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Modeling and Synergy Testing of Drug Combination Data: A Pharmacokinetic Analysis

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MODELING AND SYNERGY TESTING OF DRUG COMBINATION DATA:

A PHARMACOKINETIC ANALYSIS

by

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CERTIFICATE OF APPROVAL

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ABSTRACT

In this paper, we present and implement a method to assess the mathematical synergy of two-drug combinations based on a stochastic model. The drugs in question are two isomers that are applied to the human eye via a liquid eye drop. Techniques applied to the data in this paper can be applied to other two-drug combination studies.

We derive the mean and the variance terms of the drug combination "effects" in closed form using Ito's method of stochastic differential equations. The model fit of the data to the individual subject is examined by both statistical and graphical methods. Two estimation methods in SAS, PROC NLIN and PROC NLMIXED, are used to estimate model parameters. We perform simulation and power studies using R software to show the strengths of the proposed approach in estimating the model parameters.

From this research, we find that the combination of drugs under study is synergistic in nature. We also confirm that the proposed stochastic model is appropriate.

1. INTRODUCTION

Combining drugs in biological systems is a common practice, and the effect can be one of three types: additive, synergistic, or antagonistic. These three terms refer to the response generated by using drugs in combination. Additivity means that using the drugs in combination is equivalent to using them separately; synergy means that the response is amplified; and antagonism means that the response is inhibited.

Mathematical modeling is often used to describe the relationship of two drugs. One such model, referred to as the most suitable, is the Loewe additivity model under the assumption of no interactions in the model (Straetemans et al, 2005). This model refers to a combination of two concentrations of drug A and drug B being additive (as defined above) and the total response is equivalent to an interaction effect. Feng and Kelly (2004) have established an extension of a model-free test for synergy in multiple drug combinations. This test can detect if synergy is present for a particular combination of two drugs using a nonparametric hypothesis test.

Sen and Bell (2006) used additive models generated from stochastic differential equations for combinations of two anesthetic agents. In classical statistics, interaction is always defined by a multiplication. However, Sen and Bell suggest that interaction in a biological model can actually be defined linearly. They successfully modeled the

absorption of two chemicals – morphine and midozolam – in a two-drug combination experiment. A simplified depiction of the compartmental modeling technique they used is shown in Figure #1 below.
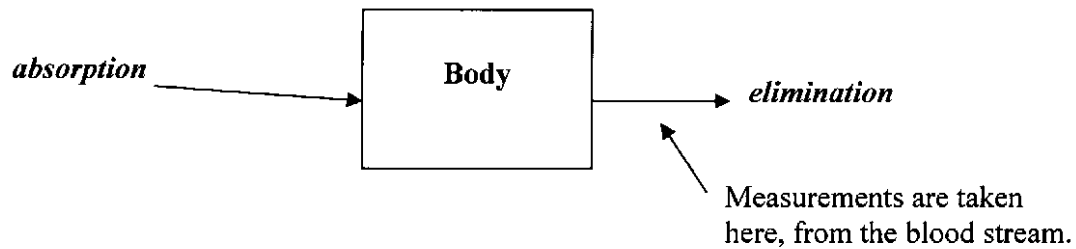
Figure # 1. Diagram depicting the flow of drugs through the human body.

Their findings suggest that their particular combination of morphine and midozolam can be defined linearly; and in fact, it can be defined as an antagonistic relationship. It is important to not that this type of stochastic modeling is a new technique that has not previously been applied to drug combination experiments.

Dr. Thomas Bradstreet performed an experiment in 1992 to determine if there was a difference in the time it took two isomers to absorb into the human body. Isomers are chemical compounds that are essentially the same, but are rotated in a way that causes them to behave differently in chemical reactions. When the rotation causes them to look like mirror images of each other, they are referred to as chiral or stereoisomers. Since the experiment was performed so long ago, there weren't many details surrounding the results. All we were given was the procedure and the final data set.

Our original intent was to apply the Sen and Bell model to a new two-drug combination experiment. Since Sen and Bell use a linear approach to modeling, we can

also apply the concepts of synergy testing to this data set. If we use the Feng and Kelly method of synergy testing, we will be able to prove if synergy is present. This means that we will be able to detect an amplified effect. From the Sen and Bell model, this can be detected by a resulting positive linear model of the drug "effects."

It is also important to note that in the Bradstreet data, the only outcomes we are given are the total concentrations of the isomers. In the morphine and midozolam data set, the actual "effects" of the drugs are given in the resulting data set. In the Sen and Bell paper, they are able to make assumptions about the biological outcomes of the experiment. However, in the Bradstreet analysis, we can only model the concentrations and prove a mathematical synergy. While this will not prove anything about the "effect" of the two isomers, it is useful for studying their behavior in the human body.

To summarize, we use the Sen and Bell approach for developing the model for data involving two chemicals (more specifically, two chiral isomers) applied in combination to a single subject. We then propose the necessary and sufficient conditions to validate the Feng and Kelly test, showing the additive nature of a particular drug combination. It is a biological synergy test, meaning that the data they intended for this type of test has some measure of the "effect" of the drugs used. However, we will use the idea behind the synergy test to justify the use of the Sen and Bell stochastic model in drug combination experiments. That is, using the methods behind the synergy test, we justify the linear model. And extension of this paper would be to apply these techniques to a two-drug combination experiment where the drug "effects" are measured, and synergy is suspected.

We develop the model in section 2. The dataset is described in section 3, followed by the description of the synergy test and the statistical methodologies. We present results in section 6 and a simulation study comparison in section 7. In Section 8, we provide the discussion of the findings.

2. DEVELOPING THE MODEL

We start with the model presented in the paper by Sen and Bell (2006),

$$dx_t = (-\alpha x + v e^{-\beta t})dt + \kappa dw \tag{1}$$

where:

$$x_t \equiv \text{the concentration,}$$

$$\alpha \equiv \text{the rate of elimination,}$$

$$v \equiv \text{the initial concentration of the chemical,}$$

$$\beta \equiv \text{the rate of absorption,}$$

and

$$\kappa \equiv \text{the coefficient associated with}$$

$$w \equiv \text{a Wiener process.}$$
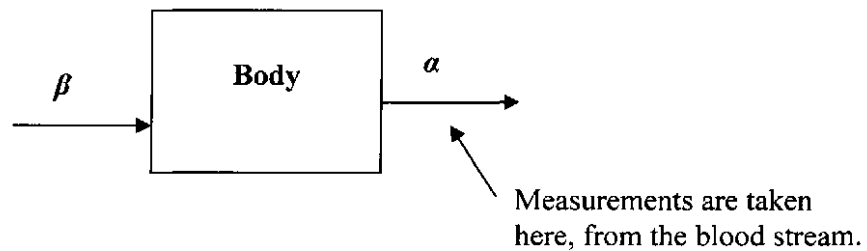
Figure # 2. Diagram depicting the flow of drugs through the human body.

We assume that the initial concentration $x(0)$ is zero and that α, β, and κ are constants. The drugs of interest, referred to from now on as isomers, are assumed to follow diffusion processes x_1 and x_2 with the same underlying Wiener process w.

First, we will develop the model for the concentration of a single isomer. Solving equation (1), we have:

$$x_t = \int_0^t \left((ve^{-\beta t} - \alpha x_t)dt + \kappa dw \right). \tag{2}$$

Now, given that $\mu_t = E(x_t)$ and equation (2), we can rewrite this as

$$E(x_t) = E\left[\int_0^t (ve^{-\beta t} - \alpha x_t)dt + \kappa dw \right],$$

and finally,

$$\mu_t = \int_0^t (ve^{-\beta t} - \alpha \mu_t)dt + 0.$$

Next, we will solve for μ_t in a closed form.

$$\mu_t' = ve^{-\beta t} - \alpha \mu_t$$

$$\mu_t' e^{\alpha t} + \alpha \mu_t e^{\alpha t} = ve^{(-\beta + \alpha)t}$$

$$\frac{d}{dt}(\mu_t e^{\alpha t}) = ve^{(-\beta + \alpha)t}$$

$$\int_0^t \frac{d}{dt}(\mu_t e^{\alpha t})dt = \int_0^t \left(ve^{(-\beta + \alpha)t} \right)dt$$

$$\mu_t e^{\alpha t} = \frac{v}{\alpha - \beta}\left(e^{(-\beta + \alpha)t} - 1 \right)$$

$$\mu_t = \frac{v}{\alpha - \beta}\left(e^{-\beta t} - e^{-\alpha t} \right) \tag{3}$$

To develop the variance, we must use techniques from Ito's calculus (Gard, 1988). Let $r_t = E(x_t^2)$. By Ito, $dwdw = dt$, $dtdt = dwdt = dtdw = 0$, and

$$d(x_t^2) = 2x_t dx_t + dx_t dx_t. \tag{4}$$

Thus, using equation (1) and (4) we have the following:

$$d(x_t^2) = 2x_t \left((ve^{-\beta t} - \alpha x_t)dt + \kappa dw_t \right) + \kappa^2 dt$$

$$E\left[d(x_t^2) \right] = E\left[2x_t \left((ve^{-\beta t} - \alpha x_t)dt + \kappa dw_t \right) + \kappa^2 dt \right]$$

