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RELATIVE IMPACT OF VARIOUS SOURCES OF PHARMACOKINETIC AND PHARMACODYNAMIC VARIABILITY ON WARFARIN RESPONSE

Shashikanth Gannu
University of Rhode Island

RELATIVE IMPACT OF VARIOUS SOURCES OF PHARMACOKINETIC AND PHARMACODYNAMIC VARIABILITY ON WARFARIN RESPONSE

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SHASHIKANTH GANNU

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SHASHIKANTH GANNU

APPROVED:

Thesis Committee

Major Professor Sara Rosenbaum

C T Rhode

Jyh-Hong Chang

Harold D Bibb

DEAN OF THE GRADUATE SCHOOL

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ABSTRACT

Warfarin is widely used oral anticoagulant and its pharmacokinetic (PK) and pharmacodynamic (PD) properties have been extensively studied. It has a narrow therapeutic index and displays poor quality of treatment due to its complex pharmacology and wide inter and intraindividual variability in the dose-response relationship.

This study developed an integrated PK-PD model using STELLA® to describe the dose-concentration-effect relationship for warfarin. This model used previously reported population PK and PD models and parameter values to generate dose-response data. A one compartment stereo-specific semi-physiological PK model with zero-order drug input was linked to an indirect PD model describing the anticoagulant effect. The indirect PD model consisted of two components: (i) the plasma concentration of S-enantiomer of warfarin (C_S) was related to synthesis of prothrombin complex activity (PCA) described by sigmoid I_{max} model (ii) conversion of PCA to prothrombin time ratio (PTR), which is further standardized in terms of INR. The model was used to study the manner in which the interindividual variability in fundamental PK parameters (intrinsic clearance, protein binding affinity constant and protein concentration), PD parameters (potency and sigmoidicity) and intraindividual variability in dose affect warfarin response. For each condition of interindividual variability studied, 100 sets of PK and PD response data were collected and % coefficient of variation (CV) was calculated. For each condition of intraindividual variability studied, 2000 data sets of PD response were collected and % CV was calculated.

For the model used in this study, variability in the response to warfarin was least sensitive to interindividual variability in protein binding affinity constant of S-warfarin (K_{a_S}), protein concentration (P) & sigmoidicity (γ) and also to intraindividual variability in dose. The PK and PD response was found to be most sensitive to interindividual variability in intrinsic clearance of S-enantiomer (CL_{int_S}) and potency (IC_{50}) parameters. Clinically, these parameters are important and their variability in population must be taken into account in order to optimize the dose and use the drug effectively and safely.

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PREFACE

This thesis has been written in the non-manuscript format option as per the guidelines issued by the Graduate School, University of Rhode Island.

Contained within is a main body of work presented as individual chapters (Introduction, Methodology, Results, Discussion and Conclusions) followed by the Appendices (ancillary data and other details pertinent to the understanding of the concepts presented in the manuscript). This thesis closes with the complete listing of all the works cited in this thesis, arranged in alphabetical order by the author's last name.

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1. INTRODUCTION

The relationship between dose and response is the cornerstone of drug therapy. Traditional PK models alone have limited applicability in understanding complete dose-response relationship for drugs that do not have direct linear relationship between drug concentration and therapeutic effect. For such drugs, thorough understanding of all of the individual processes involved from the time the dose is administered to the appearance of the clinically observed effect is very important. Overall, the intensity and duration of response to a given dose of a therapeutic drug can be considered as a function of two sequential phases: PK phase and PD phase. The PK phase describes the drug concentration-time course in body fluids (often plasma/ serum) resulting from administration of a certain dose of a drug. The PD phase, on the other hand, relates observed effect to the concentration of drug at the "effect site".

1.1. Pharmacokinetic Models:

PK models, which incorporate the rate processes of distribution, metabolism and elimination as well as absorption in the case of orally administered drugs, are derived from concentration time data. The models can then be used to observe the system under a variety of conditions. Often the PK phase is linear and as a result the dose concentration relationship is fairly predictable. Critical PK parameters that are used to describe the PK processes include volume of distribution and clearance. For orally administered drugs, additional PK parameters include absorption rate and bioavailability. The number of parameters required to describe the dose-concentration relationship depends on the complexity of the process and on the route of administration.

