CompuSyn
For Drug Combinations
User's Guide

A Computer Program for Quantitation of Synergism and Antagonism in Drug Combinations, and the Determination of IC_{50} and ED_{50} Values.

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Chapter 1

Introduction

Dose-effect relationships in biological systems, despite their complexity, follow the fundamental principle of the mass-action law. This law, not the empirical statistical functions, underlies the physical-chemical principles of pharmacodynamics. The median-effect principle (MEP), (Chou, 1976) and its extension, the combination index equation (Chou and Talalay, 1981) are derived from the mass-action law based on enzyme kinetic models and receptor binding theory via the process of mathematical induction and deduction.

When dealing with dose-effect relationships, we do not intend to draw empirical curves that best fit experimental data, but rather use the experimental data to fit the median-effect principle of the mass-action law, and then quantitate the well-defined parameters associated with it. These parameters quantitatively determine the potency (D_m), the shape (m) and conformity (r) . The D_m and m values for each drug and their combinations are then used for calculating their interactions in term of synergism, additive effect and antagonism.

Based on the records from the Institute for Scientific Information, (ISI), Philadelphia, PA., during the past twenty years, the Chou's median-effect equation and its extension, combination index (CI) equation (Chou and Talalay, 1984) have been cited by over 1,700 scientific papers published in over 260 biomedical journals. Although MEP is not among the most cited in number, it is obviously among the most widely cited in terms of broad spectrum of applications.

The present software for Windows differs from the previously available ones in the field,

containing new features such as Fa-DRI plot, Fa-Log(DRI) plot, normalized isobologram for non-constant ratio combinations, and polygonograms for three or more drug combinations. This software is designed to be easy to use and very flexible in terms of options and improved graphics and statistics.

Chapter 2

Getting Started

2.1 Computer, Memory and Printer Requirements

CompuSyn is written in Sun Java 2.0 and runs using the Sun Java Runtime Environment 1.4.2. For more information about Java, see http://java.sun.com.

For a list of hardware requirements, see Table 2.1.

Item	**Minimum**	**Recommended**
CPU	Intel Pentium Class Chip or Compatible Clone	Intel Pentium Class Chip over 450 MHz
RAM	64 MB	128 MB
Free Disk Space	80 MB	120 MB
Printer	Any Windows-Compatible Printer	-
CD-ROM Drive	Windows-Compatible CD-ROM	-

Table 2.1: Hardware Requirements

2.2 Installing CompuSyn

2.2.1 Basic Installation from CD

Installing from the CompuSyn CD is quite simple. Insert the CD into your CD-ROM drive, double click on the My Computer icon on your desktop, then the COMPUSYN CD icon. Now, double click on the program labeled Install CompuSyn or Install Compusyn.EXE. If you have a slow computer or slow CD-ROM drive, it may take a few moments before anything appears on the screen. The Installer program will bring you through the installation process.

If you do not already have the Sun Java Runtime installed on your machine, CompuSyn will not run. If this is the case, run the Java Installer provided on the CD, Install Java.EXE. After you have installed the Java Runtime Environment, try running CompuSyn again.

2.2.2 Installation Troubles

If you have problems installing CompuSyn, please report them as a bug. Include in the bug report the manufacturer and model of your computer, the version of Windows you are running and a description of the process you followed which produced the error. As always, an exact description of the error message is extremely useful. See the section on bug reporting later in this document (§6.2).

2.3 Running CompuSyn

2.3.1 Loading CompuSyn

If you installed CompuSyn with the installer, you should have a "CompuSyn" group in your Start Menu. Simply select Run CompuSyn.

Once you have loaded CompuSyn, you are presented with the Start Window. The Start Window has two buttons, New Experiment and Recall Experiment, both of which are self-explanatory. Either one will create an Experiment, and load the Main Window.

2.3.2 The Main Window

The Main Window should also be rather self-explanatory. In the upper left there are Text Fields for inputting descriptive information about the Experiment. These are provided for your convenience. They will be printed in the Report, to aid you with your record keeping.

In the center of the window, there are two lists. The top one lists all the Single Drugs in the current Experiment, the bottom lists the Drug Combos (both those at a Constant Ratio and a Non-Constant Ratio).

New Drugs and Combos are created with the New Drug and New Drug Combo buttons respectively. To edit or delete a drug, click on the drug in the list of drugs, then click the Edit Drug or the Delete Drug button. The same goes for Drug Combos.

At the bottom of the Main Window, there are buttons to Save Experiment, Generate Report, and Calculate Parameters.

Saved Experiment files end with the extension .cse. If you do not name your file with the .cse extension, CompuSyn will add it automatically.

When you choose to Calculate Parameters, the Parameter Calculation Dialog appears. In this dialog, select the parameter you wish to calculate all other parameters from, enter your effect (Fa) value, and press Enter. For example, if you wish to calculate the Doses and CIs of all Drugs and Combos at an Fa of 0.99, simply type . 9 9 in the Fa Text Field, and press Enter. You can move between the Fields with the Tab key or with the mouse.

2.3.3 Single Drug and Drug Combination Editing

All of the Data Entry Dialogs in CompuSyn are set up so that it should be very easy to enter your data quickly and effectively. All you have to do is input the requested data (where the cursor is, usually starting at Name) and press Enter. This registers the information and moves on to the next item. So, if you are entering a drug, all you have to do is type the name of the drug, press Enter, type the abbreviation, press Enter, and so on, until all the data points are entered. This will be more clear when you actually use the software.

Single Drug Editing

When you first open the Drug Editor Window, either by clicking on New Drug or Edit Drug in the Main Window, you have the chance to enter a Name, Abbreviation and Units for the Drug. The Name and Units are displayed in the Report Header, Summary, and Titles. Although these are optional, failing to enter them may make the Report look silly. The Abbreviation is used in the actual Report Section and must be between 1 and 6 characters.

Each Drug must have at least two Data Points. To enter a Data Point, simply type a Dose into the Dose field (a positive number), and press Enter. Then enter an Effect (a number between 0 and 1, exclusive, representing fraction of population affected by the treatment at the specified Dose) and press Enter. The Data Point should then appear in the Data Point List. Data Points can be Edited and Deleted with the buttons Edit Point and Delete Point.

When you add a new Data Point, it is always on the bottom of the list. If you made a mistake on a Data Point, you must click on it, then click the Edit Point button. When a point is edited, it stays in the same place on the list. If you choose to delete the point with the Delete Point button and re-add it to the list, it will be at the end of the list.

Drug Combo Editing

When you create a new Drug Combo, you must choose the Drugs which will be included in the Combo and whether or not it is at a Constant Ratio. For this purpose, the Drug Selection Dialog will appear. Click on each Drug that is in the Combo (Yes, this means you must enter all the single drugs before entering the Combo), click on the type of combo you would like it to be (select Constant (Dose) Ratio or Non-constant (Dose) ratio), then click OK.

Drug Combos are similar to Single Drugs, with a few exceptions. First of all, there are no Units on a Drug Combo, so you will only have to enter Name and Abbreviation. Drugs in a combo may have different units, CompuSyn uses only the raw numbers to do it's calculation. Also, Combos at a Non-constant Ratio can have one or more Data Points (as opposed to a minimum of two in the case of constant ratio combinations or single drugs).

For Constant Ratio Combos, you must enter the ratio at which the drugs are mixed or sequentially combined. This is accomplished in the same manner as every other Entry task,

simply type the ratio and press Enter to move on to the next field.

After entering the ratios, you proceed to entering the Data Points, much the same as a Single Drug. By default, you are prompted for the Dose of the first Drug, but you can input the Dose of any Drug or the Total Dose and CompuSyn will automatically fill in all the other Doses. You can move between Dose Entry Fields with the Tab key. So, to enter the dose of the third drug in the combo as 4.5 and the effect as .5, you would type, Tab Tab 4 . 5 Enter . 5 Enter.

Only the total dose is recorded, so if you change the ratio after inputting the data points, the individual doses will be altered so as to sum to the original total dose.

Non-Constant Ratio Combos are also quite simple. Since there is no constant ratio, you have to input the Dose of each Drug individually. Simply enter the dose, and press Enter. You will be moved to the next dose until all doses are entered, at which point you will be prompted for an effect.

2.3.4 Report Generation

To Generate a Report, click on the Generate Report button in the Main Window. You will be asked to choose which items will be included in the report (Single Drugs, Drug Combos at Constant Ratios, and Drug Combos at Non-Constant Ratios). Drugs which you do not ask to include may still be referenced in several places if they are part of a Combo that is included in the report. It is recommended that you select not more than 10 Single Drugs and Drug Combos to be included in the report as having too many items on each graph tends to make them unreadable.

After choosing the Single Drugs and Drug Combos to be included, you will be asked to choose which Sections will be included in the Report. After this the report will be created. This can take a few moments, epecially on slower computers.