Since the functions within the expectations are both continuous, we have:

$$d\left[E(x_t^2) \right] = 2\mu_t ve^{-\beta t} dt - 2\alpha r_t dt + \kappa^2 dt$$

$$dr_t = 2\mu_t ve^{-\beta t} dt - 2\alpha r_t dt + \kappa^2 dt$$

$$r_t' + 2\alpha r_t = 2\mu_t ve^{-\beta t} + \kappa^2$$

$$r_t' e^{2\alpha t} + 2\alpha r_t e^{2\alpha t} = 2\mu_t ve^{(2\alpha-\beta)t} + \kappa^2 e^{2\alpha t}$$

$$\frac{d}{dt}\left(r_t e^{2\alpha t} \right) = \frac{2v^2}{\alpha - \beta}\left(e^{-\beta t} - e^{-\alpha t} \right)e^{(2\alpha-\beta)t} + \kappa^2 e^{2\alpha t}$$

$$\int_0^t \frac{d}{dt}\left(r_t e^{2\alpha t} \right)dt = \int_0^t \left(\frac{2v^2}{\alpha - \beta}e^{(2\alpha-2\beta)t} - \frac{2v^2}{\alpha - \beta}e^{(\alpha-\beta)t} + \kappa^2 e^{2\alpha t} \right)dt .$$

Since $r_t(0) = 0$, this is evaluated as:

$$r_t e^{2\alpha t} = \frac{2v^2}{2(\alpha - \beta)^2}\left(e^{(2\alpha-2\beta)t} - 1 \right) - \frac{2v^2}{(\alpha - \beta)^2}\left(e^{(\alpha-\beta)t} - 1 \right) + \frac{\kappa^2}{2\alpha}\left(e^{2\alpha t} - 1 \right)$$

$$r_t = \frac{v^2}{(\alpha - \beta)^2}\left(e^{-2\beta t} - e^{-2\alpha t} \right) - \frac{2v^2}{(\alpha - \beta)^2}\left(e^{(-\alpha-\beta)t} - e^{-2\alpha t} \right) + \frac{\kappa^2}{2\alpha}\left(1 - e^{-2\alpha t} \right)$$

$$r_t = \frac{v^2}{(\alpha - \beta)^2}\left(e^{-2\beta t} - 2e^{(-\alpha-\beta)t} + e^{-2\alpha t} \right) + \frac{\kappa^2}{2\alpha}\left(1 - e^{-2\alpha t} \right) \tag{5}$$

Using the variance formula $V(x_t) = E(x_t^2) - (E(x_t))^2$ and equations (4) and (5),

we have:

$$V(x_t) = r_t - \mu_t^2$$

$$V(x_t) = r_t - \frac{v^2}{(\alpha - \beta)^2}\left(e^{-2\beta t} - 2e^{(-\alpha - \beta)t} - e^{-2\alpha t}\right)$$

$$V(x_t) = \frac{\kappa^2}{2\alpha}\left(1 - e^{-2\alpha t}\right) \tag{6}$$

We use the linear combination

$$x \equiv c_1 x_1 + c_2 x_2 \tag{7}$$

to represent the total response produced by using the two isomers in combination.

Starting with equation (7), it follows immediately that the mean is

$$E(x) = c_1 \mu_1 + c_2 \mu_2$$

$$E(x) = \frac{c_1 v_1}{\alpha_1 - \beta_1}\left(e^{-\beta_1 t} - e^{-\alpha_1 t}\right) + \frac{c_2 v_2}{\alpha_2 - \beta_2}\left(e^{-\beta_2 t} - e^{-\alpha_2 t}\right). \tag{8}$$

Now, let $c_t = E(x_1 x_2)$. We will use the variance formula

$$V(x_t) = c_1^2 V(x_1) + c_2^2 V(x_2) + 2c_1 c_2 \, \text{cov}(x_1, x_2) \tag{9}$$

and covariance formula

$$\text{cov}(x_1, x_2) = E(x_1 x_2) - E(x_1)E(x_2) \tag{10}$$

to solve for the variance of the combination described in equation (7) in closed form.

By Ito,

$$d(x_1 x_2) = x_2 dx_1 + x_1 dx_2 + dx_1 dx_2. \tag{11}$$

Thus, using equation (1) we have the following:

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$$d(x_1 x_2) = x_2((ve^{-\beta_1 t} - \alpha_1 x_1)dt + \kappa_1 dw) + x_1((ve^{-\beta_2 t} - \alpha_2 x_2)dt + \kappa_2 dw) + \kappa_1 \kappa_2 dt$$

$$E(d(x_1 x_2)) = E(x_2 ve^{-\beta_1 t}dt - \alpha_1 x_1 x_2 dt + \kappa_1 dw + x_1 ve^{-\beta_2 t}dt - \alpha_2 x_1 x_2 dt + \kappa_2 dw + \kappa_1 \kappa_2 dt)$$

$$d(E(x_1 x_2)) = \mu_2 ve^{-\beta_1 t}dt - c_t \alpha_1 dt + \mu_1 ve^{-\beta_2 t}dt - c_t \alpha_2 dt + \kappa_1 \kappa_2 dt$$

$$d(c_t) = \mu_2 ve^{-\beta_1 t}dt + \mu_1 ve^{-\beta_2 t}dt - c_t(\alpha_1 + \alpha_2)dt + \kappa_1 \kappa_2 dt$$

$$c_t^{'} + c_t(\alpha_1 + \alpha_2) = \mu_2 ve^{-\beta_1 t} + \mu_1 ve^{-\beta_2 t} + \kappa_1 \kappa_2$$

$$c_t^{'} e^{(\alpha_1 + \alpha_2)t} + c_t(\alpha_1 + \alpha_2)e^{(\alpha_1 + \alpha_2)t} = \mu_2 ve^{(\alpha_1 + \alpha_2 - \beta_1)t} + \mu_1 ve^{(\alpha_1 + \alpha_2 - \beta_2)t} + \kappa_1 \kappa_2 e^{(\alpha_1 + \alpha_2)t}$$

$$\frac{d}{dt}\left(c_t e^{(\alpha_1 + \alpha_2)t}\right) = \frac{v^2}{\alpha_2 - \beta_2}\left(e^{-\beta_2 t} - e^{-\alpha_2 t}\right)e^{(\alpha_1 + \alpha_2 - \beta_1)t} + \frac{v^2}{\alpha_1 - \beta_1}\left(e^{-\beta_1 t} - e^{-\alpha_1 t}\right)e^{(\alpha_1 + \alpha_2 - \beta_2)t} + \kappa_1 \kappa_2 e^{(\alpha_1 + \alpha_2)t}$$

$$\int_0^t \frac{d}{dt}\left(c_t e^{(\alpha_1 + \alpha_2)t}\right)dt = \int_0^t \left\{ \begin{array}{l} \dfrac{v^2}{\alpha_2 - \beta_2}\left(e^{(\alpha_1 + \alpha_2 - \beta_1 - \beta_2)t} - e^{(\alpha_1 - \beta_1)t}\right) \\ + \dfrac{v^2}{\alpha_1 - \beta_1}\left(e^{(\alpha_1 + \alpha_2 - \beta_1 - \beta_2)t} - e^{(\alpha_2 - \beta_2)t}\right) + \kappa_1 \kappa_2 e^{(\alpha_1 + \alpha_2)t} \end{array} \right\} dt$$

$$c_t e^{(\alpha_1 + \alpha_2)t} = v^2\left(e^{(\alpha_1 + \alpha_2 - \beta_1 - \beta_2)t} - 1\right)\left(\frac{1}{(\alpha_2 - \beta_2)(\alpha_1 + \alpha_2 - \beta_1 - \beta_2)} + \frac{1}{(\alpha_1 - \beta_1)(\alpha_1 + \alpha_2 - \beta_1 - \beta_2)}\right)$$
$$+ \frac{v^2}{(\alpha_1 - \beta_1)(\alpha_2 - \beta_2)}\left(e^{(\alpha_1 - \beta_1)t} + e^{(\alpha_2 - \beta_2)t} - 2\right) + \frac{\kappa_1 \kappa_2}{\alpha_1 + \alpha_2}\left(e^{(\alpha_1 + \alpha_2)t} - 1\right)$$

$$c_t = \frac{v^2}{(\alpha_1 - \beta_1)(\alpha_2 - \beta_2)}\left(e^{-(\beta_1 + \beta_2)t} + e^{-(\alpha_1 + \alpha_2)t} - e^{-(\alpha_2 + \beta_1)t} - e^{-(\alpha_1 + \beta_2)t}\right) + \frac{\kappa_1 \kappa_2}{\alpha_1 + \alpha_2}(1 - e^{-(\alpha_1 + \alpha_2)t})$$

Substituting the above result into equation (10) yields the covariance defined in the equation below.

$$\text{cov}(x_1, x_2) = c_t - \mu_1 \mu_2$$

$$\text{cov}(x_1, x_2) = \frac{\kappa_1 \kappa_2}{\alpha_1 + \alpha_2}(1 - e^{-(\alpha_1 + \beta_2)t}) \tag{12}$$

Finally, substituting into the variance formula described in equation (9), we have:

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$$V(x) = c_1^2 \sigma_1^2(t) + c_2^2 \sigma_2^2(t) + \frac{2c_1 c_2 \kappa_1 \kappa_2}{\alpha_1 + \alpha_2} \left(1 - e^{-(\alpha_1 + \alpha_2)t} \right)$$

$$V(x) = \frac{c_1^2 \kappa_1^2}{2\alpha_1} \left(1 - e^{-2\alpha_1 t} \right) + \frac{c_2^2 \kappa_2^2}{2\alpha_2} \left(1 - e^{-2\alpha_2 t} \right) + \frac{2c_1 c_2 \kappa_1 \kappa_2}{\alpha_1 + \alpha_2} \left(1 - e^{-(\alpha_1 + \alpha_2)t} \right). \qquad (13)$$

Using the mean and variance in equations (8) and (13), the full model used for

programming is defined as (Sen and Bell, 2006):

$$L \equiv \frac{E(x)}{\sqrt{V(x)}} \qquad (14)$$

In equations (8) and (13), v_1 and v_2 are initial amounts, α_1 and α_2 are the rates

of eliminations, β_1 and β_2 are the rates of absorptions for the two isomers, κ_1 and κ_2

represent the variability coefficients within each process, and c_1 and c_2 are two constants

as used in equation (2). In this paper, we refer to α's and β's as the main parameters for

the model, κ's as the variance parameters, and c's as the synergy parameters.