Over the last two decades the knowledge of pharmacokinetics has increased greatly and a variety of mathematical models and software have been successfully developed. The pharmacokinetics of most of the drugs and the factors influencing the PK processes are well understood and documented.

1.2. Pharmacodynamic Models:

PD models are used to characterize the relationship between drug concentrations at the site of action and the pharmacological effect [1] and are used to develop mathematical expressions to describe the drug response as a function of the concentration time profile. Although the concentration at the receptor site drives the response, owing to the difficulties associated with measuring this value, plasma concentrations are usually used for PD models in vivo. Critical PD parameters include efficacy (E_{max}/I_{max}), potency (EC_{50}/IC_{50}) and sigmoidicity (γ). The efficacy represents the maximum effect that occurs when all the receptors are occupied. The potency is the concentration at 50% of the maximum effect and γ is the number of drug molecules bound to each receptor and it determines the steepness of the concentration-effect relationship. If the drug has stimulatory action the PD parameters are E_{max} and EC_{50} and if the drug has inhibitory action, I_{max} and IC_{50} are used to represent efficacy and potency respectively. When the PK steady-state conditions exist and the pharmacological effect is easily measured, concentration-effect relationships can be described by simple PD models such as fixed effect model, linear model, log-linear model, E_{max}-model, and sigmoid E_{max}-model [1]. The selection of model basically depends on many factors such as (a) the drug used (b)

the response to be measured (c) the effect observed after administration of drug and of placebo (d) the degree of linearity in the effect-concentration curve (e) potential for achieving the maximum possible response. However, the non-linear E_{max} and sigmoid E_{max} models are very commonly used to describe the PDs of many drugs. The sigmoid E_{max} model is a modification of the E_{max} model, which accounts for the probability that more than one drug molecule binds to each receptor by using the term sigmoidicity (γ). The sigmoid E_{max} model is derived from the Hill equation [2]. In some cases, additional components are required to accommodate distribution lag times and/or indirect drug effects, which often complicate the concentration-response relationship. In the case of a distributional delay [3], plasma concentration Vs effect plots indicate pronounced hysteresis. Such relationship can be simplified by considering hypothetical effect compartment to account for the time lag between concentration and response [1,4], and using steady-state conditions. Owing to the complex and non-linear nature of the plasma concentration-effect relationships, it is often difficult to predict how the system may behave under a variety of situations. Thus, simulation studies can be very valuable.

The PDs of relatively few drugs has been extensively studied due to the difficulty in measuring clinically relevant responses. In the absence of PD information it is difficult to appreciate and understand the impact of altered PKs on the drug response.

1.3. Combined Pharmacokinetic-Pharmacodynamic models to understand the dose-response relationships:

In understanding dose-response relationships, an integrated approach involving combined pharmacokinetic and pharmacodynamic modeling has proved tremendously helpful [5,6,7]. The objective for PK-PD modeling is to link PK and PD phases of the drug to establish and evaluate dose-concentration-response relationships and subsequently describe and predict the effect-time courses resulting from given dose of drug. In general, PK-PD modeling based on the underlying physiological process should be preferred whenever possible [5]. This approach has provided significant insight into the pharmacology of various drugs under conditions of normal and abnormal physiology. Furthermore, to characterize and appropriately describe the time course of drug action under non steady-state conditions, PKs and PDs of the drug have to be adequately linked to predict dose-concentration relationship and concentration-effect relationship. This link can be established, when the plasma concentration is substituted for concentration in PD equations with an assumption that the concentration at the site of action is in equilibrium with plasma. This assumption may be valid, if the drug effect is direct, receptor site rapidly equilibrates with plasma and the drug-receptor interaction in relation to the response occurs rapidly.