Once the report has been created, it will automatically open in your default web browser, likely Internet Explorer or Mozilla. At this point, you are no longer in the CompuSyn application.

Significant Figures

CompuSyn does not calculate significant figures. All numbers are stored and calculated using 80 bit IEEE standard double floating point precision. This means the largest gap between representable numbers is about 2×10^{-16}.

On the other hand, when numbers are displayed in the Report, they are rounded. All numbers are rounded such that they are 7 characters long, including the decimal point. Very large numbers and very small numbers are converted into scientific notation. If "NaN" (Not a Number) appears in place of a number, this indicates either a mathematical error (e.g., a divide by zero error) or a rounding error. "NaN" errors should be reported like any other bug (See §6.2).

Printing the Report

To print a report, simply choose Print from your web browser's file menu. The exact dialog box and options vary from browser to brower.

Report Options

In the Main Window there is a Report Options button. This button brings up the Preferences Window which has all sorts of settings.

You can set the effect levels (Fa) to be examined in the Isobologram, the Summary Table, and the Polygonogram.

If you set the Fa to 0 as a Summary Table effect, that section will not be included. For example, if you only want three sections at the end of the Summary table detailing Fa = .71, .92, and .99, you would enter .71, .92, .99, 0, and 0 as the five summary table effect levels.

For the Polygonogram, the effect level is the level at which the CI to determine the thickness of the lines is calculated at. This number is not directly displayed on the report. Synergism is shown as a green solid line and antagonism is shown as a red dashed line. See Table 4.1.

There are also a number of Check Boxes which control features of the report.

S.D.A. for CI in Report tells CompuSyn to perform Sequential Deletion Analysis in the CI Table and Fa-CI Plot. This may slow down report generation.

S.D.A. Is a measure of variablity of CI values at a given effect level. For more information on Sequential Deletion Analysis and determination of experimental variability of CI values see §4.3.

Use Total Dose for Text in Report controls the printing of the dosage for Combos at a Constant Ratio. If it is checked, the sum of the doses will be printed. Otherwise, by default, the dose of the first drug in combo will be printed, followed by a '+' character to indicate an incomplete display. This does *not* affect any calculations or plots, only the display in text based components.

Split Large Graphs (on by default) causes the Dose Effect Plot and the Median Effect Plot to be split into two graphs to avoid crowding if there are more than two Drugs and two Combos. Note that the scaling of the graphs is unaffected.

Use Color in Report does exactly what it says, controls whether or not color is used in generating the Report. Turning this off may significantly speed report generation on slow computers.

The Scales button brings up the Scaling Window, a window devoted to report scales. By default, everything is set to 0 which tells CompuSyn to do the scaling automatically. But if CompuSyn does something wrong with the scaling, or you wish to use a custom scale on one of the graphs, you can manually override CompuSyn by entering something in the scale entry for said graph. Note that for plots with multiple frames, such as the Isobologram, the scale on all frames is affected.

2.4 The Console Window

When loaded in debug mode from the Start Menu (start the program csdebug.exe from the Start Menu) CompuSyn outputs progress information to the Console Window as it runs. This is intended to provide feedback and progress information to the user. As various operations, such as Report Generation and Report Export, tend to take a long time, especially on slower computers, it may be useful to watch the progress of CompuSyn in the Console Window, so one can be sure CompuSyn is working correctly. If an error does occur, details of the error will be

printed in the Console Window. For more information of what to do in case of an error, see §6.1.

Chapter 3

Theoretical Background

3.1 Theoretical Basis

The median-effect principle (MEP) introduced by Chou in 1976 is based on the physico-chemical principle of the mass-action law. This basic approach is distinct from the empirical statistical approach, although statistics is widely used for end-result analysis such as probability, variability, correlation or significance estimate. The median-effect equation (Chou, 1976) is derived via mathematical induction and deduction of several hundred derived equations from enzyme kinetic models with different reaction mechanisms in the presence of an inhibitor. The median-effect equation is the basis of the median-effect plot, which provides the foundation of the subsequent developments.

The validity of the median-effect equation is confirmed by the fact that it can be used to derive four major equations in biochemistry, namely, the Michaelis-Menten equation of enzyme kinetics, the Hill equation of receptor occupancy, the Scatchard equation of receptor binding, and the Henderson-Hesselbalch equation of pH ionization (Chou, 1977).

By using multiple inhibitors of different types and mechanisms of inhibition on enzyme kinetic models, again, several hundred equations have been derived which can then be reduced to a "general equation" by comparing theoretical biological activities in the presence and absence of multiple inhibitors. These mathematical work lead to the term "combination index" (CI) (Chou

and Talalay, 1981). The CI equation is the basis for the Fa-CI plot of Chou-Talalay.

The isobologram concept was conceived intuitively more than one hundred years ago. But it was followed by very confusing and conflicting discussions over the decades by various investigators. Among them are Loewe, Berenbaum, Steel and others. The explicit derivation of the isobol equation was achieved by Chou and Talalay in 1984. With the isobol equation available, the automated construction of isobologram can be done in seconds. The isobologram equation is, in fact, a special case expressing combination index equation. The Fa-CI plot is "effect oriented" whereas isobologram is "dose oriented." Both methods of graphics should yield quantitatively identical conclusion of synergism or antagonism since both methods are based on the same multiple drug effect equation (Chou and Talalay, 1981). However, it should be noted that the Chou-Talalay plot for Fa-CI has several practical advantages over the isobol. They are:

1. Fa-CI plot covers all effect levels (e.g., for 1%-99% inhibition) whereas isobologram cover only a few effect levels. Beyond three effect levels, isobol becomes over-crowded and difficult to read.

2. Fa-CI plot is represented by actual combination data points as well as their simulated curve which is never over-crowded. In contrast, isobol uses actual doses on x- and y-axes which tend to be out-of-scale when strong antagonism occurs or difficult to read (overcrowded) when very strong synergism occurs. Although Chou-Talalay's Fa-CI plot may be out-of-scale when strong antagonism occurs, it can be easily overcome by the automated Fa-Log(CI) plot.

3. Fa-CI plot can handle both constant ratio and non-constant ratio combinations in which constant ratio combo shows both the actual data points and the simulated curve, and non-constant ratio combos shows actual data points only. In contrast, the classic isobol can handle only the constant ratio combo with actual dose scales. For non-constant ratio combo, the isobol has to use normalized doses for each component drugs ($\frac{D_1}{Dx_1}$ and $\frac{D_2}{Dx_2}$ with a scale of 0 to 1 for both x- and y- axes), which we call "normalized isobol".

4. The most serious limitation of the isobologram is that it is limited to two drugs. The three dimensional isobol for three drug combos proves to be too messy to read. In contrast, for

the CI equation and for the Fa-CI plot there is no limit to the number of drugs that can be displayed.

All of the above graphics are presented in the present work, in which the normalized isobol is illustrated for the first time with a computer program.

3.2 Glossary of Terms

Median-effect equation

$\frac{f_a}{f_u} = (\frac{D}{D_m})^m$; a general equation for dose-effect relationship derived from the mass-action law principle that takes into account both the potency (D_m) and the shape (m) of dose-effect curve, where f_a and f_u are the fractions affected and unaffected, respectively (Chou, 1976).

Median-effect plot (Chou plot)

A plot $x = \log(D)$ *vs* $y = \log(\frac{f_a}{f_u})$. Since $f_a + f_u = 1$, $f_u = 1 - f_a$.

Median-effect dose (D_m)

The dose that produces 50% effect such as IC_{50}, ED_{50}, or LD_{50}. It is potency parameter and is obtained from the antilog of the x- intercept of the median-effect plot (Chou, 1976). Also see Eq. 3.5.

m Value

The shape parameter for dose-effect curve. The m value is the slope of the median-effect plot. $m = 1$, $m > 1$ and $m < 1$ indicate hyperbolic, sigmoidal, and flat sigmoidal, respectively.

r Value

The conformity parameter for goodness of fit. It is the linear correlation coefficient by the median-effect plot.

Combination Index (CI)

A quantitative measure of the degree of drug interaction in terms of synergism and antagonism for a given endpoint of the effect measurement. (Chou and Talalay, 1981).

Synergism ($CI < 1$)

Greater than expected additive effect.

Additive effect ($CI = 1$)

The combined effect predicted by the mass-action law principle in the absence of synergism or antagonism.

Antagonism ($CI > 1$)

Smaller than expected additive effect.

Fa-CI Plot (Chou-Talalay Plot)

A plot of CI on y- axis as a function of effect level (f_a) on the x- axis.

Potentiation

A condition in which one of two drugs is not effective by itself, but increases the effect of the other drug. It is usually described by percent potentiation or fold potentiation.