3. DESCRIPTION OF DATA

This study focuses on the concentrations of two chiral stereoisomers, S and R, in the human bloodstream. Isomers are essentially identical chemical substances that differ only in their stereochemistry. That is, the elemental make-up of the substances is exactly the same, but the 3-dimensional positioning is different. Specifically for S and R isomerism, certain components of the compound are arranged in a different circular pattern about the center. S isomers indicate "left-hand" arrangements while R isomers indicate "right-hand" arrangements. These differences are often compared to mirror images or the direction of spokes on a wheel (McMurry, 1988).

When the experiment begins, a blood sample is taken from each of seven volunteers (referred to as subjects) at the same location on the body. It is determined that the isomers are not present in any of the initial blood samples. That is, the concentration at time zero is 0 ng/mL (Bradstreet, 1992). A mixture containing equal parts of both isomers is applied via eye drops to the subjects at a rate of one drop per minute for three minutes. At the five minute time mark and eleven intervals thereafter, blood samples are taken from each subject and the concentration (in ng/mL) is recorded. We define the response of the single dose (either R or S) by the concentration amount of the isomer over

eleven time points. We also define the response of the combination dose by using the sum

of the S and R concentrations over the corresponding eleven time points.

Table # 1. Original Data Set (Bradstreet, 1992).

Concentrations (ng/ml) of S- and R-Isomers													
Subject	Isomer	Minutes Post-Dosing											
		0	5	10	15	30	45	60	90	120	240	360	480
1	S	0	9	18	26	33	39	46	52	37	54	61	66
	R	0	9	18	12	26	28	31	24	33	29	23	17
2	S	0	22	47	78	104	142	167	160	173	173	210	186
	R	0	26	49	69	100	101	113	126	98	75	57	40
3	S	0	23	29	49	57	69	85	85	107	148	159	174
	R	0	15	25	39	55	66	71	69	56	64	59	60
4	S	0	14	35	44	97	108	160	134	146	169	161	150
	R	0	24	58	85	99	102	109	103	99	74	47	36
5	S	0	25	38	52	77	86	86	96	131	134	141	144
	R	0	14	31	46	56	56	52	52	64	50	32	21
6	S	0	41	73	75	112	64	145	148	182	183	224	188
	R	0	38	65	73	76	105	99	105	95	53	38	30
7	S	0	27	37	42	61	86	110	148	140	205	205	202
	R	0	31	48	67	98	99	112	125	98	73	57	39

4. A STUDY FOR SYNERGY

Laska et al (1994) proposed a synergy test of two-drug combinations that does not require modeling a response surface. They have used a model-free test to establish a sufficient condition for synergy at a combination dose. The Loewe definition of additivity implies that the mathematical equation of the form below should be satisfied.

$$\sum_{i=1}^{n} x_i / x_{ie} = 1 \tag{15}$$

Here, n is the number of drugs used in combination, x_i is the dose amount of the ith drug, and x_{ie} is the dose amount of the ith drug that, given alone, would produce e, the magnitude of the response of the drug combination. The value $\sum_{i=1}^{n} x_i / x_{ie}$ in equation (15) is referred to Berenbaum's Interaction Index. When Berenbaum's Interaction Index is less than 1, the combination is synergistic. When it is greater than 1, the combination is antagonistic. When it is equal to 1, the combination is additive.

By definition, e is unknown and determined by a unique function g, the mapping function between dose and response. In this study, the g-function is the link between the initial dose amounts (either individual or combined), and the response produced (either individual or combined).

It is also important to define what is meant by the term "response." Response, in the Sen and Bell (2006) study, is measured by the "effect" of the drugs used in combination. In this study, response is measured by the concentration of the isomers in the bloodstream.

The equation (15) defines the theoretical line of dose additivity, where $x = (x_1, x_2)$ lies on an e-isobole (Laska et al 1994), a theoretical line describing a sampling of the dose combinations that produce synergistic, antagonistic, or additive responses. An example of an isobologram is given in Figure # 3.
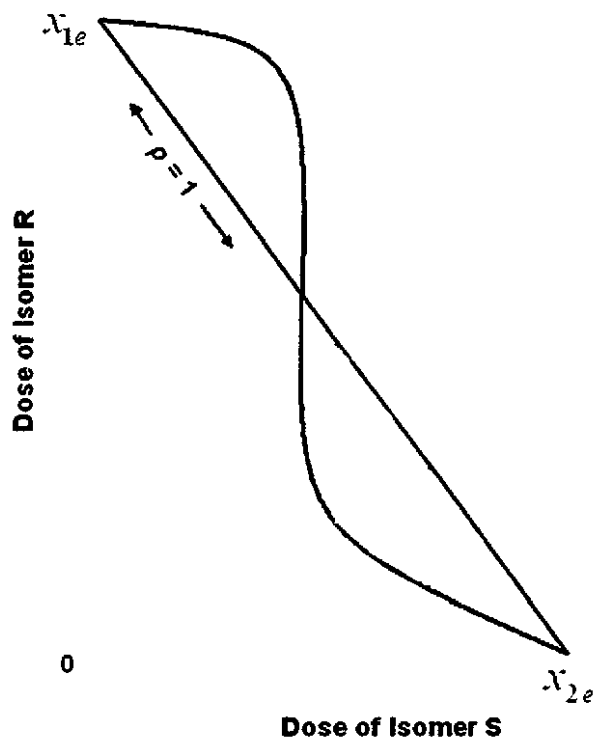
Figure # 3. A hypothetical isobologram.

The endpoints of the e-isobole lie on the e-theoretical line - the line connecting the dose points of x_{1e} to x_{2e}. At all points on the e-theoretical line of additivity, those dose combinations produce e. When a dose combination on the e-isobole produces a response that is above the e-theoretical line, the combination is defined as antagonistic. When the response is below the line, it is synergistic. The authors' proposed method for establishing a sufficient condition for a synergy test uses an arbitrary line through (x_1^*, x_2^*) given by $r(x_1 - x_1^*) + (x_2 - x_2^*) = 0$ where $x^* = (x_1^*, x_2^*)$ is a given dose point.

In the fixed ratio design of two drugs (Tallarida, 2000), the proportion between two chemicals used in combination is kept constant by controlling the dose amount. In other words, for two levels of drugs we look at g-function inequalities of the form $\rho g(x_R, x_S) + g(x_R, 0)$ or $\rho g(x_R, x_S) + g(0, x_S)$, compared to one. In the expression, $g(x_R, x_S)$ is a function of two drugs, $g(x_R, 0)$, and $g(0, x_S)$ are the functions of one drug in the absence of the other and ρ is the correlation coefficient that depends on the ratio of the combination of the two drugs and is defined using Berenbaum's Interaction Index.

For the synergy test, we use the null hypothesis, H_o of the format $rg(x_R^*, x_S^*) \geq g(x_R^*, 0)$ or $rg(x_R^*, x_S^*) \geq g(0, x_S^*)$ versus hypothesis of synergy H_a as $rg(x_R^*, x_S^*) < g(x_R^*, 0)$ and $rg(x_R^*, x_S^*) < g(0, x_S^*)$ respectively for a specified value of r. A necessary condition for this hypothesis test is that the power for the specified value of r is at least 0.5. Also, since correlation is time dependent and expected to change as concentration combination changes, we establish a bound on r for significant results of the synergy tests for all subjects.

5. STATISTICAL METHODOLOGY

The NLIN procedure in SAS uses the mean and variance calculated from the stochastic model without any assumptions about the parametric form of the distribution. The procedure uses the least squares method to fit the curve to the observation and estimate the model parameters. The process requires the first derivatives of the equation with respect to each model parameter to be estimated. Mean Squared Error (MSE) is calculated as a result of convergence of the NLIN procedure.

The NLMIXED procedure in SAS requires that the mean and the variance expressions for the equations be supplied as the initial input with the assumption that model has a normal distribution with the stated parameters. The procedure uses maximum likelihood estimates of the parameters while fitting the model to the observations. It includes information of the mean and variance for the combined data, and hence we estimate the combination coefficients c_1 and c_2 of the drug efficacies. We use individual data for later analyses where c_1 and c_2 are considered to be known constants.

Akaike's information criterion (AIC) is calculated for each selected model as

$$AIC = n * \ln\left(\frac{SSE}{n}\right) + 2k,$$ where k is the number of parameters to be estimated and SSE stands for sum of squared errors. A low value for AIC indicates a better fit (Sen, 2004).

The value of AIC is computed after the convergence of the NLMIXED procedure. The value of AIC is calculated for NLIN procedure from the respective MSE values.