The direct correlation of pharmacological response to drug concentration is not always possible with all drugs. Sometimes intermediate steps are involved in the mechanism of action of the drug that is more complex than is assumed in the model. For example, dose-response relationships can be complicated when the drug action is indirect and/or irreversible. Four basic physiologic indirect response models proposed by Jusko et al. may be used in PK-PD modeling to describe the pharmacodynamics of drugs that have

indirect mechanisms such as inhibition/stimulation of the production or degradation of endogenous substances/mediators, which control the measured response [8, 9, 10]. These PD models when coupled with the PK models of the drug help in simplifying the relationship between the dose administered and the clinically observed response. For example, warfarin (anticoagulant) exerts an indirect action and the resultant delay in response makes a direct correlation of the anticoagulant activity to the plasma drug concentration impossible. Therefore, plasma warfarin level is correlated with inhibition of the prothrombin complex production rate, which is then linked to the pharmacological response (anticoagulation). The application of PK-PD modeling to understand dose-response relationship in the case of warfarin is described in detail in the following sections of this thesis.

1.4. Sources of Variability

Biological variability is an inherent feature of drug action. Variability in a response arises in drug therapy when a standard dose or dosing regimen evokes differing responses in various individuals (referred to as interindividual variability) or in a given individual at different times (referred to as intraindividual variability). However, interindividual variability was identified to be major source of variability for many drugs [11].

Intra and interindividual variability in the dose response relationship can arise from two sources: PK variability and PD variability. Clinically, PK variability commonly arises from variability in the PK parameters describing the rate and extent of absorption, distribution, metabolism, and elimination of drugs. Some drugs show greater

pharmacokinetic variability than others do. Variability in the PK characteristics of drug is well recognized and understood due to the ease of monitoring and control of therapeutic plasma concentrations. But, little is known about PD variability due to the difficulty of measuring clinically relevant responses for most drugs and limited research activity in this field [11]. The other reason for poor understanding of PD variability is because of difficulty in distinguishing between PD and PK variability. For example, if a certain pharmacologic effects arises, wholly or in part, from a minor metabolite of a drug, then a twofold or threefold increase in the formation of that metabolite could cause a substantial increase in pharmacologic effect without any apparent change in the drug's PKs. The increased pharmacological effect may be interpreted as due to PD rather than PK variability if the role of the quantitatively minor metabolite has not been recognized [12]. The magnitude of variability in the PK and PD parameters may be of varying amounts depending on the drug and the pathological condition of the patient. Over the last decade enormous work have been done to understand PK and PD variability and the factors influencing this variability. Factors such as age [3, 13, 14], gender [3, 13, 15], disease state, nutrition, genetics, environment, and concurrent drug therapy [6, 16] may affect the patient's physiologic functions and lead to variation in the pharmacokinetic and/or pharmacodynamic parameters.

The interindividual differences in the relationship between drug plasma concentration and pharmacological effect intensity have been reported to be mainly due to various factors. These factors include: (i) receptor density and affinity (ii) the formation and elimination kinetics of endogenous ligands (iii) postreceptor transduction processes (iv) homeostatic

responses and (v) the kinetic characteristics of transporters involved in drug transfer between fluids of distribution and the biophase [11]. Usually PD variability is more pronounced than PK variability. Mandema et al. reported large interindividual PD variability in response as compared to interindividual PK variability in the case of ketorolac [17].

1.5. Warfarin

Warfarin, the most commonly used anticoagulant, is an example of a drug that displays wide-variability in the dose-response relationship in the population [6, 7, 18-22, 23]. Since both sub-therapeutic and large doses are associated with serious clinical consequences [24], the PK and PD of warfarin have been extensively studied. These studies have been conducted in order to provide insight into dose optimization and identification of factors that influence dose-response relationship. Additionally, since the effect of warfarin is easily measured, there is much information in the literature on pharmacodynamics of warfarin.