Augmentation

Another term for potentiation.

Enhancement

Yet another term for potentiation.

Isobologram (ED_{50}-isobol, ED_{75}-isobol, ED_{90}-isobol, etc.)

A graph indicating the equipotent combinations of various doses of two drugs. It can be used to illustrate additive, synergism, or antagonism.

Classic Isobol

An equipotent graph with the doses of Drug_1 and Drug_2 on x- and y- axes, respectively.

Normalized Isobol

An equipotent graph with the normalized dose of Drug_1 as $[\frac{D_1}{(Dx)_1}]$ and Drug_2 as $[\frac{D_2}{(Dx)_2}]$ on the x- and y- axes, respectively.

Dose-Reduction Index (DRI)

A measure of how many folds the dose of each drug in a synergistic combination may be reduced at a given effect level when compared with the doses of each drug alone.

Fa-DRI Plot (Chou-Martin Plot)

A plot of DRI on y- axis as a function of effect level (f_a) on the x- axis.

Therapeutic Index (TI)

The ratio of the median-effect dose for toxicity in relation for the dose for therapeutic effect.

Sequential Deletion Analysis (S.D.A.)

An iterative sequential deletion of one dose (or concentration) of a drug at a time for repetitive CI calculations. This is followed by calculating the mean $\pm$ 95% confidence interval at each specified effect levels.

Mutually Exclusive Drugs

Two (or more) drugs with similar basic modes of action are considered mutually exclusive drugs. The mutually exclusive condition is the general assumption of the classic isobologram and its equations are accepted as the golden standard for calculating the CI and DRI values.

Mutually Non-exclusive Drugs

Two (or more) drugs with totally independent modes of action analogous to the binding of one ligand to the receptor will not at all affect the binding of other ligands at the different sites. In the ideal situation, even if D_1 and D_2 alone give parallel lines on the median-effect plot, $D_1 + D_2$ in combination will give a non-parallel concave upward curve. Thus, $D_1 + D_2$ in combination contains an element of "intrinsic synergism" which may contribute to the

overall synergism. If mutually non-exclusive condition is assumed for the CI calculation, it will contain the third term (i.e., the product of the first two terms) in the CI equation. Consequently, CI value will be greater (i.e., synergism will be less) and thus it was termed conservative synergism (Chou and Talalay, 1984). In real life, partial exclusivity may occur that would be difficult to quantize. This is especially so if the combination includes more than two drugs, since different pairs of drugs could have different degrees of exclusivity. In order to be consistent with the classic isobologram assumption and for simplicity of calculation, the present software uses only the mutually exclusive assumption and does not use the mutually non-exclusive assumption for drug combinations (i.e., the intrinsic synergism, if it exists, is incorporated as a norm in drug combination analysis).

Polygonogram

A polygonal graphic representation depicting synergism (solid line), additive effect (thin line) and antagonism (broken line) for 3- (triangular), 4- (tetrahedral), 5- (pentagonal) or more drug combinations. The degree of boldness (thickness) of the line represents the degrees of synergism or antagonism. The "Component Drugs" in pairs or triplets, etc. in the polygonogram may be considered dissectional components presented in the same graph (Chou et al., 1994) (Chou, 1998).

In the current software, only drug pairs are represented. That is, only combinations (at a constant ratio) with two member drugs are shown on the polygonogram. The polygonogram provides a simple visual presentation for complicated multi-drug combinations. It also provides a rational for projecting the outcome of synergism or antagonism for the multi-drug combination experiments not yet conducted.

3.3 Equations and Definitions

3.3.1 The Median-Effect Equation of Chou and its Rearrangements

$$\frac{f_a}{f_u} = (\frac{D}{Dm})^m \tag{3.1}$$

[The dose-effect relationships as depicted by the mass-action law: On the right is dose and on

the left is effect.]

$$\log(\frac{f_a}{1-f_a}) = m\log(D) - m\log(D_m) \tag{3.2}$$

[The dose effect curves can be linearized by the median-effect plot with $x = \log(D)$ and $y = \log(\frac{f_a}{f_u})$.]

$$f_a = \frac{1}{1+(\frac{D}{D_m})^m} \tag{3.3}$$

[From a given dose (D) you can calculate the effect (f_a) when the m value (slope) and the D_m value (antilog of x-intercept) are determined from the median effect plot.]

$$D = D_m[\frac{f_a}{1-f_a}]^{\frac{1}{m}} \tag{3.4}$$

[From a give effect (f_a) you can calculate the dose (D) when the m and D_m values are determined from the median effect plot.]

Where

f_a: the fraction affected

f_u: the fraction unaffected, $(1 - f_a) = f_u$

D: the dose of drug

D_m: the dose that is required to produce a median-effect (e.g., IC_{50}, ED_{50}, or LD_{50}). The D_m value can be obtained from the antilog of the x-intercept of the median-effect plot.

m: the slope of the median-effect plot signifying the shape of the dose-effect curve.

$m = 1$: hyperbolic

$m > 1$: sigmoidal

$m < 1$: negative (flat) sigmoidal

3.3.2 The Median-Effect Plot of Chou

Based on the logarithmic form of the median-effect equation (Eq. 3.2):

$$\log[\frac{f_a}{f_u}] = m\log(D) - m\log(D_m)$$

thus, in the form of $y = ax + b$ for a straight line, giving:

$y : \log[\frac{f_a}{f_u}]$

$x : \log(D)$

a (slope) : m

b (y − intercept) : $-m\log(D_m)$

At the median-effect dose, $f_a = f_u = 0.5$, and thus $\log(\frac{f_a}{f_u}) = 0$, therefore, $\log(D) = \log(D_m)$. Hence, the x-intercept of the median-effect plot gives $\log(D_m)$, therefore, D_m can be obtained from the antilog of the x-intercept.

$$D_m = 10^{-(\frac{\text{y-intercept}}{m})} \tag{3.5}$$

r: the linear correlation coefficient of the median-effect plot indicates goodness of fit or the conformity of data to the mass-action law.

$r = 1.0$ indicates perfect conformity of the dose-effect data with respect to the median-effect principle of the mass-action law.

$r < 1.0$ indicates less than perfect correlation.

3.3.3 Combination Index (CI) Equation of Chou-Talalay

Combination Index Equation for Two Drugs

$$\frac{(D)_1}{(D_x)_1} + \frac{(D)_2}{(D_x)_2} = CI \tag{3.6}$$

Where in the denominators, $(D_x)_1$ is the doses of Drug_1 alone that inhibits $x\%$. Likewise, $(D_x)_2$ is the dose of Drug_2 alone that inhibits $x\%$. In the numerators, $(D)_1$ is the portion of Drug_1 in combination $(D)_1 + (D)_2$ also inhibits $x\%$. Again, likewise $(D)_2$. Thus $(D)_1 + (D)_2$ also inhibits $x\%$.

When Eq. (3.6) = 1 it indicates "additive effect," which is derived from the median-effect principle of the mass-action law using kinetic models.

The dimensionless value as specified by Eq. (3.6) was termed the "Combination Index" or "CI" (Chou and Talalay, 1981).

Thus:

$CI = 1$ indicates additive effect in the absence of synergism or antagonism.

$CI < 1$ indicates synergism.

$CI > 1$ indicates antagonism.

Based on the median-effect equation, (D_x) for each drug can be determined by Eq. (3.4). Thus, when $D_1 : D_2 = P : Q$ for $D_{1,2} = D_1 + D_2$, we obtain the two-drug combination index algorithm:

$$CI = \frac{D_{1,2}(\frac{P}{P+Q})}{(D_m)_1[(\frac{f_a}{f_u})^{\frac{1}{(m)_1}}]} + \frac{D_{1,2}(\frac{Q}{P+Q})}{(D_m)_2[(\frac{f_a}{f_u})^{\frac{1}{(m)_2}}]} \tag{3.7}$$

It should be noted that when $CI = 1$, it also describes the classic isobologram for two-drug combination as illustrated in Chou and Talalay (1981).

It should also be noted that Eq. (3.6) is derived under the assumption that D_1 and D_2 are mutually exclusive in their effects (e.g., similar basic modes of action). If based on the mutually non-exclusive assumption (e.g., purely independent modes of action) then, the additive effect for CI = 1 in Eq. (3.6) includes a third term $\frac{(D)_1(D)_2}{(D_x)_1(D_x)_2}$ which is the product of the first two terms. Thus, the higher CI value should indicate lower degree of synergism. We called it the conservative estimation of synergism for the conservative isobologram. If all the CI calculations are under the mutually exclusive assumption, then the mutually non-exclusive case should have *intrinsic synergistic effect* which should contribute to the *over-all synergism* determined. For simplicity and for the consistency with the classic isobologram concept, we have employed mutually exclusive assumption in all the CI calculations in the present software.