As suggested by Laska and Meisner (1989) we use the Wilcoxon Rank Test for the hypotheses that establishes a sufficient condition for synergy with a 0.05 significance level. Since the distribution of the time data for each subject is unknown, the nonparametric min test is appropriate. We perform the power study using the WILCOX.TEST procedure in R for a simulation size of 5000 data sets. For the synergy hypotheses, we test seven pairs of hypotheses against one sided alternatives, one pair per subject on each isomer type for combined and individual data.

For simulations, we use R software to generate 20,000 data sets for each patient. Using the estimated parameters from both the NLIN and NLMIXED procedures, we supply the initial estimates of the parameters and use the NLS function to check the convergence of the model parameters to their initial values.

6. RESULTS

The initial dose amounts are considered known and used as constants throughout the analysis. We utilize the combined data for seven subjects to estimate the parameters in equation (14). We use the equations (8) and (13) to input the mean and variance required in the procedure. The NLMIXED procedure was necessary to incorporate the subjects as blocking variables. This allowed us to analyze the data in its entirety and estimate the synergy parameters c_1 and c_2 as given in Table # 2. This capability is not available from the NLIN procedure. In Table # 2 below, the only meaningful parameters are c_1 and c_2. The others are not applicable to a larger model since parameters such as absorption are intended to be studied for one subject rather than a group.

Table # 2. Parameter estimates from PROC NLMIXED using the full model, using combined data.

Parameter	α_1	β_1	c_1	κ_1	α_2	β_2	c_2	κ_2
Estimate +/- standard error	0.09299 +/- 0.1077	0.02499 +/- 0.06809	1.113 +/- 1.0098	-10.640 +/- 24.4854	0.02475 +/- 0.01893	0.00028 +/- 0.00056	1.107 +/- 0.825	17.0014 +/- 6.0380

We use the individual data to estimate mean and variance parameters for seven subjects using both the NLIN and NLMIXED procedures. The results are presented in Tables # 3 - 4, using the given coefficients of c_1 and c_2 from Table # 2.

Table # 3. Parameter estimates for subjects 1-7 from PROC NLIN using individual data. (Standard Errors are in parenthesis.)

Subject	PROC NLIN					
	α_1	β_1	κ_1	α_2	β_2	κ_2
1	0.0116	0.0001	-1.6259	0.0304	0.0590	2.7249
	(0.0138)	(0.00083)	(0.9421)	(0.0101)	(0.1688)	(1.0368)
2	0.0078	0.0010	-0.4173	0.0260	0.0239	0.7696
	(0.0184)	(0.00272)	(0.4009)	(0.0135)	(0.0530)	(0.4214)
3	0.0476	0.0427	1.0600	0.0103	-0.0003	-0.4560
	(0.0296)	(0.1007)	(0.0748)	(0.0110)	(0.00085)	(0.1791)
4	0.0626	-0.0016	-0.6967	0.0046	0.0050	0.2330
	(0.0788)	(0.00206)	(0.4343)	(0.0426)	(0.0176)	(0.5246)
5	0.1163	-0.0018	1.0275	0.0109	0.0016	-0.3480
	(0.0927)	(0.0209)	(0.2282)	(0.0608)	(0.0173)	(0.4120)
6	0.0195	0.0508	-0.2631	-0.0119	0.0572	0.0470
	(0.0130)	(0.2504)	(0.0850)	(0.0322)	(0.1945)	(0.0927)
7	0.1702	-0.2898	2.6484	-0.2928	-0.2646	-0.5329
	(4.9554)	(4.7923)	(42.5844)	(4.7738)	(4.7690)	(4.3407)

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Table # 4. Parameter estimates for subjects 1 - 7 from PROC NLMIXED using

individual data. (Standard Errors are in parenthesis.)

Subject	PROC NLMIXED					
	α_1	β_1	κ_1	α_2	β_2	κ_2
1	0.0796	-0.0005	0.0001	7.8761	3.2148	-18.3960
	(0.00324)	(0.00015)	(4.8522)	(5.1508)	(0.00001)	(4.4106)
2	0.0231	0.0230	2.7994	0.0209	0.0003	-0.5299
	(0.01068)	(0.01063)	(36.5755)	(0.00203)	(0.0003)	(35.7244)
3	0.0346	-0.0009	1.3451	0.0251	0.3338	-3.0440
	(0.00173)	(0.00015)	(8.0622)	(0.01983)	(0.1183)	(7.5456)
4	0.0410	0.0008	-3.4502	0.0008	0.0397	0.0162
	(0.02101)	(0.00196)	(1.2728)	(0.00191)	(0.02022)	(0.5374)
5	0.2167	0.0001	7.6815	0.0373	0.0000	-4.7880
	(0.09124)	(0.00335)	(4.3065)	(0.00514)	(0.0006)	(1.5228)
6	0.0730	0.0004	15.7446	0.0272	0.0005	-4.6469
	(0.03567)	(0.00588)	(9.8340)	(0.00892)	(0.00233)	(9.0839)
7	0.0926	0.0229	4.5984	0.0005	0.0183	0.0762
	(0.0531)	(0.02304)	(2.1488)	(0.00029)	(0.00161)	(0.6630)

The above two procedures both generated parameter estimates with reasonably

low standard errors. Of course, the variance parameters are expected to have larger

standard errors due to their nature.

Our next point of comparison is to look at the AIC values generated by each

procedure. The AIC values in Table # 5 show lower numbers for the NLIN procedure in

every subject as compared to the NLMIXED procedure, and points towards a better fit of

the data.

Table # 5. AIC comparisons for the individual subjects using two SAS procedures.

SUBJECT	NLMIXED	NLIN
1	79.2	35.8
2	93.6	68.5
3	88.5	54.3
4	98.0	71.8
5	94.9	63.0
6	115.8	78.5
7	97.7	72.1

Finally, using the parameter estimates obtained from the data in previous tables, we fit the estimated equations to the individual data as shown in Figure # 4. The fitted curves below indicate an extremely good fit of the model to the data by both the procedures. A careful look at the fitted curves by the NLIN procedure confirms that it follows the data for the individual subjects slightly better than the curves fitted by the NLMIXED procedure.
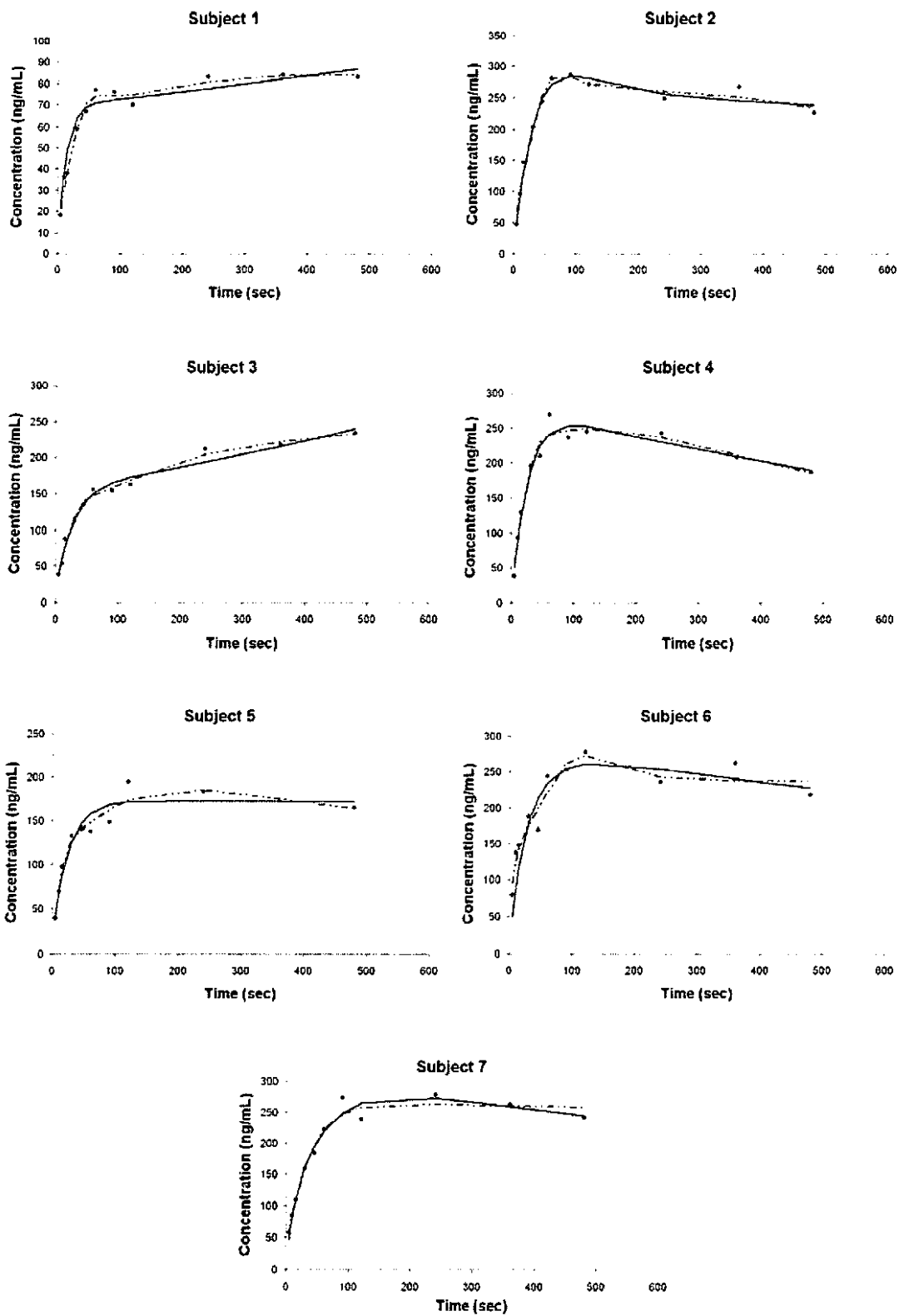
· obs --- NLIN — NLMIXED

Figure # 4. (previous page) Concentration versus time for individual subjects plotted with estimated curves by NLIN (dotted lines) and NLMIXED (solid lines) methods on the observed values.