Warfarin is administered clinically as a racemic mixture of two enantiomers, R- and S-warfarin. The disposition and pharmacological action of the both the enantiomers are qualitatively similar but quantitatively quite different [25]. The differences in the anticoagulant activities and metabolism of the S- and R- isomers of warfarin are very large. As a result, it is necessary to consider these isomers separately in PK & PD model for warfarin.

1.5.1. Pharmacokinetics

The pharmacokinetics of warfarin has been extensively studied, and several reviews of warfarin pharmacokinetics have been published [2, 21, 26, 27]. Warfarin is rapidly and completely absorbed from the gastrointestinal tract. Warfarin does not exhibit dose dependency in the rate or extent of absorption, and enantiomer specific differences in absorption patterns have not been reported. Warfarin is highly plasma protein bound drug and binds to albumin at site I. The bound fraction of racemic warfarin ranged from 97.4 to 99.9% under normal physiological conditions [6, 28]. In early 1975, Yacobi et al demonstrated significant intersubject variability in the extent of protein binding at therapeutic concentrations; intrasubject variability was much less substantial [29]. Variations in albumin concentrations in plasma occur as result of altered synthesis, loss, or a shift of albumin from the intravascular to extravascular spaces. Physiologic conditions such as age, pregnancy, and nutritional status cause decrease in albumin concentration [30]. Pathologic conditions include renal disease, hepatic disease, acute myocardial infraction, cancer, sever burn injury, diabetes mellitus, thyroid disease, and cystic fibrosis lead to decreased plasma protein binding due to altered protein concentrations, or qualitative changes in protein molecules [30]. Concurrent administration of warfarin with other highly protein bound drugs having affinity to site I on albumin may lead to changes in binding of warfarin. The principal route of warfarin elimination is hepatic metabolism and renal excretion was reported to be very negligible [31]. The metabolic elimination of the pharmacologically more potent S-enantiomer is mediated by cytochrome P450 CYP2C9 isoform which steroselectively converts S-warfarin to the inactive phenolic metabolite, S-7-hydroxywarfarin. The rate of elimination of the two

isomers differs substantially. Warfarin undergoes restrictive hepatic clearance [32]. Thus its clearance is approximated by the following equation:

$$CL_H = CL_{int} * f_u \quad (1)$$

Where,

CL_H is the Hepatic clearance of warfarin;

CL_{int} is the Intrinsic clearance of warfarin; and

f_u is the fraction of warfarin unbound to plasma proteins;

It can be seen from equation (1), an increase in "free" fraction in an individual would lead to a substantial increase in hepatic clearance and thus total body clearance. The variations in intrinsic clearance of warfarin are usually associated to interindividual differences in the activity of the drug metabolizing enzyme systems due to genetic and environmental effects. The hepatic metabolism was reported to be the major determinant of intrasubject variability in the warfarin dose-concentration-response relationship.

1.5.2. Pharmacodynamics

Unlike the direct concentration-effect relationships identified for most drugs, the relationship between warfarin's anticoagulant effect and the drug's concentration in serum or plasma is nonlinear, complex and indirect. The anticoagulant action of warfarin is mediated by inhibition of vitamin K reductase linked to the vitamin-K dependent carboxylation of glutamic acid residues on certain coagulation proteins like prothrombin, clotting factors II, VII, IX, X and protein C [33]. The overall effect can be characterized in terms of the degree of inhibition of the synthesis rate of prothrombin-complex activity

(PCA). The reduction in the activity of these clotting factors results in a prolongation of clotting time, an effect easily measured clinically.