Combination Index Equation for Three or More Drugs

Using the same kinetic model as above in 3.3.3, it was further shown that Eq. 3.6 can be extended to:

$$\frac{(D)_1}{(D_x)_1} + \frac{(D)_2}{(D_x)_2} + \frac{(D)_3}{(D_x)_3} = CI \tag{3.8}$$

for 3-drug combination. Similarly,

$$\frac{(D)_1}{(D_x)_1} + \frac{(D)_2}{(D_x)_2} + \frac{(D)_3}{(D_x)_3} + \cdots + \frac{(D)_n}{(D_x)_n} = CI \tag{3.9}$$

or

$$\sum_{i=0}^{n} \frac{(D)_i}{(D_x)_i} = CI \tag{3.10}$$

for the n drug combination.

3.3.4 Dose-Reduction Index (DRI) Equation of Chou

From the reciprocal of each term of Eq. 3.6 (and Eqs. 3.8 – 3.10), we obtain DRI value for the $x\%$ inhibition as shown below:

$$(DRI)_1 = \frac{(D_x)_1}{(D)_1} \tag{3.11}$$

$$(DRI)_2 = \frac{(D_x)_2}{(D)_2} \tag{3.12}$$

$$(DRI)_3 = \frac{(D_x)_3}{(D)_3} \tag{3.13}$$

and

$$(DRI)_n = \frac{(D_x)_n}{(D)_n} \tag{3.14}$$

Where $(D_x)_1$, $(D_x)_2$, $(D_x)_3$, ..., $(D_x)_n$ alone each inhibit $x\%$ and n drugs in combination, $D_{1,2,3,\ldots,n} = [(D)_1 + (D)_2 + (D)_3 + \cdots + (D)_n]$ inhibit $x\%$

3.3.5 Therapeutic Index (TI) Equation

$$TI = \frac{TD_{50}}{ED_{50}} \tag{3.15}$$

for the therapeutic index at the median-effect dose level.

For the general case, TI at any effect level (e.g., x% inhibition), Eq. 3.14 becomes:

$$TI = \frac{TD_x}{ED_x} \tag{3.16}$$

3.4 References for Theoretical Background

Note: These refer to entries in the Bibliography. See Page 48.

3.4.1 Single Drugs (Theory)

(Chou, 1974), (Chou, 1976), (Chou, 1977), (Chou, 1980).

3.4.2 Drug Combinations (Reviews)

(Chou and Talalay, 1977), (Chou and Talalay, 1981), (Chou and Talalay, 1983), (Chou and Talalay, 1984), (Chou and Talalay, 1987), (Chou, 1987), (Chou et al., 1991a), (Chou, 1991b), (Chou et al., 1994), (Bertino and Chou, 1997), (Chou, 1998).

3.4.3 Computer Software

(Chou and Chou, 1987), (Chou, 1991a), (Chou and Hayball, 1996), (Chou and Martin, 2004).

Chapter 4

Scope of Applications

4.1 Single Drug and Dose-Effect Parameters

4.1.1 Calculation of Potency (D_m value)

Calculation of the median-effect concentration (IC_{50}) or the median-effect dose (ED_{50}) is a common practice in biomedical sciences. The old arbitrary method was to draw the empirical best-fit curve and then predict IC_{50} or ED_{50} value from the empirical curve. Instead of drawing empirical curves to fit experimental data, the median-effect principle (MEP) uses the data to fit the basic principle of the mass-action law. The median-effect equation takes into account both the "shape" (the m value) and the "potency" (the D_m value) simultaneously, and takes into account every data points equally, not just the data point(s) near the IC_{50} value (or near the ED_{50} value).

Based on the logarithmic form of the median-effect equation (Eq. 3.2) we obtain

$$\log(\frac{f_a}{f_u}) = m\log(D) - m\log(D_m)$$

which represents a straight line $y = ax + b$ when we designate $y = \log(\frac{f_a}{f_u})$ vs $x = \log(D)$, which we call the median-effect plot. The resulting line should have a slope of m and a y-intercept of $\log(Dm)$. At the median-effect dose, we have $f_a = f_u$, therefore, $\log(\frac{f_a}{f_u}) = 0$. Hence

$\log(D) = \log(D_m)$. Thus, the D_m value can be determined from the antilog of the x- intercept. (See §3.3.1).

4.1.2 Calculation of Shape (m value)

As indicated above, the m value can be easily obtained from the "slope" of the median-effect plot. Substitute this m value into the median-effect equation: the dose-effect curve represented by $y = f_a$ *vs* $x = D$ yields various shapes depending on the m values:

$m = 1$: hyperbolic

$m > 1$: sigmoidal

$m < 1$: negative (flat) sigmoidal

4.1.3 Conformity to Rule (r value)

When experimental data follow the median-effect principle of the mass-action law, the resulting median-effect plot should yield a straight line with a characteristic m and D_m values. The goodness of fit to the straight line can be determined by the linear correlation coefficient (r value) of the median-effect plot. The perfect fit will yield $r = 1$. The conformity as described by the r value, however, is subjective. For practice purposes, we usually set higher standard for *in vitro* studies on enzyme, or receptor binding studies (e.g., $r \geq 0.95$), or tissue culture studies (e.g., $r \geq 0.90$), and less stringent standard for the *in vivo* studies in animals (e.g., $r \geq 0.85$).

4.2 Multi-Drug Combination Dose-Effect Analysis

4.2.1 Experimental Design

Dose Density and Dose Range

It is desirable to spread experimental data points below and above the ED_{50} value. Usually the approximated ED_{50} is obtained from the preliminary studies or from relevant literature references. When approximate ED_{50} is identified, more focused dose range and dose-density can be designed.

For example: If Drug 1 (D_1) has an ED_{50} of 2 uM, it is desirable to make a stock solution of 8 uM and then make a 2-fold serial dilutions such as:

8 uM	4 uM	2 uM	1 uM	0.5 uM	0 uM
$4(ED_{50})$	$2(ED_{50})$	(ED_{50})	$\frac{1}{2}(ED_{50})$	$\frac{1}{4}(ED_{50})$	Control

In this case, the dose-range is from 0.5 uM - 8 uM or from $\frac{1}{4}(ED_{50})$ to 4 (ED_{50}).

The dose density for this drug is five doses (5 concentrations) plus a control.

The dose range or density can be increased or decreased depending on the experimental systems (e.g., increase for *in vitro* experiments and decrease for *in vivo* experiments).

Serial 10x or 5x dilutions are not recommended since this may generate inaccurate extreme data points (e.g., near 0% or near 100% inhibition).

Constant Ratio Combination Design

The constant ratio design provides the most useful information while minimizing the number of drug combination data points. It is most efficient and cost-effective and, therefore, highly recommended. This is particularly important for *in vivo* animal or clinical studies (i.e. diagonal rather than checker-board design).

This design allows auto-simulation of Fa-CI plot and Fa-DRI plot at all effect levels, and auto construction of classic isobologram.

Example for 2-drug constant ratio combination:

Assuming:

D_1 has IC_{50} of 1 uM

D_2 has IC_{50} of 5 uM

The recommended combination ratio is: $D_1 : D_2 = 1 : 5$

This is at equipotency ratio so that the contribution to the effect of each drug would be about equal.

You may design experiment in any other ratios if you have any reason to do so; e.g.,

1. One drug has severe toxicity and you want to minimize its contribution in the mixture, or in the combination.

2. One drug is very weak by itself (e.g., modulator or regulatory ligand) and you don't want to use it at too high concentrations.

Actual Design: 2-fold serial dilution, 1:5 ratio.

D_1	D_2
0 uM	
0.25 uM	
0.5 uM	
1 uM	
2 uM	
4 uM	
	0 uM
	1.25 uM
	2.5 uM
	5 uM
	10 uM
	20 uM
0.25 uM	1.25 uM
0.5 uM	2.5 uM
1 uM	5 uM
2 uM	10 uM
4 uM	20 uM

Non-constant Ratio Combination Design

Some researchers design their drug combination in non-constant ratios for no apparent reason. Even so, the Chou-Talalay method can still be used to analyze this type of data set.

For example: If

IC_{50} for $D_1 = 5$ uM

IC_{50} for $D_2 = 10$ uM

One possible experiment would be random combination ratios:

D_1	**D_2**	ratio
0 uM		
1 uM		
3 uM		
5 uM		
7 uM		
9 uM		
	0 uM	
	5 uM	
	7 uM	
	10 uM	
	12 uM	
	15 uM	
0.5 uM	2 uM	(1 : 4)
1 uM	5 uM	(1 : 5)
3 uM	7 uM	(3 : 7)
5 uM	10 uM	(1 : 2)
7 uM	12 uM	(7 : 12)
12 uM	17 uM	(12 : 17)

As long as D_1 and D_2 alone dose-effect curves are available (i.e., the D_m, m and r value can be determined), the CI value(s) for the combination at any ratios and with any number of data point(s) (including only one data point) can be determined with the Chou-Talalay's CI equation.

