboratory methodology

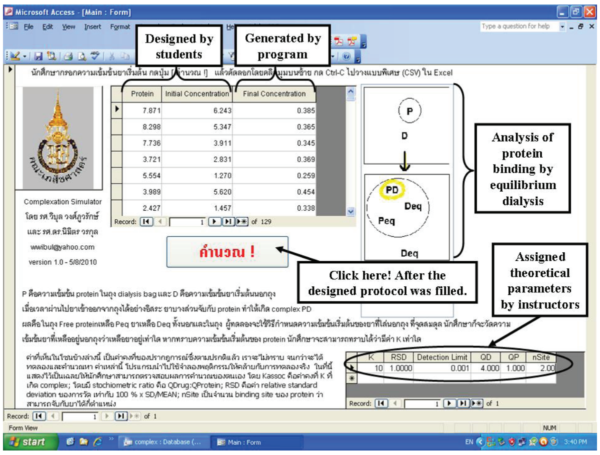
The 39 fourth-year pharmacy students of the Faculty of Pharmaceutical Sciences, Prince of Songkla University attended the lecture of 'complexation' topic in the course of "581-401 Pharmaceutical Preparation III" in Semester 1/2010. In this lecture, the students had been introduced about the concept of complexation. Classification of complexes, applications of complexation in pharmaceutics, method of analysis, protein binding, and thermodynamic treatment of stability constants had been informed. Subsequently, the students were assigned to plan an experimental design for studying protein binding between a model drug and a model protein. The instructors suggested the students to produce the concentration profile of a drug and protein with randomized and fixed maximum values in a spreadsheet of Microsoft Excel^{TM}. For example, if the students would like to use drug concentrations from 0 to 10 moles, they could create the concentrations with "=10*RAND()". Afterwards, the data were copied and then pasted on the spreadsheet of Microsoft Access^{TM} to generate concentrations of free drug according to the assigned theoretical parameters. The students could design their own experiment with the computer simulation program posted on http://mail.pharmacy.psu.ac.th/~wwibul/complex.mdb. Moreover, they could study and review the topic of complexation by themselves at any times.

Evaluation of satisfaction of the students

All students received a questionnaire asking about the degree of their satisfaction with studying by the computer simulation program. The questionnaire was five-choice of satisfaction level with the statement, i.e., excellence (score = 5), good (score = 4), fair (score = 3), poor (score = 2), and very poor (score = 1). The students were asked to return the completed questionnaire after their first using of the program in the computer center of the faculty.

Results and Discussion

Figure 1 shows an example of spreadsheet in Microsoft Access^{TM} simulation program. The designed protocol, i.e., [P] and [D], was filled in columns of "Protein" and "Initial Concentration", respectively. The generated concentrations of free drug ([D_f]) after complexation according to the assigned theoretical parameters appeared in column of "Final Concentration". Afterwards, the students could use the obtained data for further calculation in computer spreadsheet software, e.g., Microsoft Excel^{TM}. The values of [D_f]/[D], [PD], r and r/[D_f] could be computed from the simulated dataset, where [PD] and r are defined in Eq. 3 and Eq. 5, respectively. The data after equilibrium or saturation point obtained from the plot between [P] and [D_f]/[D] were selected for further creating a Scatchard plot. The equilibrium or saturation point could be observed when [D_f]/[D] was near zero. A Scatchard plot could be created using r as x-axis and r/[D_f] as y-axis. The linear equation was then estimated by simple linear regression. For the interpretation according to Eq. 7, K was equal to minus slope and *nSite* was equal to y-intercept over K. If the students used appropriate concentrations of drug and protein, they could obtain the experimental parameters similar to the assigned theoretical parameters. If not, the generated data would not provide a Scatchard plot, due to the reason that any extreme values of drug or protein used would be very sensitive to noise effect in the Scatchard transformation. However, the students could repeat all over again with different experimental conditions.