Overall, we found that for this data set, the NLIN procedure in SAS fit the data the best. For the next part of our research, we studied the necessary and sufficient conditions for the synergy test. The necessary condition involved a power study. The sufficient conditions are verified using a hypothesis test.

We performed a power study using R software to determine the significant ranges for r for each subject. The results are given in Table # 6. The lower bound for r is the maximum possible power (i.e. it is equal to 1.00). The upper bound for r is the first estimate that generated an acceptable power (i.e. it is above 0.5). Using these results, we perform the synergy tests on the observed data.

Table # 6. Power study for the synergy tests using individual data.

Subject	Result for r	
	Lower Bound	Upper Bound
1	0.08	0.23
2	0.17	0.26
3	0.19	0.27
4	0.20	0.29
5	0.12	0.23
6	0.34	0.71
7	0.21	0.28

In Table # 6, Subject 6 shows the maximum strength of maintaining the power. The synergy tests for individuals based on the choice of correlation coefficient between 0.08 and 0.23 (over all data) gives p-values less than 0.05 for each subject when performed for both the isomers. The W-Score for the Wilcoxon Rank-Sum statistics and the p-values are shown in Tables # 7 – 8.

Table # 7. Synergy tests for hypothesis $H_0 : r * g(x_R^*, x_S^*) \geq g(x_R^*, 0)$ for subjects 1 – 7 using isomer R.

Subject		Wilcoxon Rank-Sum Results	
		Lower Bound	Upper Bound
1	W	121	97
	p-value	<0.0001	0.009025
2	W	121	92
	p-value	<0.0001	0.01999
3	W	118	101
	p-value	<0.0001	0.003317
4	W	121	97
	p-value	<0.0001	0.009025
5	W	119	89
	p-value	<0.0001	0.03291
6	W	121	90
	p-value	<0.0001	0.02837
7	W	121	97
	p-value	<0.0001	0.009025

Table # 8. Synergy test for hypothesis $H_0 : r * g(x_R^*, x_S^*) \geq g(0, x_S^*)$ for subjects $1 - 7$ using isomer S.

Subject		Wilcoxon Rank-Sum Results	
		Lower Bound	Upper Bound
1	W	121	110
	p-value	<0.0001	0.0006443
2	W	119	104
	p-value	<0.0001	0.002369
3	W	121	104
	p-value	<0.0001	0.002369
4	W	113	96
	p-value	<0.0001	0.009615
5	W	121	110
	p-value	<0.0001	0.0006443
6	W	121	114
	p-value	<0.0001	<0.0001
7	W	121	97
	p-value	<0.0001	0.009025

Using the ratio of $\frac{c_1}{c_1 + c_2}$ and $\frac{c_2}{c_1 + c_2}$ in Table # 2 we get an estimate of ρ as

close to 0.5 over all subjects. The estimate of ρ from Table # 2 is a crude estimate of the

ratio of the combination of two chemicals acting simultaneously for the combined data.

The variability of seven individuals entered into the calculation and is considered to have

inflated the estimate. Therefore, we establish a bound for r, which is attuned to our data

set, between 0.08 and 0.23 using the individual subjects. In this range, all of the above

tests still remain significant establishing the sufficient condition for synergy.

A description of the estimated values for the correlation coefficient is shown

below in the isobologram in Figure # 5. The theoretical isobole described in Laska et al

(1994) illustrates two hypothetical doses with a generic placement of the synergistic

locations. The straight line represents the e-theoretical line of additivity connecting

x_{1e} and x_{2e}. In Figure # 4, we use the same line setup and place the correlation bounds to

show the synergistic power and possible location for the dose combination of the data.
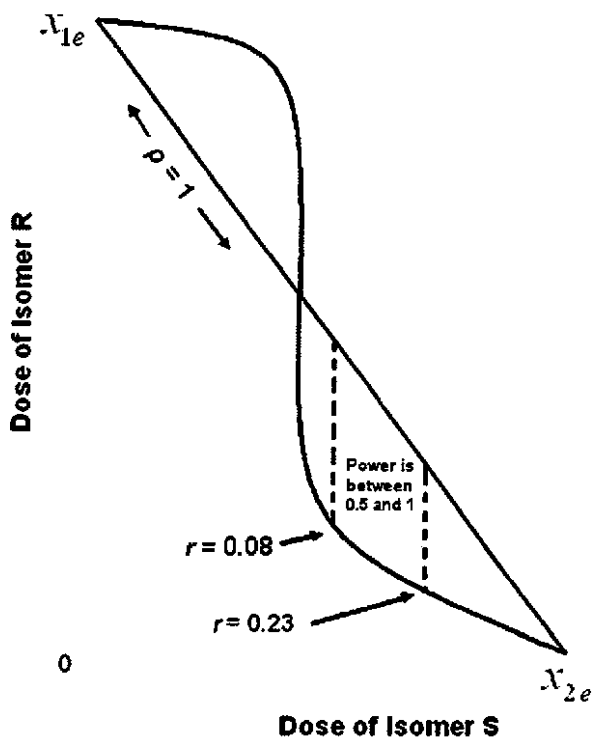
Figure # 5. A hypothetical isobologram with the estimated bounds for the correlation

coefficient and the corresponding power is shown here. Note that the isobole curve

represents only a sampling of the possible dose combinations.

7. SIMULATIONS AND EFFICIENCY COMPARISON FOR PARAMETER ESTIMATIONS

A Bootstrapping Monte Carlo simulation study was conducted for individual subjects to compare the inferential performance of the NLIN and NLMIXED procedures described in section 5. We considered the parameter estimates from the data as the initial starting points. After 20,000 iterations, the equations converged to assigned criteria and produced the average of each parameter value with the corresponding standard deviation. We used the known estimates of c_1 and c_2 in the simulation to be consistent with the synergy test.

In most cases in Tables # 9 - 10 we get extremely close to the true parameters in Tables # 3 - 4. Difference tables (shown in Tables # 11-12) indicate that some of the variance (κ_1, κ_2) parameters were incorrectly estimated by the data, though the main parameter estimates were quite close. Only one indication of a large difference was detected by the simulation. Table # 12, the NLMIXED difference table, shows a large difference for two main parameter estimates for Subject 1.

Table # 9. Simulation results for subjects 1 – 7 using parameters of PROC NLIN

procedures. (Standard Errors are in parenthesis.)

Subject	PROC NLIN					
	α_1	β_1	κ_1	α_2	β_2	κ_2
1	0.0116	0.0001	-1.6270	0.0304	0.0593	2.7250
	(0.00003)	(0.000002)	(0.00185)	(0.00002)	(0.00033)	(0.00204)
2	0.0078	0.0010	-0.4174	0.0260	0.0239	0.7696
	(0.00001)	(0.000001)	(0.00017)	(0.00001)	(0.00002)	(0.00018)
3	0.0477	0.0426	1.0600	0.0103	-0.0003	-0.4561
	(0.00002)	(0.00008)	(0.00006)	(0.00001)	(0.000001)	(0.00014)
4	0.0626	-0.0016	-0.6967	0.0046	0.0050	0.2330
	(0.00003)	(0.000001)	(0.00019)	(0.00002)	(0.00001)	(0.00023)
5	0.1162	-0.0018	1.0280	0.0109	0.0016	-0.3479
	(0.00005)	(0.00001)	(0.00012)	(0.00003)	(0.00001)	(0.00022)
6	0.0195	0.0509	-0.2632	-0.0118	0.0571	0.0470
	(0.000004)	(0.00007)	(0.00002)	(0.00001)	(0.00006)	(0.00003)
7	0.1699	-0.2899	2.6495	-0.2929	-0.2647	-0.5330
	(0.00215)	(0.00208)	(0.01848)	(0.00207)	(0.00207)	(0.00188)

Table # 10. Simulation results for subjects 1 – 7 using parameters of PROC NLMIXED

procedures. (Standard Errors are in parenthesis.)

Subject	PROC NLMIXED					
	α_1	β_1	κ_1	α_2	β_2	κ_2
1	0.0323	-0.0005	-4.0940	0.0985	0.0197	9.7950
	(0.00031)	(0.00001)	(0.05381)	(0.00094)	(0.00055)	(0.0706)
2	0.0231	0.0230	2.7990	0.0209	0.0002	-0.5299
	(0.01237)	(0.01327)	(16.7000)	(0.0005)	(0.00001)	(16.8000)
3	0.0346	-0.0009	1.2730	0.0248	0.3344	-2.9730
	(0.00075)	(0.000002)	(0.1415)	(0.00108)	(0.00771)	(0.1348)
4	0.0419	0.0008	-3.5496	0.0008	0.0418	0.1042
	(0.00257)	(0.01442)	(0.36869)	(0.01535)	(0.01326)	(0.39592)
5	0.2163	0.0004	7.6820	0.0373	0.0000	-4.7880
	(0.00035)	(0.00038)	(0.01632)	(0.00022)	(0.00005)	(0.01551)
6	0.0718	0.0004	15.5999	0.0271	0.0005	-4.5510
	(0.0019)	(0.00088)	(0.21123)	(0.00016)	(0.00034)	(0.22363)
7	0.0947	0.0253	4.6770	0.0005	0.0187	0.0122
	(0.00143)	(0.00189)	(0.07197)	(0.000005)	(0.00028)	(0.06414)

Table # 11. Difference of true parameter values from estimated values with NLIN.