Another possible experimental design for the non-constant ratio combination would be to keep D_1 at a fixed concentration and vary D_2 or *vice versa*.

With this type of data set, we can obtain:

1. Dose-effect curves and the median-effect plots for each drug alone but not for the combination.

2. We will get the following parameters: $(D_m)_1$, m_1, and r_1; $(D_m)_2$, m_2, and r_2.

3. Actual combination data points will be shown on plot but without simulated curve for the dose-effect relation nor the simulated line for the median-effect plot.

4. Fa-CI Table, Fa-CI plot, Fa-Log(CI) plot, Fa-DRI Table, Fa-DRI plot and Fa-log(DRI) plot will show actual combination data points but no simulated curves in the plots are possible.

Combinations of More than Two Drugs

The same rules as above can also be applied for three or more drug combinations except no isobologram will be generated and, instead, a polygonogram can be made available. However, there will be some increased complications due to the increased number of drugs involved. For example, if D_1, D_2 and D_3 are combined at the ratio of $a : b : c$, it is suggested (but not obligated) that the combination of their component drug combinations be included at the same time, at $D_1 : D_2 = a : b$, $D_2 : D_3 = b : c$, and $D_1 : D_3 = a : c$. These extended studies will have the following benefits:

1. Dissection of the 3-drug combinations by the component 2-drug combinations, e.g., D_1+D_2 synergism, D_2+D_3 antagonism, and D_1+D_3 synergism. For more than 3 drugs, the same thing applies.

2. The each-drug-alone dose-effect curves and their parameters may be shared by all the component drug combinations. It is more economical in terms of the number of assays, time, effort and cost. It also avoids variations due to different experiments under different conditions.

The above examples show that MEP and the Chou-Talalay method is versatile, quantitative and very easy to use. The citations of these methods in over 260 biomedical journals lend support to this conclusion.

4.2.2 Determination of Synergism and Antagonism (CI value)

In all cases where CI value can be determined, (e.g., CI Table, Fa-CI Plot or Fa-Log(CI) Plot) the following diagnostic rule apply:

$CI < 1$ indicates *Synergism*
$CI = 1$ indicates *Additive Effect*
$CI > 1$ indicates *Antagonism*

In conjunction with the CI values, the Chou-Talalay plots provide visual illustration of synergism or antagonism.

The Fa-CI Plot (Chou-Talalay's Plot)

Data points or simulated curves	**Illustrated Diagnosis**
Below the $CI = 1$ horizontal line	*Synergism*
Above the $CI = 1$ horizontal line	*Antagonism*
On the $CI = 1$ horizontal line	*Additive Effect*

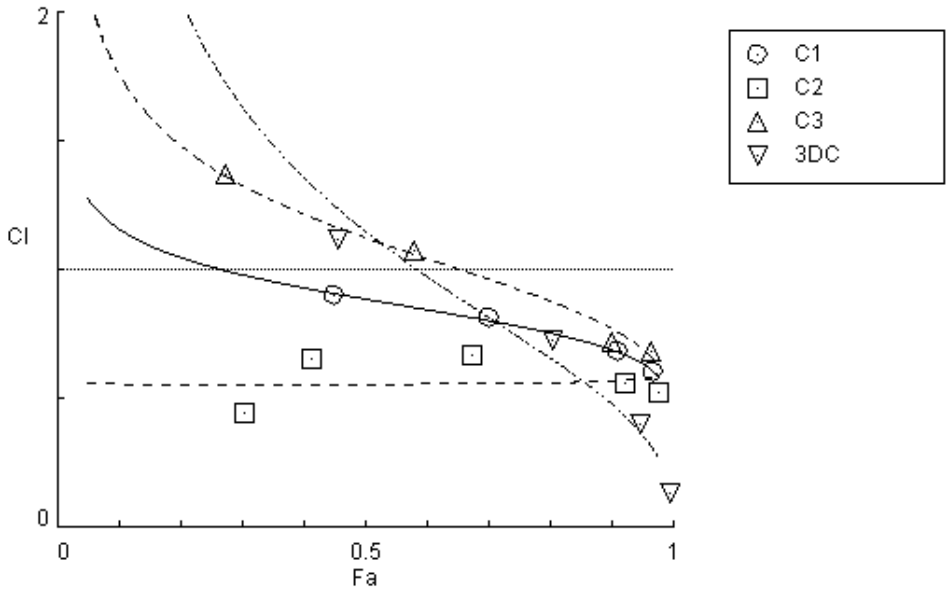

Figure 4.1: An Example Fa-CI Plot (Chou-Talalay Plot).

Fa-Log(CI) Plot

Data points or simulated curves	**Illustrated Diagnosis**
Below the $\log(CI) = 0$ horizontal line	*Synergism*
Above the $\log(CI) = 0$ horizontal line	*Antagonism*
On the $\log(CI) = 0$ horizontal line	*Additive Effect*

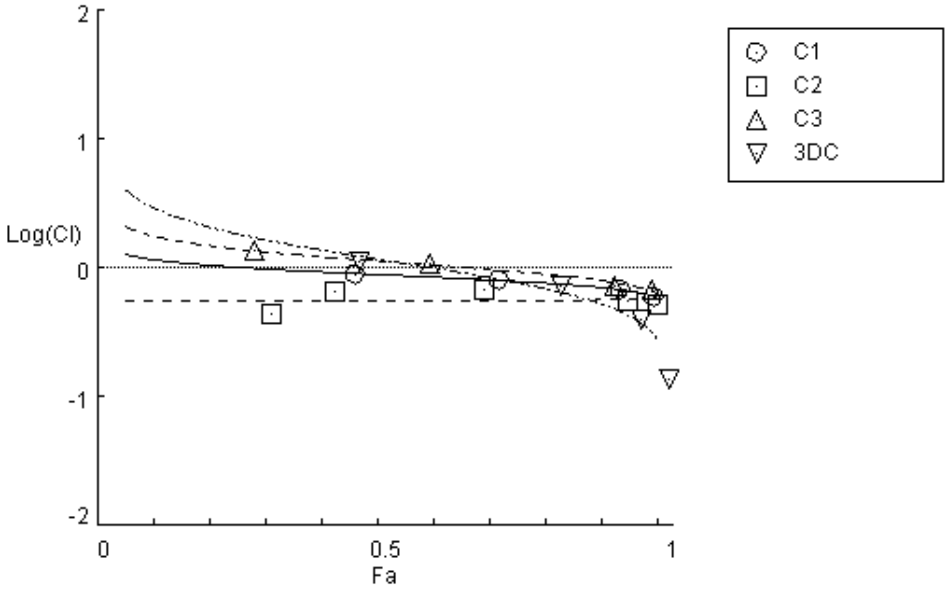

Figure 4.2: An Example Fa-Log(CI) Plot.

4.2.3 Construction of Isobologram

The constant ratio combination design for two drugs allows automated construction of classic isobologram whereas the non-constant ratio combination design for two drugs allows the automated construction of the normalized isobologram.

Isobologram

Data points	**Illustrated Diagnosis**
On the lower-left of the hypotenuse	*Synergism*
On the upper-right of the hypotenuse	*Antagonism*
On the hypotenuse	*Additive Effect*