Warfarin is administered as a mixture of R- and S- enantiomer. However, the anticoagulant activity of the S-enantiomer has been reported to be 3 to 6 times as greater as that of the R-enantiomer [34]. The pharmacodynamics of warfarin have been described using a mechanism-based indirect model [2, 10, 33]. This consists of a sub-model for synthesis and degradation of clotting factors, and the inhibitory action of warfarin on clotting factors synthesis. Changes in the amount of clotting factors alter the PCA, which can be clinically assessed using prothrombin clotting time (PT). Owing to the dependency of the PT on the particular thromboplastin used in the test an additional thromboplastin-specific parameter, International Sensitivity Index (ISI), is required to relate a thromboplastin dependent PT to the standardized international normalized ratio (INR) [35, 36, 37]. Although the INR system is unreliable during the initiation of warfarin therapy, it does provide an advantage over the reporting of the results as PT ratio [36, 38]. Thomas et al. indicated that oral anticoagulant therapy monitored with the INR is associated with lower bleeding complications than therapy monitored with the PT ratio, and the rate of thromboembolic events using the INR is acceptably low [39].

Factors that have been reported to influence sensitivity to warfarin include (i) disease conditions: congestive heart failure [25], thyroid disease [40] (ii) age [41] (iii) hepatic insufficiency [42] (iv) differences in the hemostatic response to given concentrations of warfarin and (v) concomitant administration of other drugs [20]. Factors that influence

warfarin resistance include (i) Patient non-compliance (ii) Excessive intake of vitamin K (iii) Co-administration with other drugs that induces cytochrome P450 2C9 enzyme system and (vi) hereditary resistance which may require increased doses of warfarin [43, 44, 45].

Several approaches have been proposed in the literature to describe the anticoagulant response of warfarin using a combined pharmacokinetic-pharmacodynamic model [2]. However, previous attempts have not considered the warfarin anticoagulant response in terms of INR. In the present study, INR component was considered in the integrated PK-PD model to report the pharmacodynamic effects of warfarin.

The effect of variability in PK parameters and PD parameters play an important role in causing the variability in therapeutic response among population. Therefore, these simulation studies were conducted to compare the individual and combined effect of PK and/or PD parameters of warfarin on PK response data from a one compartment model with zero order absorption and PD response arising from an indirect sigmoid Imax model.

The objectives of this study include:

1. To create an integrated PK-PD model for warfarin that takes into account individual enantiomers of warfarin. The model developed in STELLA® use average population values of the parameters from previous investigations [6, 7]. PK model includes a sub-model for clearance based on well-stirred venous equilibrium component. This sub-model helps in understanding the effect of parameters such as fraction of S-warfarin

bound to plasma proteins and intrinsic clearance of S-enantiomer (CL_{int_S}) on plasma concentration of S-enantiomer (C_S) and anticoagulant effect of warfarin in terms of INR.

2. Perform computer simulations:

   (i). To evaluate and compare the manner in which different sources of variability in PK parameters such as CL_{int_S}, protein binding affinity constant (K_{a_S}) and plasma albumin concentration (P) and PD parameters such as IC_{50} and γ affect the dose-response relationship of warfarin.

   (ii). To investigate the relative impact of intraindividual variability in dose on C_S, and INR.

   All the simulations were performed using computerized integrated PK-PD model.

2. METHODOLOGY

The integrated PK-PD model describing the complete dose-response relationship for warfarin was constructed in STELLA® (High Performance Systems, Hanover, NH). This model was used throughout the study.

2.1. Pharmacokinetic Model

A one-compartment stereo-specific semi-physiological pharmacokinetic model, which adequately describes the warfarin dose-concentration relationship, was used [6]. Hignite [46] and Chan [6] reported that the anticoagulant activities and metabolism of warfarin enantiomers are different in many aspects. For these reasons, the PK model in this study took into consideration the individual isomers of warfarin as separate drugs. Constant, zero order oral administration was used in all the simulations. The bioavailability of warfarin was assumed to be 100% [2] and the dose was assumed to be absorbed rapidly and completely [42] at a constant rate over a 24 hour dosing interval. Zero order input was assumed in all the simulations so it would facilitate the understanding of different sensitivity of the warfarin response to various different factors.