Subject	PROC NLIN					
	α_1	β_1	κ_1	α_2	β_2	κ_2
1	0.0000	0.0000	0.0011	0.0000	-0.0004	-0.0001
2	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000
3	0.0130	0.0436	-0.2851	-0.0148	-0.3341	2.5880
4	0.0215	-0.0024	2.7535	0.0038	-0.0347	0.2168
5	-0.1004	-0.0019	-6.6540	-0.0265	0.0016	4.4400
6	-0.0535	0.0504	-16.0077	-0.0391	0.0567	4.6939
7	0.0776	-0.3127	-1.9500	-0.2933	-0.2829	-0.6091

Table # 12. Difference of true parameter values from estimated values with NLMIXED.

Subject	PROC NLMIXED					
	α_1	β_1	κ_1	α_2	β_2	κ_2
1	0.0796	-0.0005	-1.0000	7.8645	3.2147	-16.7690
2	0.0231	0.0230	0.7994	0.0131	-0.0008	-0.1125
3	0.0346	-0.0009	-1.6549	-0.0226	0.2912	-4.1040
4	0.0410	0.0008	-7.4502	-0.0617	0.0414	0.7129
5	0.2167	0.0001	2.6815	-0.0789	0.0018	-5.8160
6	0.0730	0.0004	9.7446	0.0076	-0.0504	-4.3837
7	0.0926	0.0229	-2.4016	-0.1694	0.3082	-2.5733

NLIN converged faster than NLMIXED and the NLIN parameter estimates are closer to the actual values obtained from the experimental data. We encountered problems of occurring singular values using NLMIXED.

The variance parameters have large standard errors, but this was expected. When data is generated by a simulation technique, it is common to see inflated variance estimates. However, the bulk of the convergence was smooth giving precise estimates with very small standard errors. We suspect that NLIN performs better due to the lack of a distribution assumption. To perform calculations for parameter values, we suggest the NLIN procedure in SAS.

8. DISCUSSION

Pharmacology studies deal with combined data that are interactive in nature. In mathematical terms, an interaction is usually represented by multiplication of terms. However, it is not quite clear for drug interactions if the combined effect is additive, multiplicative, or inhibitive in nature. Combination of isomers in drugs administered to the eye gives a reason to test for synergy to assert the researchers' effort to show an additive effect.

In this paper, we find that our proposed model shows a positive linear relationship between the concentrations of the two isomers. We also have enough evidence to conclude that, by the Feng and Kelly synergy test, that the two drugs are indeed synergistic in nature. As a result of these findings, we show that the linear stochastic model, as proposed by Sen and Bell, is appropriate for modeling drug combination data.

The proposed model can be analyzed with simple techniques from differential equations to determine the model mean and variance in closed form, and it is useful in the data analysis. The two different methods of statistical procedures, NLIN and NLMIXED, are used here to confirm that our model has the flexibility required for use by practitioners.

The proposed model uses a limited number of dose combinations since we were restricted to using the existing data set; however, the data collection can be done based on the recommendations in the paper of Laska et al (1994). Necessary and sufficient conditions should be tested before implementing the modeling techniques presented in this paper.

Many methods of drug assessment and model building are available. Some focus on testing for a biological synergy based on the effects generated by a particular drug combination. Since no biological "effects" are measured in the Bradstreet (1992) data, the model we propose tests for a mathematical synergy instead of a biological synergy, and biological synergy is not an implied result. However, if biological effects data is collected based on the combined dose levels as suggested by other researchers, we can test for synergy as shown in this paper.

APPENDIX II: PROGRAMMING ALGORITHMS

Note: The code listed is representative of the code used to generate results; however, redundant iterations have been removed and data tables have been shortened.

Algorithm 1. Fitting the Model in SAS

Step 1. Input data.

```
options nodate linesize=80;
data isomerorig;
input t res isomer $; v=5;
cards;
     5    18 ONE
    10    36 ONE
    15    38 ONE
    30    59 ONE
;run;
```

Step 2. Run PROC NLMIXED using entire dataset with all parameters unknown.

```
proc nlmixed data=isomerorig maxiter=1500;
parms   a = 0.8  b = -0.005 c=0.8 k=2.1 w=.04 x=-0.0002 y=1.4 z=.9;
mean=c*v*(exp(-b*t)-exp(-a*t))/(a-b)+y*v*(exp(-x*t)-exp(-w*t))/(w-x);
var = c*c*k*k*(1-exp(-2*a*t))/(2*a)+y*y*z*z*(1-exp(-2*w*t))/(2*w)
+ 2*c*y*k*z*(1-exp(-a*t-w*t))/(a+w);
MODEL res~normal(mean,var);
SUBJECT=isomer;
PREDICT mean out=z;
run;
```

Step 3. Using c_1, c_2 from Step 2, input data for NLIN procedure.

```
data isomer2;
input t S1 R1 S2 R2 S3 R3 S4 R4 S5 R5 S6 R6 S7 R7;v=5; c=1.1134;
y=1.1072;
cards;
    5    9    9   22   26   23   15   14   24   25   14   41   38   27   31
   10   18   18   47   49   29   25   35   58   38   31   73   65   37   48
   15   26   12   78   69   49   39   44   85   52   46   75   73   42   67
   30   33   26  104  100   57   55   97   99   77   56  112   76   61   98
;
options nodate linesize = 80;
run;
```

Step 4. Calculate combination concentration.

```
data isomer1;
set isomer2;
ONE=S1+R1;
TWO=S2+R2;
THREE=S3+R3;
FOUR=S4+R4;
FIVE=S5+R5;
SIX=S6+R6;
SEVEN=S7+R7;
run;
```

Step 5. Evaluate model parameters using PROC NLIN.

```
proc nlin data=isomer1 converge = .00001 convergeparm = .00001
method = marquardt;
parms   a = 0.05  b = -0.0005 k=-4.1  w=.02 x=-0.0004 z=5.01;
model ONE =2*(c*v*(exp(-b*t)-exp(-a*t))/(a-b)+y*v*(exp(-x*t)-exp(-
w*t))/(w-x))/(2*c**2*k**2*(1-exp(-2*a*t))/a+2*y**2*z**2*(1-exp(-
2*w*t))/w+8*c*y*k*z*(1-exp(-a*t-w*t))/(a+w))**(1/2);
der.a = 2*(c*v*t*exp(-a*t)/(a-b)-c*v*(exp(-b*t)-exp(-a*t))/(a-
b)**2)/(2*c**2*k**2*(1-exp(-2*a*t))/a+2*y**2*z**2*(1-exp(-
2*w*t))/w+8*c*y*k*z*(1-exp(-a*t-w*t))/(a+w))**(1/2)-(c*v*(exp(-b*t)-
exp(-a*t))/(a-b)+y*v*(exp(-x*t)-exp(-w*t))/(w-x))*(4*c**2*k**2*t*exp(-
2*a*t)/a-2*c**2*k**2*(1-exp(-2*a*t))/a**2+8*c*y*k*z*t*exp(-a*t-
w*t)/(a+w)-8*c*y*k*z*(1-exp(-a*t-w*t))/(a+w)**2)/(2*c**2*k**2*(1-
exp(-2*a*t))/a+2*y**2*z**2*(1-exp(-2*w*t))/w+8*c*y*k*z*(1-exp(-a*t-
w*t))/(a+w))**(3/2);
der.w = 2*(y*v*t*exp(-w*t)/(w-x)-y*v*(exp(-x*t)-exp(-w*t))/(w-
x)**2)/(2*c**2*k**2*(1-exp(-2*a*t))/a+2*y**2*z**2*(1-exp(-
2*w*t))/w+8*c*y*k*z*(1-exp(-a*t-w*t))/(a+w))**(1/2)-(c*v*(exp(-b*t)-
```

exp(-a*t))/(a-b)+y*v*(exp(-x*t)-exp(-w*t))/(w-x))*(4*y**2*z**2*t*exp(-
2*w*t)/w-2*y**2*z**2*(1-exp(-2*w*t))/w**2+8*c*y*k*z*t*exp(-a*t-
w*t)/(a+w)-8*c*y*k*z*(1-exp(-a*t-w*t))/(a+w)**2)/(2*c**2*k**2*(1-
exp(-2*a*t))/a+2*y**2*z**2*(1-exp(-2*w*t))/w+8*c*y*k*z*(1-exp(-a*t-
w*t))/(a+w))**(3/2);
der.b = 2*(-c*v*t*exp(-b*t)/(a-b)+c*v*(exp(-b*t)-exp(-a*t))/(a-
b)**2)/(2*c**2*k**2*(1-exp(-2*a*t))/a+2*y**2*z**2*(1-exp(-
2*w*t))/w+8*c*y*k*z*(1-exp(-a*t-w*t))/(a+w))**(1/2);
der.x = 2*(-y*v*t*exp(-x*t)/(w-x)+y*v*(exp(-x*t)-exp(-w*t))/(w-
x)**2)/(2*c**2*k**2*(1-exp(-2*a*t))/a+2*y**2*z**2*(1-exp(-
2*w*t))/w+8*c*y*k*z*(1-exp(-a*t-w*t))/(a+w))**(1/2);
der.k = -(c*v*(exp(-b*t)-exp(-a*t))/(a-b)+y*v*(exp(-x*t)-exp(-w*t))/(w-
x))*(4*c**2*k*(1-exp(-2*a*t))/a+8*c*y*z*(1-exp(-a*t-
w*t))/(a+w))/(2*c**2*k**2*(1-exp(-2*a*t))/a+2*y**2*z**2*(1-exp(-
2*w*t))/w+8*c*y*k*z*(1-exp(-a*t-w*t))/(a+w))**(3/2);
der.z = -(c*v*(exp(-b*t)-exp(-a*t))/(a-b)+y*v*(exp(-x*t)-exp(-w*t))/(w-
x))*(4*y**2*z*(1-exp(-2*w*t))/w+8*c*y*k*(1-exp(-a*t-
w*t))/(a+w))/(2*c**2*k**2*(1-exp(-2*a*t))/a+2*y**2*z**2*(1-exp(-
2*w*t))/w+8*c*y*k*z*(1-exp(-a*t-w*t))/(a+w))**(3/2);
output out = ONE
p=pred r = resid PARMS=a b k w x z;
run;