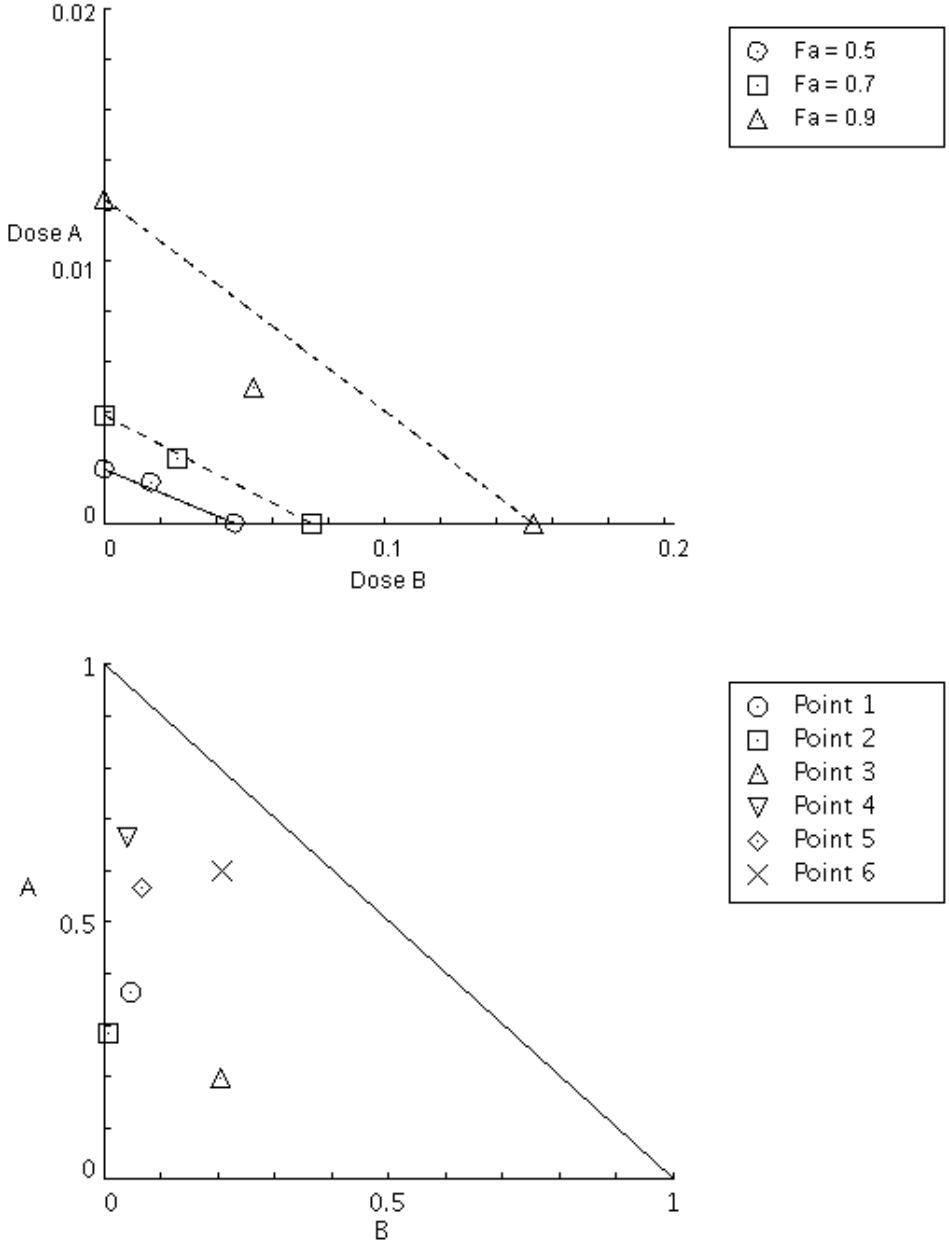

Figure 4.3: Example Isobols, Classic and Normalized.

4.2.4 Calculation of Dose-Reduction Index (DRI value)

One major aim for achieving synergy in drug combination is that it allows dose-reduction for the therapeutic effect. The beneficial consequence of dose-reduction is the reduced toxicity toward the host. Dose-reduction Index (DRI) denotes how many fold of dose-reduction is allowed for each drug due to synergism when compared with the dose of each drug alone.

Dose-reduction Index (Chou, 1998) can be readily obtained from the reciprocal of each term of the CI equation (Chou and Talalay, 1984). Thus,

$$(DRI)_1 = \frac{(Dx)_1}{(D)_1}$$

$$(DRI)_2 = \frac{(Dx)_2}{(D)_2}$$

An example of the DRI values at different-effect levels (Chou-Martin Plot, 2004 as illustrated for this manual) is shown below:

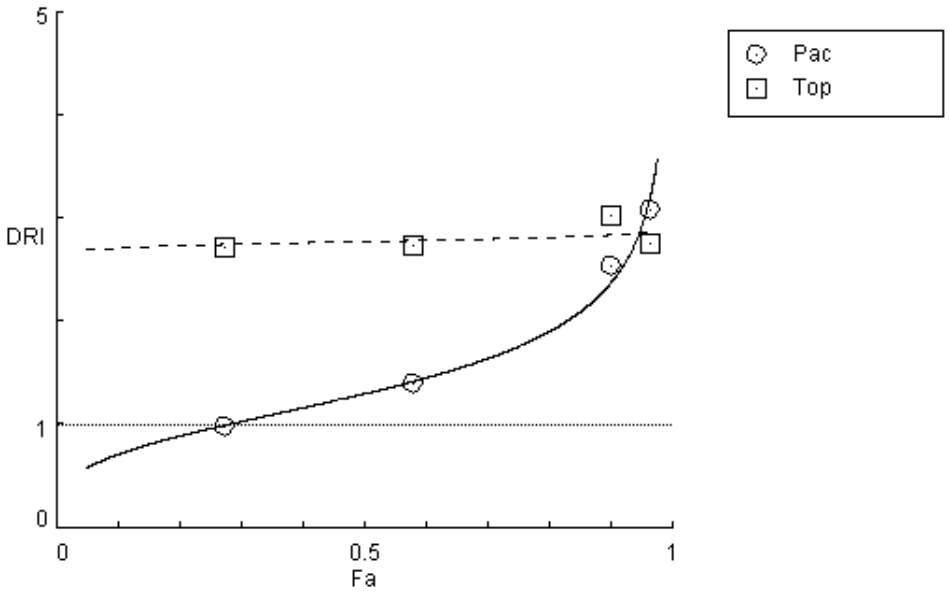

Figure 4.4: An Example Fa-DRI Plot.

In order to avoid points being out of scale when DRI has a large value, the Fa-Log(DRI) plot

was devised to condense the scale. An example of this is shown in Figure 4.5:

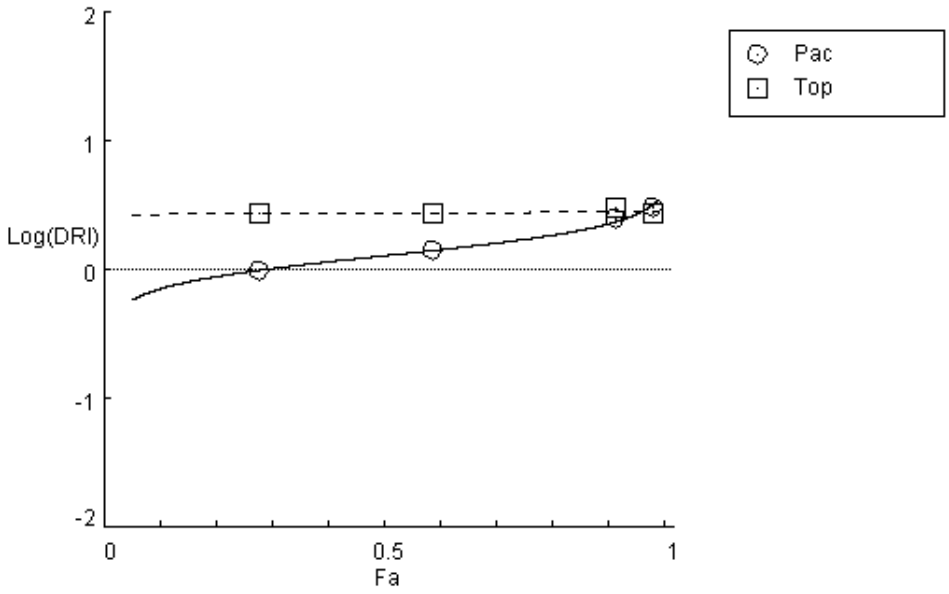

Figure 4.5: An Example Fa-Log(DRI) Plot.

4.2.5 Description of Complex Synergism and Antagonism

Verbal and Symbolic Descriptions

The CI (Combination Index) quantifies the degree of synergism in a combination. However, as indicated in many Fa-CI plots, different effect levels (different f_a values) may have different degrees of synergism or antagonism (e.g., antagonism at $f_a = 0.3$ and synergism at $f_a = 0.9$, etc). Frequently, there is no simple way to describe synergism or antagonism. Furthermore, how much synergism is a real synergism? Does $CI = 0.98$ indicates synergism and $CI = 1.05$ indicates antagonism? Depending on biological variability, accuracy of experimental assays, r values, and other statistics, it is difficult to have absolute criteria of making conclusion in marginal cases.

Since the scale for synergism is $0<CI<1$, and the scale for antagonism is $1<CI<\infty$, logarithmic values of CI can be useful to represent the relative degrees of synergism or antagonism. Based on these premises, alternative verbal descriptions, graded symbols and graphic symbols

for synergism or antagonism are recommended, as in Table 4.1:

CI	**Description**	**Symbol**
< 0.1	Very Strong Synergism	
0.1–0.3	Strong Synergism	
0.3–0.7	Synergism	
0.7–0.85	Moderate Synergism	
0.85–0.90	Slight Synergism	
0.90–1.10	Nearly Additive	
1.10–1.20	Slight Antagonism	
1.20–1.45	Moderate Antagonism	
1.45–3.3	Antagonism	
3.3–10	Strong Antagonism	
> 10	Very Strong Antagonism	

Table 4.1: Symbols for Synergism and Antagonism using CI analysis

Polygonogram (for 3- or More Drug Combinations)

Polygonographic showing of synergism or antagonism was first used by Chou et al. in 1994, and formerly presented as "Polygonogram" by Chou in 1998.