In 1993, Pitsiu et al. performed a population PK and PD study of warfarin in 48 normal, healthy young volunteers [7]. Another population study was conducted by Chan et al. in 1994 [6]. The population PK and PD parameter values reported by these studies were used in the present investigation. These parameters were chosen due to the following reasons: the study represented an integrated PK-PD analysis and thus both PK and PD parameters were derived in the same analysis; PK and PD data were available on both S- and R-

enantiomer of warfarin and since these isomers were reported to display different pharmacokinetics and pharmacodynamics [46, 48], it was considered that each isomer should be represented individually in the model; a true population approach using NONMEM was used and the estimates were also in agreement with those reported in the previous studies [49].

The primary route of warfarin elimination was reported as hepatic metabolism [50]. Hepatic clearance was calculated using the well-stirred venous equilibrium model [32] with the equation.

$$CL_H = E * Q \quad (2) \text{ and}$$

$$E = (f_u * CL_{int}) / (Q + f_u * CL_{int}) \quad (3)$$

Where,

CL_H is the hepatic clearance of warfarin;

E is the extraction ratio;

Q is the hepatic plasma flow, 40.5 L/h [51];

f_u is the fraction of drug unbound to plasma proteins; and

CL_{int} is the intrinsic hepatic clearance of warfarin

Warfarin was assumed to undergo first order elimination [7]. Mean population PK parameter values obtained from the warfarin literature of Pitsiu et al. were used. The parameter values were well in agreement with other investigations [6]. First order elimination rate constants of S- and R- enantiomer used in the model were 0.0254 and 0.0193 h^{-1} respectively. The terminal half-lives of S- and R- enantiomer were calculated to be 27.3 and 35.9 hours respectively which means it takes about a week to reach steady-

state conditions. The volume of distribution was 11.8 and 10.5 L for S- and R- warfarin enantiomers respectively. The unbound fraction (f_u) for S- and R- enantiomer of warfarin was assumed to be 0.51 and 0.62 % respectively [6].

Finally, intrinsic hepatic clearance of S- and R- enantiomer were calculated and set at 59.21 and 32.85 L/h respectively.

Effect of Protein Binding:

Concentration-dependent protein binding occurs with highly protein bound drugs such as warfarin. The unbound fraction of warfarin can be mathematically described as

$$f_u = 1 / [1 + K_a * P] \quad (4)$$

Where,

K_a is the affinity constant for protein binding; and

P is the concentration of albumin plasma protein ($6.5 * 10^{-4}$ Molar);

The values of affinity constants for S- and R- enantiomer were calculated using the above equation and were initially set at $3.0 * 10^5$ and $2.466 * 10^5$ $Molar^{-1}$ respectively.

Initially, the amounts of both the warfarin enantiomers in the model were set to steady state levels. Thus, the combined and individual enantiomer steady-state plasma concentrations were expressed using equation

$$Cp_{ss} = D / (CL * \tau) \quad (5)$$

Where,

Cp_{ss} represents the steady-state concentration of warfarin;

D is the dose of warfarin;

CL represents the total body clearance; and

τ is the dosing interval;

2.2. Pharmacodynamic Model

The simple linear PD models that were usually used to describe the direct relationship between plasma concentration and the response are inadequate to describe the pharmacodynamics of warfarin. As described earlier, PDs of warfarin is best described using an indirect inhibitory model in which, warfarin concentrations are related to clotting factor synthesis but only indirectly related to the observed therapeutic effect (INR). This indirect PD model consists of two sub-components:

(i). Relationship between warfarin concentration and PCA/PT

(ii). Conversion of PCA to INR

2.2.1. Relationship between warfarin concentration and PCA/PT

In this sub-component of the PD model the plasma concentration of S- enantiomer was related to the PCA. Both synthesis and degradation of the clotting factors determine the hypoprothrombinemic effect, yet warfarin will only affect synthesis. A physiologic effect model describing the direct relationship between warfarin inhibition of epoxide reductase and clotting factor synthesis as proposed by Nagashima et al. [52] was used. The prothrombin complex activity in the plasma represents the net effect of the synthesis of various clotting factors such as II, VII, IX, X and their normal degradation. This model assumes time course of PCA after warfarin administration as a function of rate of

synthesis and degradation of clotting factors. This relationship can be mathematically described as:

$$dPCA/dt = R_s - R_d \quad (6)$$

Where,

R_s is the Rate of PCA synthesis; and

R_d is the rate of PCA degradation;