Step 6. Evaluate model parameters using PROC NLMIXED.

```
data zpiece;
set isomerorig;
where isomer="ONE";
c=1.1134; y=1.1072;
run;

proc nlmixed data=zpiece maxiter=1500;
parms   a = 0.1   b = 0.005 k=-1.2 w=-.02 x=-0.0002 z=-1.9;
mean=c*v*(exp(-b*t)-exp(-a*t))/(a-b)+y*v*(exp(-x*t)-exp(-w*t))/(w-x);
var = c*c*k*k*(1-exp(-2*a*t))/(2*a)+y*y*z*z*(1-exp(-2*w*t))/(2*w)
+ 2*c*y*k*z*(1-exp(-a*t-w*t))/(a+w);
MODEL res~normal(mean,var);
SUBJECT=isomer;
PREDICT mean out=zpiece1;
run;
```

Step 7. Generate plots comparing NLMIXED and NLIN procedures.

```
data new2;
set ONE zpiece1;
run;

data new3;
set new2;
if ONE <.01 then p1=pred;
if ONE >.01 then p2=pred;
run;

proc plot;
plot ONE*t = 'o' p1*t = 'm' p2*t='l'/overlay;
title 'Comparing Pred and Obs for Patient ONE';
run;
```

Step 8. Gather results for all subjects and report.

Algorithm 2. Simulation Study in R

Step 1. Define model function and initial values.

```
test.model1 <- function(v1,c1,y1,a,b,k,w,x,z,t) {
response<-2*(c1*v1*(exp(-b*t)-exp(-a*t))/(a-b)+y1*v1*(exp(-x*t)-exp(-
w*t))/(w-x))/ sqrt(2*c1**2*k**2*(1-exp(-2*a*t))/a+2*y1**2*z**2*(1-
exp(-2*w*t))/w+8*c1*y1*k*z* (1-exp(-a*t-
w*t))/(a+w))+rnorm(1,mean=0,sd=sqrt(t))
return(response) }
v<-5.0000
c<-1.1134
y<-1.1072
ctl.obj <- nls.control(maxiter=500, tol=1e-02, minFactor = 1/9000)
sim<-5000
crit<-0.01
flag1<-0
flag2<-0
flag3<-0
flag4<-0
flag5<-0
flag6<-0
flag7<-0
flag11<-0
flag22<-0
flag33<-0
flag44<-0
flag55<-0
flag66<-0
flag77<-0
```

```
a1<- 0.01157 ;b1<- 6.51E-05 ;k1<- -1.6259   ;w1<- 0.030414 ;x1<- 0.058949 ;z1<- 2.724914
a11<-0.07963 ;b11<- -0.0005 ;k11<- 0.00005 ;w11<- 7.8761   ;x11<- 3.2148   ;z11<- -18.396
a2<- 0.00784 ;b2<- 0.001003 ;k2<- -0.41729 ;w2<- 0.025968 ;x2<- 0.02389   ;z2<- 0.769501
a22<-0.02308 ;b22<- 0.02301 ;k22<- 2.7994   ;w22<- 0.02094 ;x22<-0.000247 ;z22<- -0.5299
```

Step 2. Generate simulation data set.

```
R<-0
T<-0
i<-1
for (i in 1:sim){
R[1+11*(i-1)]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,5)
R[2+11*(i-1)]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,10)
R[3+11*(i-1)]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,15)
R[4+11*(i-1)]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,30)
R[5+11*(i-1)]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,45)
R[6+11*(i-1)]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,60)
R[7+11*(i-1)]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,90)
R[8+11*(i-1)]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,120)
R[9+11*(i-1)]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,240)
R[10+11*(i-1)]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,360)
R[11+11*(i-1)]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,480)
T[1+11*(i-1)]<-5
T[2+11*(i-1)]<-10
T[3+11*(i-1)]<-15
T[4+11*(i-1)]<-30
T[5+11*(i-1)]<-45
T[6+11*(i-1)]<-60
T[7+11*(i-1)]<-90
T[8+11*(i-1)]<-120
T[9+11*(i-1)]<-240
T[10+11*(i-1)]<-360
T[11+11*(i-1)]<-480
}
```

Step 3. Run NLS procedure to determine the new parameter estimates.

```
Test.matrix<-data.frame(T, R)
fit1<-nls(R ~ 2*(c*v*(exp(-b*T)-exp(-a*T))/(a-b)+y*v*(exp(-x*T)-exp(-
w*T))/(w-x))/ sqrt(2*c**2*k**2*(1-exp(-2*a*T))/a+2*y**2*z**2*(1-
exp(-2*w*T))/w+8*c*y*k*z*(1-exp(-a*T-w*T))/(a+w)),
data = Test.matrix,
start = list(a=a1, b=b1, k=k1, w=w1, x=x1, z=z1))
```

Step 4. Check the difference between initial parameter values and new estimates.

```
atest<-abs(coef(fit1)[1]-a1)
btest<-abs(coef(fit1)[2]-b1)
ktest<-abs(coef(fit1)[3]-k1)
wtest<-abs(coef(fit1)[4]-w1)
xtest<-abs(coef(fit1)[5]-x1)
ztest<-abs(coef(fit1)[6]-z1)
if(atest<crit && btest<crit && ktest<crit && wtest<crit && xtest<crit
&& ztest<crit){
flag1<-1
}
(summary(fit1))
(flag1)
```

Step 5. Gather results for all subjects and report.

Algorithm 3. Power Study in R

Step 1. Define the models.

```
test.model1 <- function(v1,c1,y1,a,b,k,w,x,z,t,p) {
response<-p*(2*(c1*v1*(exp(-b*t)-exp(-a*t))/(a-b)+y1*v1*(exp(-x*t)-
exp(-w*t))/(w-x))/ sqrt(2*c1**2*k**2*(1-exp(-
2*a*t))/a+2*y1**2*z**2*(1-exp(-2*w*t))/w+8*c1*y1*k*z*
(1-exp(-a*t-w*t))/(a+w))+rnorm(1,mean=0,sd=sqrt(t)))
return(response) }

test.modelR <- function(v1,c,d,e,t) {
response<- ((v1*(exp(-d*t)-exp(-c*t)))/(c - d))/sqrt(((e**2)/(2*c))*(1-
exp(-2*c*t)))+rnorm(1,mean=0,sd=sqrt(t))
return(response) }

test.modelS <- function(v1,f,g,h,t) {
response<- ((v1*(exp(-g*t)-exp(-f*t)))/(f - g))/sqrt(((h**2)/(2*f))*(1-exp(-
2*f*t)))+rnorm(1,mean=0,sd=sqrt(t))
return(response) }
```

Step 2. Define initials and input data.

```
sim<-5000
    a1<-  0.01157    ;b1<-  6.51E-05   ;k1<-  -1.6259
    a2<-  0.00784    ;b2<-  0.001003   ;k2<-  -0.41729
    a3<-  0.047611   ;b3<-  0.042653   ;k3<-  1.059978
    a4<-  0.062561   ;b4<-  -0.00162   ;k4<-  -0.69668
    a5<-  0.116269   ;b5<-  -0.0018    ;k5<-  1.027519
v<-5.0000
c<-1.1134
y<-1.1072
power1<-1
power2<-1
power3<-1
power4<-1
power5<-1
power6<-1
power7<-1
p<-0.20
```

Step 3. Use a while loop to determine the bound on *r*. (In this program, $r = p$.)

```
while(power1=power2&&power2=power3&&power3=power4&&power4
=power5&&power5=power6&&power6=power7&&power7=power1){
ptest<-0
```