For example, five major cancer chemotherapeutic agents used worldwide with different mechanisms of action were used for the combination index analysis. Among taxol, vinblastine, cisplatin, topotecan and etoposide, (Chou et al., 1994) tried to determined which 2 or 3 of these drugs in combination would produce more synergism or antagonism than others. There are many possible combinations of these drugs ($C_2^5 + C_3^5 + C_4^5 + C_5^5 = 26$) and Chou and coworkers used the polygonographic presentations for some of them, later called "polygonograms" as shown in Figure 4.6.

Since high effect levels are more relevant to cancer chemotherapy, the polygonogram constructions shown in Figure 4.6 used $[CI]_{90}$ instead of the more conventional $[CI]_{50}$.

Similarly, four drug combinations can be represented by the individual solid or broken tetrahedral, and five drug combination can be represented by pentagonal, etc. (Note that the current software does not have this functionality. Instead, the component two drug combinations are presented in the polygonogram.)

This method has a great condensation and summarizing power. If we consider seven drugs

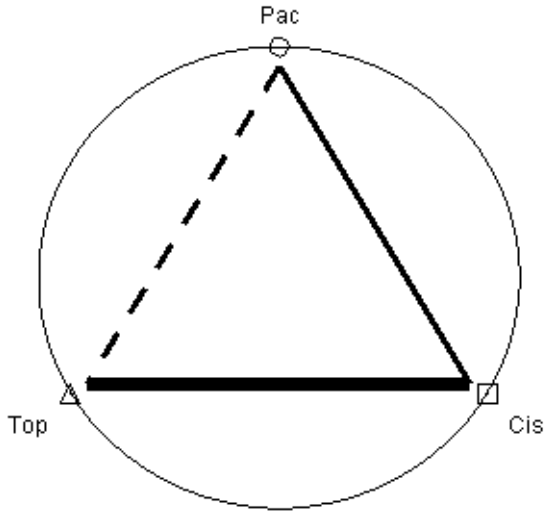

Figure 4.6: An Example Polygonogram.

for combinations, there will be $C_2^7 + C_3^7 + C_4^7 + C_5^7 + C_6^7 + C_7^7 = 120$ possible combinatory combinations. The polygonogram method should have utility in this complicated situation.

4.3 Variability: Sequential Deletion Analysis (S.D.A.)

In the design of dose density for an experiment, there is no absolute rule as to exactly how many points (or concentrations) should be used for each drug. To use 4, 5, 6 or 7 concentrations for each drug is a rather arbitrary choice. In order to determine variability of effects at different dose levels, we employed a sequential (serial) deletion method. For example, if we use 5 concentrations, A, B, C, D and E for Drug_1, and if for Drug_2 we use 5 concentrations, a, b, c, d, and e, for calculating the variability of parameters D_m, m and r, we conduct serial deletion: e.g., first round with no deletion, 2^{nd} round we delete A for Drug_1, 3^{rd} round we delete B for Drug_1, ... and 12^{th} round we delete e for Drug_2. For each round, the CI values at different effect levels will be simulated. This iteration process allows the 95% confidence interval around the mean at each effect levels to be determined. A typical Fa-CI Plot analyzed by the Sequential Deletion Analysis is shown in Figure 4.7. Note that in many cases the variability is greater at low effect

levels (e.g., $f_a < 0.3$) and at high effect levels (e.g., $f_a > 0.9$) whereas the variability is smallest near $f_a = 0.5$. It should be noted that variability can also be studied with other methods, e.g. *i*, repeat the experiment several times and conduct statistical analyses; and *ii*, conduct Monte Carlo analysis or algebraic approximation (Chou and Hayball, 1996).

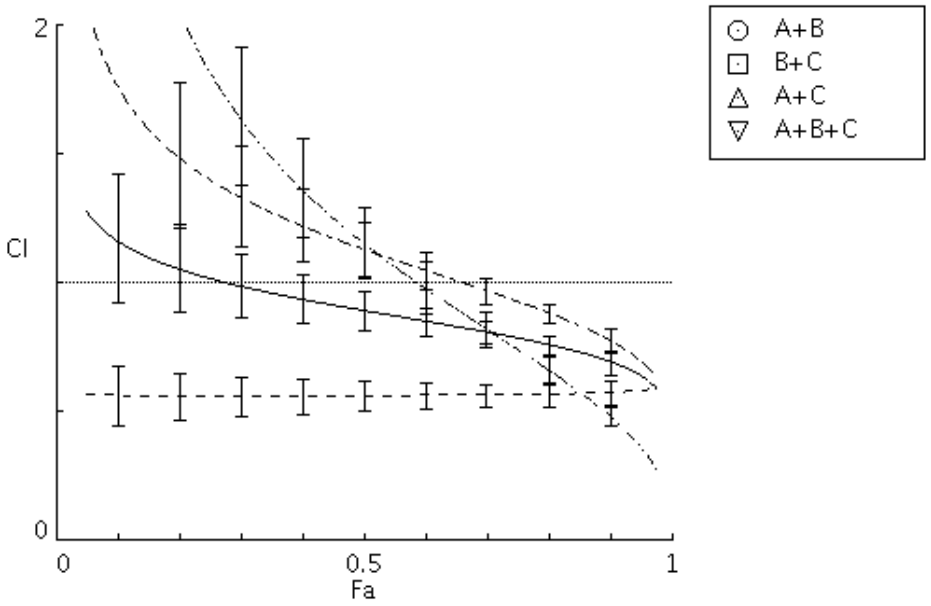

Figure 4.7: An Example Fa-CI Plot with Sequential Deletion Analysis.

Chapter 5

Example of Applications

In this chapter, numerical experimental data from Chou et al, J. Nat'l Cancer Inst. 86:1517-1524, 1994 are used as examples of 2- and 3- drug combinations and analysed with CompuSyn. In the tables the left column explains what is being done while the right column tells what actions must be taken to accomplish the task at hand. This only details the most basic use of CompuSyn operation and should be used as a starting point for further exploration.

Dose (μM)			*Effect*
Paclitaxel	**Cisplatin**	**Topotecan**	F_a
Single Drugs			
0.002			0.429
0.004			0.708
0.005			0.761
0.01			0.882
0.02			0.932
	0.05		0.055
	0.1		0.233
	0.2		0.301
	0.5		0.559
	1.0		0.821
	2.0		0.953
		0.01	0.069
		0.02	0.213
		0.05	0.373
		0.1	0.785
		0.2	0.940
		0.5	0.991
Two Drug Combos			
0.001	0.1		0.450
0.002	0.2		0.701
0.005	0.5		0.910
0.01	1.0		0.968
	0.05	0.005	0.304
	0.1	0.01	0.413
	0.2	0.02	0.675
	0.5	0.05	0.924
	1.0	0.1	0.977
0.001		0.01	0.274
0.002		0.02	0.579
0.005		0.05	0.901
0.01		0.1	0.965
Three Drug Combo			
0.001	0.1	0.01	0.456
0.002	0.2	0.02	0.806
0.003	0.3	0.03	0.947
0.005	0.5	0.05	0.995

Data used in Examples 1-5. (Chou et al., 1994)

5.1 The Median-Effect Equation of Chou

5.1.1 Example 1: Dose-Effect Curve, Median-Effect Plot, m, D_m, and r Values

- Dose-effect curve and the median-effect plot
- Calculation of D_m (ED_{50}), m, and r values.

DESCRIPTION	ACTION
Load CompuSyn	
First, load CompuSyn. See §2.3	**Start** > Programs > CompuSyn > Run CompuSyn
Open a New Experiment.	**New Experiment**
Enter Experiment Info	
Select existing title ("Untitled").	Click and drag from one side of the title to the other.
Type new Title	S a m p l e Enter
Enter the Date	0 1 / 0 1 / 0 0 Enter
Enter a Description	E x a m p l e Space 1
Enter First Drug[1]	
Create new Drug	Click **New Single Drug**
Enter Name	P a c l i t a x e l Enter
Enter Abbreviation	P A C Enter
Enter Units	u M Enter
Enter Data Point 1	. 0 0 2 Enter . 4 2 9 Enter
Data Point 2	. 0 0 4 Enter . 7 0 8 Enter
Data Point 3	. 0 0 5 Enter

Continues on next page...