Microsoft Access - [Main : Form]
File
Edit
View
Insert
Format
He
Type a question for help
Designed by students
Generated by program
Analysis of protein binding by equilibrium dialysis
P
D
PD
Deq
Peq
Deq
คำนวณ !
Click here! After the designed protocol was filled.
Assigned theoretical parameters by instructors
Form View
NUM
start
complex : Database (...
Main : Form
EN
3:40 PM

นักศึกษากรอกความเข้มข้นยาเริ่มต้น กดปุ่ม [คำนวณ !] แล้วคัดลอกโดยคลิกมุมบนซ้าย กด Ctrl-C ไปวางแบบพิเศษ (CSV) ใน Excel

Protein	Initial Concentration	Final Concentration
7.871	6.243	0.385
8.298	5.347	0.365
7.736	3.911	0.345
3.721	2.831	0.369
5.554	1.270	0.259
3.989	5.620	0.454
2.427	1.457	0.338

Record: 1 of 129

Complexation Simulator
โดย รศ.วิบูล วงศ์ภูวรักษ์
และ รศ.ดร.นิมิตร วรกุล
wwibul@yahoo.com
version 1.0 - 5/8/2010

P คือความเข้มข้น protein ในถุง dialysis bag และ D คือความเข้มข้นยาเริ่มต้นนอกถุง
เมื่อเวลาผ่านไปยาเข้าออกจากถุงได้อย่างอิสระ ยาบางส่วนจับกับ protein ทำให้เกิด complex PD
ผลคือในถุง Free proteinเหลือ Peq ยาเหลือ Deq ทั้งนอกและในถุง ผู้ทดลองจะใช้วิธีกำหนดความเข้มข้นเริ่มต้นของยาที่ใส่นอกถุง ที่จุดสมดุล นักศึกษาก็จะวัดความ
เข้มข้นยาที่เหลืออยู่นอกถุงว่าเหลือยาอยู่เท่าใด หากทราบความเข้มข้นเริ่มต้นของ protein นักศึกษาจะสามารถทราบได้ว่ามีค่า K เท่าใด

ค่าที่เห็นในโซนข้างล่างนี้ เป็นค่าคงที่ของปรากฏการณ์ซึ่งตามปรกติแล้ว เราจะไม่ทราบ จนกว่าจะได้ทดลองและคำนวณหา ค่าเหล่านี้ โปรแกรมนำไปใช้จำลองพฤติกรรมให้คล้ายกับการทดลองจริง ในที่นี้แสดงไว้เป็นเฉลยให้นักศึกษาสามารถตรวจสอบผลการคำนวณของตนเอง โดย Kassoc คือค่าคงที่ K ที่เกิด complex; โดยมี stochiometric ratio คือ QDrug:QProtein; RSD คือค่า relative standard deviation ของการวัด เท่ากับ 100 % x SD/MEAN; nSite เป็นจำนวน binding site ของ protein ว่าสามารถจับกับยาได้กี่ตำแหน่ง

K	RSD	Detection Limit	QD	QP	nSite
10	1.0000	0.001	4.000	1.000	2.00

Record: 1 of 1

Record: 1 of 1

Figure 1 A spreadsheet of Microsoft Access^{TM} computer simulation.

The total of 38 questionnaires (97.44%) were answered and returned from 39 students of one class. The numbers of each answer for each question were presented in Table 1. The results exhibited that the method of learning was satisfactory. The students averagely rated above 3 from 5 for their satisfaction on this simulation tool. The majority of the students thought that the learning method was useful, helped them to study by themselves, and reduced the time of studying. Computer simulation experiments offered possibility to practice data analysis in complexation topic within a very short period of time.

Table 1 The numbers of the answers and average scores on the questionnaires about the opinion of 38 students on the studying with the computer simulation program of complexation.