Step 3.1. Use a for loop to generate the simulated data set. Inside this loop, use the WILCOX.TEST procedure to determine p-values for the corresponding value of *r*. If the p-value is significant, increment a counting variable by one unit.

```
for (i in 1:sim){
RS<-0
R<-0
S<-0
fit1<-0
fit2<-0
RS[1]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,5,p)
RS[2]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,10,p)
RS[3]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,15,p)
RS[4]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,30,p)
RS[5]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,45,p)
RS[6]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,60,p)
RS[7]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,90,p)
RS[8]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,120,p)
RS[9]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,240,p)
RS[10]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,360,p)
RS[11]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,480,p)
R[1]<-test.modelR(v,c1,d1,e1,5)
R[2]<-test.modelR(v,c1,d1,e1,10)
R[3]<-test.modelR(v,c1,d1,e1,15)
R[4]<-test.modelR(v,c1,d1,e1,30)
R[5]<-test.modelR(v,c1,d1,e1,45)
R[6]<-test.modelR(v,c1,d1,e1,60)
R[7]<-test.modelR(v,c1,d1,e1,90)
R[8]<-test.modelR(v,c1,d1,e1,120)
R[9]<-test.modelR(v,c1,d1,e1,240)
R[10]<-test.modelR(v,c1,d1,e1,360)
R[11]<-test.modelR(v,c1,d1,e1,480)
S[1]<-test.modelS(v,f1,g1,h1,5)
S[2]<-test.modelS(v,f1,g1,h1,10)
S[3]<-test.modelS(v,f1,g1,h1,15)
S[4]<-test.modelS(v,f1,g1,h1,30)
S[5]<-test.modelS(v,f1,g1,h1,45)
S[6]<-test.modelS(v,f1,g1,h1,60)
S[7]<-test.modelS(v,f1,g1,h1,90)
```

```
S[8]<-test.modelS(v,f1,g1,h1,120)
S[9]<-test.modelS(v,f1,g1,h1,240)
S[10]<-test.modelS(v,f1,g1,h1,360)
S[11]<-test.modelS(v,f1,g1,h1,480)

fit1<-wilcox.test(R,RS,alternative="greater")
fit2<-wilcox.test(S,RS,alternative="greater")

if(fit1$p.value<0.05&&fit2$p.value<0.05){
ptest<-ptest+1
}
}
```

Step 3.2. Calculate the estimated power using the number of significant p-values found during the simulation divided by the simulation size.

```
power1<-ptest/sim
```

Step 4. Repeat the above procedure to find the upper bound on r.

```
power1<-0
power2<-0
power3<-0
power4<-0
power5<-0
power6<-0
power7<-0

p<-0.5

while(power1<0.5&&power2<0.5&&power3<0.5&&power4<0.5&&pow
er5<0.5&&power6<0.5&&power7<0.5){

ptest<-0
for (i in 1:sim){
RS<-0
R<-0
S<-0
fit1<-0
fit2<-0
RS[1]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,5,p)
RS[2]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,10,p)
RS[3]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,15,p)
RS[4]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,30,p)
RS[5]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,45,p)
RS[6]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,60,p)
```

```
RS[7]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,90,p)
RS[8]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,120,p)
RS[9]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,240,p)
RS[10]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,360,p)
RS[11]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,480,p)
R[1]<-test.modelR(v,c1,d1,e1,5)
R[2]<-test.modelR(v,c1,d1,e1,10)
R[3]<-test.modelR(v,c1,d1,e1,15)
R[4]<-test.modelR(v,c1,d1,e1,30)
R[5]<-test.modelR(v,c1,d1,e1,45)
R[6]<-test.modelR(v,c1,d1,e1,60)
R[7]<-test.modelR(v,c1,d1,e1,90)
R[8]<-test.modelR(v,c1,d1,e1,120)
R[9]<-test.modelR(v,c1,d1,e1,240)
R[10]<-test.modelR(v,c1,d1,e1,360)
R[11]<-test.modelR(v,c1,d1,e1,480)
S[1]<-test.modelS(v,f1,g1,h1,5)
S[2]<-test.modelS(v,f1,g1,h1,10)
S[3]<-test.modelS(v,f1,g1,h1,15)
S[4]<-test.modelS(v,f1,g1,h1,30)
S[5]<-test.modelS(v,f1,g1,h1,45)
S[6]<-test.modelS(v,f1,g1,h1,60)
S[7]<-test.modelS(v,f1,g1,h1,90)
S[8]<-test.modelS(v,f1,g1,h1,120)
S[9]<-test.modelS(v,f1,g1,h1,240)
S[10]<-test.modelS(v,f1,g1,h1,360)
S[11]<-test.modelS(v,f1,g1,h1,480)

fit1<-wilcox.test(R,RS,alternative="greater")
fit2<-wilcox.test(S,RS,alternative="greater")

if(fit1$p.value<0.05&&fit2$p.value<0.05){
ptest<-ptest+1
}
}
power1<-ptest/sim
```

Step 5. Using the same logic, find the bounds on r for each subject.

```
power1<-1
p<-plow
while(power1=1){
ptest<-0
for (i in 1:sim){
RS<-0
R<-0
```

```
S<-0
fit1<-0
fit2<-0
RS[1]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,5,p)
RS[2]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,10,p)
RS[3]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,15,p)
RS[4]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,30,p)
RS[5]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,45,p)
RS[6]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,60,p)
RS[7]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,90,p)
RS[8]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,120,p)
RS[9]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,240,p)
RS[10]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,360,p)
RS[11]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,480,p)
R[1]<-test.modelR(v,c1,d1,e1,5)
R[2]<-test.modelR(v,c1,d1,e1,10)
R[3]<-test.modelR(v,c1,d1,e1,15)
R[4]<-test.modelR(v,c1,d1,e1,30)
R[5]<-test.modelR(v,c1,d1,e1,45)
R[6]<-test.modelR(v,c1,d1,e1,60)
R[7]<-test.modelR(v,c1,d1,e1,90)
R[8]<-test.modelR(v,c1,d1,e1,120)
R[9]<-test.modelR(v,c1,d1,e1,240)
R[10]<-test.modelR(v,c1,d1,e1,360)
R[11]<-test.modelR(v,c1,d1,e1,480)
S[1]<-test.modelS(v,f1,g1,h1,5)
S[2]<-test.modelS(v,f1,g1,h1,10)
S[3]<-test.modelS(v,f1,g1,h1,15)
S[4]<-test.modelS(v,f1,g1,h1,30)
S[5]<-test.modelS(v,f1,g1,h1,45)
S[6]<-test.modelS(v,f1,g1,h1,60)
S[7]<-test.modelS(v,f1,g1,h1,90)
S[8]<-test.modelS(v,f1,g1,h1,120)
S[9]<-test.modelS(v,f1,g1,h1,240)
S[10]<-test.modelS(v,f1,g1,h1,360)
S[11]<-test.modelS(v,f1,g1,h1,480)

fit1<-wilcox.test(R,RS,alternative="greater")
fit2<-wilcox.test(S,RS,alternative="greater")

if(fit1$p.value<0.05&&fit2$p.value<0.05){
ptest<-ptest+1
}
}
power1<-ptest/sim
p=p+0.01
```

```
}
plow1<-p-0.01

power1<-0
p<-pup
while(power1<0.5){
ptest<-0
for (i in 1:sim){
RS<-0
R<-0
S<-0
fit1<-0
fit2<-0
RS[1]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,5,p)
RS[2]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,10,p)
RS[3]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,15,p)
RS[4]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,30,p)
RS[5]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,45,p)
RS[6]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,60,p)
RS[7]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,90,p)
RS[8]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,120,p)
RS[9]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,240,p)
RS[10]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,360,p)
RS[11]<-test.model1(v,c,y,a1,b1,k1,w1,x1,z1,480,p)
R[1]<-test.modelR(v,c1,d1,e1,5)
R[2]<-test.modelR(v,c1,d1,e1,10)
R[3]<-test.modelR(v,c1,d1,e1,15)
R[4]<-test.modelR(v,c1,d1,e1,30)
R[5]<-test.modelR(v,c1,d1,e1,45)
R[6]<-test.modelR(v,c1,d1,e1,60)
R[7]<-test.modelR(v,c1,d1,e1,90)
R[8]<-test.modelR(v,c1,d1,e1,120)
R[9]<-test.modelR(v,c1,d1,e1,240)
R[10]<-test.modelR(v,c1,d1,e1,360)
R[11]<-test.modelR(v,c1,d1,e1,480)
S[1]<-test.modelS(v,f1,g1,h1,5)
S[2]<-test.modelS(v,f1,g1,h1,10)
S[3]<-test.modelS(v,f1,g1,h1,15)
S[4]<-test.modelS(v,f1,g1,h1,30)
S[5]<-test.modelS(v,f1,g1,h1,45)
S[6]<-test.modelS(v,f1,g1,h1,60)
S[7]<-test.modelS(v,f1,g1,h1,90)
S[8]<-test.modelS(v,f1,g1,h1,120)
S[9]<-test.modelS(v,f1,g1,h1,240)
S[10]<-test.modelS(v,f1,g1,h1,360)
S[11]<-test.modelS(v,f1,g1,h1,480)
```

```
fit1<-wilcox.test(R,RS,alternative="greater")
fit2<-wilcox.test(S,RS,alternative="greater")

if(fit1$p.value<0.05&&fit2$p.value<0.05){
ptest<-ptest+1
}
}
power1<-ptest/sim
p=p-0.01
}
pup1<-p+0.01
```

Step 6. Gather results for all subjects and report.

Algorithm 4. Synergy Test in R

Step 1. Set initial values and input data.

```
P<- .25
R1<-0
S1<-0
RS1<-0
R1<-scan()
9 18 12 26 28 31 24 33 29 23 17
S1<-scan()
9 18 26 33 39 46 52 37 54 61 66
```

Step 2. Calculate combination dose data set.
```
for(i in 1:11){
RS1[i]<-P*(R1[i]+S1[i])
}
RS1
```

Step 3. Run WILCOX.TEST procedure.

```
wilcox.test(R1,RS1,alternative="greater")
wilcox.test(S1,RS1,alternative="greater")
```

Step 4. Gather results for all subjects and report.

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