[1]Data from Journal of the National Cancer Institute, Vol. 86, No. 20, 1517-1524, 1994

DESCRIPTION	ACTION
	. 7 6 1 Enter
Data Point 4	. 0 1 Enter . 8 8 2 Enter
Data Point 5	. 0 2 Enter . 9 3 2 Enter
Finish Drug	Click **Finished**
Enter Second and Third Drug. *Follow Same Procedure as Drug 1*	
Generate Report	
Begin Report Generation	Click **Generate Report**
Select all Drugs (default)	Click **OK**
Accept default relevant sections	Click **OK**
Report Generation is now complete. The resulting report contains calculations of m, D_m, and r values for each of the Drugs, as well as Dose-Effect and Median-Effect plots.	

5.1.2 Example 2: Calculation of Dose from Effect or Effect from Dose

DESCRIPTION	ACTION
Load CompuSyn and enter Drugs *Follow Same Procedure as Example 1*	
Open Parameter Calculation Dialog	Click **Calculate Parameters**
Calculate ED_{70} for each drug	Select (click and drag) Fa ("0.5") Type . 7 Enter
Dose of each drug required for effect of 0.7 is shown in Dose column	
Calculate Fractional Effect at a dose of 4.25	Select dose of Drug A Type 4 . 2 5 Enter
Continues on next page...	

Description	Action
Effect resulting from dose of 4.25 units of Drug A is shown in Fa Box. Dose of each drug required for said effect is shown in Dose column	

5.2 The Combination Index Equation of Chou-Talalay

5.2.1 Example 3: Two Drug Combination at Constant Ratios

- CI and DRI values
- Fa-CI Plot and Fa-DRI plot (with simulation)
- Classic Isobol

Description	Action
Load CompuSyn and enter Drugs *Follow Same Procedure as Example 1 (for Single Drugs)*	
Enter First Drug Combo[2]	
Create new Drug Combo	Click **New Drug Combo**
Select Drugs to be included	Click **Drug 1** **Drug 2** **O.K.**
Enter Name	C o m b o Space 1
Enter Abbreviation	C 1 Enter
Enter Ratio	1 Enter 1 0 0 Enter
Enter Data Point 1	. 0 0 1 Enter . 4 5 Enter
Enter Data Point 2 (using total dose[3])	Tab Tab . 2 0 2 Enter
Continues on next page...	

[2]Data from Journal of the National Cancer Institute, Vol. 86, No. 20, 1517-1524, 1994

[3]Using total dose is optional. One can enter the dose of any Single Drug or the total dose and all other doses with be calculated. Total dose is used in this case as an example

DESCRIPTION	ACTION
	. 7 0 1 Enter
Enter Data Point 3 (using dose B)	Tab . 5 Enter . 9 1 0 Enter
Enter Data Point 4	. 0 1 Enter . 9 6 8 Enter
Finish Drug Combo	Click **Finished**
Enter Second and Third Drug Combo. *Follow Same Procedure as Drug Combo 1*	
Generate Report	
Begin Report Generation	Click **Generate Report**
Select all Single Drugs (default)	Click **OK**
Select all Drug Combos (default)	Click **OK**
Accept default relevant sections	Click **OK**
Report Generation is now complete. The resulting report contains all the D_m, m and r calculations as seen in Example 1, as well as calculations of CI and DRI in both graphical and tabular form. Specifically, the default report will contain CI and DRI tables (one per Drug Combo), as well as Fa-CI and Fa-DRI Plots. Also of note is the Isobologram. In this case a "Classical Isobologram" is displayed, as the input is a Constant-ratio Combo. Also provided is a Summary Table, ideally suited for quick filing and record keeping.	

5.2.2 Example 4: Two Drug Combination at Non-constant Ratios

- CI and DRI values
- Fa-CI Plot and Fa-DRI plot (without simulation)
- Normalized Isobol

DESCRIPTION	ACTION
Continues on next page...	

DESCRIPTION	ACTION
Load CompuSyn and enter Drugs *Follow Same Procedure as Example 1*	
Enter Drug Combo 1 at a Non-constant ratio [4]	
Create new combo	Click **New Drug Combo**
Select Non-Constant Ratio	Click **Non-Constant Ratio**
Select Single Drugs to be included	Click **Drug 1** **Drug 2** **OK**
Enter Name	N C - C o m b o Space 1
Enter Abbreviation	N C 1 Enter
Enter Data Point 1	. 0 1 Enter . 0 1 Enter . 3 7 9 Enter
Enter Data Point 2	. 0 1 Enter . 0 2 Enter . 4 4 1 Enter
Enter Data Point 3	. 0 2 Enter . 0 1 Enter . 4 1 3 Enter
Enter Data Point 4	. 0 2 Enter . 0 3 Enter . 6 3 6 Enter
Enter Data Point 5	. 0 4 Enter . 0 7 Enter . 7 9 5 Enter

Continues on next page...

[4] Data are entirely fictional.

DESCRIPTION	ACTION
Generate Report	
Begin Report Generation	Click **Generate Report**
Select all Single Drugs (default)	Click **OK**
Select all Combos at a Non-Constant Ratio (default)	Click **OK**
Accept default relevant sections	Click **OK**
Report Generation is now complete. The resulting report contains all the D_m, m and r calculations as seen in Example 1, as well as calculations of CI and DRI as in Example 3. However, since the ratio is not constant, no simulation can occur. Thus, all graphs and tables are restricted to actual experimental points. Instead of the Classical Isobologram, a Normalized Isobologram is generated.	

5.2.3 Example 5: Three Drug Combination at Constant Ratios

- CI and DRI values
- Fa-CI Plot and Fa-DRI plot (with simulation)
- Classic Isobol for each drug pair
- Polygonogram

DESCRIPTION	ACTION
Load CompuSyn and enter Drugs *Follow Same Procedure as Example 1*	
Enter Three Drug Combo[5]	
Create new Drug Combo	Click **New Drug Combo**
Select Drugs to be included	Click **Drug 1** **Drug 2** **Drug 3**

Continues on next page...

[5]Data from Journal of the National Cancer Institute, Vol. 86, No. 20, 1517-1524, 1994

DESCRIPTION	ACTION
	O.K.
Enter Name	3 D Space C o m b o Space 1
Enter Abbreviation	3 C 1 Enter
Enter Ratio	1 Enter 1 0 0 Enter 1 0 Enter
Enter Data Point 1	. 0 0 1 Enter . 4 5 6 Enter
Enter Data Point 2 (using total dose[6])	Tab Tab . 2 2 2 Enter . 8 0 6 Enter
Enter Data Point 3 (using dose B)	Tab . 3 Enter . 9 4 7 Enter
Enter Data Point 4	. 0 0 5 Enter . 9 9 5 Enter
Finish Drug Combo	Click **Finished**
Generate Report	
Begin Report Generation	Click **Generate Report**
Select all Single Drugs (default)	Click **OK**
Select all Drug Combos (default)	Click **OK**
Accept default relevant sections	Click **OK**
Report Generation is now complete. The resulting report contains all the features as seen in Example 3, with a few exceptions. Since CompuSyn does not handle three-dimensional graphics, Isobolograms are limited to drug pairs. Three or more drug combos will still appear in Fa-CI and Fa-DRI plots and tables, but will be excluded from Isobolograms and Polygonograms.	

[6]Using total dose is optional. One can enter the dose of any Single Drug or the total dose and all other doses will be calculated using the already specified combination ratio. Total dose is used in this case as an example

Chapter 6

Bug Reporting and Troubleshooting

6.1 Troubleshooting

Since CompuSyn is a limited scale application environment, its built-in troubleshooting capabilities are limited. CompuSyn will print various status messages in the Console Window when run in debug mode. If something fails to work as expected, the first thing to do is try to repeat the error in the debug application, csdebug.exe and look in the Console Window to see if it is a known error.

If the Console Window does not show an error message, you should save your Experiment, quit CompuSyn, restart your computer, and reload CompuSyn. If the exact same error occurs, file a bug report (See §6.2).

If the Console Window does show an error, and the solution is not obvious, try restarting CompuSyn. If this does not help, report it as a bug and be sure to include *all* of the error messages printed on the Console.

6.2 Bug Reporting

Even though CompuSyn has been completed, tested, and released, there is no guarantee that it is completely bug-free. Reporting bugs and inconsistencies you find greatly increases the ability of ComboSyn staff to fix the problem.

You can email bug reports to bugs@nimlabs.org. Please include in the body of the e-mail a description of the problem and any error messages you receive as well as attaching the experiment file that produced the error. The last few lines shown in the Console Window are also very helpful. All bug reports are confidential.

6.3 Contacting ComboSyn

ComboSyn can be contacted in several ways. They are listed in order of preferability.

E-mail	compusyn@nimlabs.org
US Mail	ComboSyn, Inc.
	599 Mill Run
	Paramus, NJ
	07653, USA

Note that for bug reports please use the procedure detailed in §6.2 for Contacting ComboSyn.

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