No.	**Question**	**Satisfaction level***					**Average Scores**
		5	**4**	**3**	**2**	**1**	
1	The computer simulation was remarkable.	13	18	4	1	2	4.03
2	The studying with computer simulation supported the student to understand the subject in a relative short period of time.	8	12	11	6	1	3.53
3	The computer simulation could be applied to use in studying of other topics.	9	17	10	2	0	3.87
4	Using computer simulation in this study provided the student to comprehend the subject of "complexation".	8	11	14	5	0	3.58
5	Listening to the lecture in this study provided the student to understand the subject of "complexation".	5	18	12	3	0	3.66
6	Searching information by your own in this study provided the student to understand the subject of "complexation".	1	11	19	6	1	3.13
7	The software program of computer simulation using in this study was effortless to apply.	5	16	14	1	2	3.55
8	Using computer simulation helped the student to understand the subject of "complexation" more than only listening to the lecture.	9	15	11	1	2	3.74

Note: *Score: 5 = excellence, 4 = good, 3 = fair, 2 = poor, 1 = very poor.

Conclusions

Studying requires a proactive approach by both the instructors and the students. Developing new tools such as computer simulations allows the instructors to offer the students new avenues to study. The students not only develop their knowledge but also develop their ability to study by themselves. The results suggested that the proposed simulation program for complexation was a satisfactory studying tool.

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Appendix

The source code for implemented in the software was a user-defined function that could be used to calculate the equilibrium drug concentration from assigned values of binding constants and the input values of total protein concentration and initial drug concentration.

```
UPDATE Drug, [Default] SET Drug.Dfree
=ConcEquilibrium(Drug!P,Drug!D,Default!K,De
fault!RSD,Default!QD,Default!QP,Default!nSite)
WHERE (((Drug.P) Is Not Null) AND ((Drug.D) Is
Not Null));
```

The default value for K, QD, QP, nSite and RSD of noise to be simulated are given in a table [Default] and initial value of P and D are given in a table [Drug].

By execute such SQL code, the new value of equilibrium drug will be updated in a column *Dfree* in table [Drug]. This is the simulated dataset that can be further processed in spreadsheet software.

```
'Source code start here
Function NormalDistribution(xbar, sd) As Double
    'This is log-and-trig formula (Daykin et al.,
1994)
    Do
    r1 = Rnd(Timer)
    r2 = Rnd(Timer)
    Loop Until r1 > 0
    x1 = (Cos(2 * Pi * r2)) * Sqr(-2 * Log(r1))
    If Rnd(Timer) > 0.5 Then
      NormalDistribution = xbar + x1 * sd
    Else
      NormalDistribution = xbar - x1 * sd
    End If
End Function
Function AverageBindingSite(maxsite) As Double
    If maxsite > 1 Then
      N = CInt(maxsite)
    ElseIf maxsite = 1 Then
      N = 1
    Else
      N = CInt(1 / maxsite)
    End If
    sump = 0
    For i = 1 To N
      sump = sump + 1 / N ^ i
    Next i
    If maxsite >= 1 Then
      AverageBindingSite = 1 / sump
    Else
      AverageBindingSite = sump
    End If
End Function
Function ConcEquilibrium(P, D, K, RSD, QD, QP, nSite)
On Error GoTo xx
    If P / QP < D / QD Then
       UpperboundComplex = P / QP
    Else
       UpperboundComplex = D / QD
       Minf = 0
    Maxf = 1
    For i = 1 To 60
      f = (Minf + Maxf) / 2
      cpx = f * UpperboundComplex
      myK = cpx / (((D - cpx * QD) ^ QD) * ((P
- cpx * QP / AverageBindingSite(nSite)) ^ QP))
      If myK > K Then Maxf = f Else If myK <
K Then Minf = f Else Exit For
    Next i
    De = D - cpx * QD
    'Now add error term to the result
    ConcEquilibrium = NormalDistribution(De,
RSD * De / 100)
    Exit Function
xx:
Resume Next
End Function
```