The R_s and R_d values in the equation are expressed in terms of per cent of normal activity. The rate of PCA degradation was calculated from it's first order rate of degradation (K_d) and can be expressed as:

$$R_d = K_d * PCA \quad (7)$$

The effect of warfarin on PCA synthesis can be expressed in terms of its fractional effect

$$R_s = K_d * PCA_{normal} * I(t) \quad (8)$$

Under normal circumstances the system is assumed to be at steady state and PCA has its maximum value (100 %)

$$R_s = K_d * 100\% \quad (9)$$

A sigmoid I_{max} model [2, 10] was used to relate warfarin concentrations to the inhibitory action on PCA synthesis rate. Previous investigations [6, 7] indicated that R-enantiomer have negligible effect on clotting factor activity that it can be neglected in the PD model. Therefore, in this study, it was assumed that only S-enantiomer had pharmacological activity and its concentration was linked to the PD model. The degree of inhibition of clotting factor synthesis by S-enantiomer is expressed as:

$$I(t) = [1 - (C_s^{\gamma} / (IC_{50}^{\gamma} + C_s^{\gamma}))] \quad (10)$$

Which may be simplified and rewritten as

$$I(t) = [1 / (1 + (C_s^{\gamma} / IC_{50}^{\gamma}))] \quad (11)$$

Where,

I(t) is the inhibitory function of warfarin concentration that predicts the synthesis rate as a percentage of the baseline value;

C_s is the plasma concentration of S-warfarin;

IC_{50} is the plasma concentration of S-warfarin that produces 50% inhibition of clotting factor synthesis; and

γ is the sigmoidicity describing the steepness of the concentration-effect curve;

As indicated before the values reported for warfarin population analysis by Pitsiu et al. [7] were used for the PD and physiological model parameters. First order rate of degradation, K_d was set at 0.094 h^{-1}, and the half-life of K_d was calculated to be 7.4 hours. The values of IC_{50} and n were initially set in the model as 0.394 and 1.0 respectively [7].

2.2.2. Conversion of PCA to INR

When monitoring treatment with warfarin, it is common practice to assess PCA by determining PT and the therapeutic range is usually specified in terms of the prothrombin time ratio (PTR). The PTR is the patient's PT divided by the laboratory's control or normal PT. By using the PTR instead of PT alone, part of the technical variation in the PT test is eliminated, since the ratio is unaffected if both the patient's and the normal PT vary in the same proportion. The regression equation described by Chan et al. [6] and obtained from serial dilutions of normal plasma was used to convert PCA to PTR

$$PTR = (426 + PCA * 7.75) / (PCA * 12) \quad (12)$$

However, still systematic variation may be observed in PTR determinations due to the considerable variability in the sensitivities of thromboplastin from different species, manufacturer to manufacturer and lot to lot. As a consequence of the variability in response of different thromboplastin reagents, PTR results are not comparable from laboratory to laboratory without knowing the sensitivity of the thromboplastin. As a result this variation could produce potential problems for anticoagulation control.

The need for standardizing the measurement of PTRs has long been recognized [53]. In early 1980, the term INR was introduced to standardized the PTR by adjusting for variability in thromboplastins with different sensitivities. Finally, the World Health Organization has urged that all medical staff and health auxiliaries involved in controlling anticoagulant treatment in patients should use the INR. INR system is based on International Sensitivity Index (ISI) values derived from the plasma of patients stabilized on a regimen of anticoagulant treatment for at least 6 weeks. The expression used to convert PTR to INR can be described as [37]

$$INR = [PTR]^{ISI} \qquad (13)$$

The INR would be equal to PTR if a thromboplastin with an ISI of unity were used in the test. In this study, PTR was converted to INR using ISI of 2.2 since this is the estimated ISI of the thromboplastin used by Chan et al [6, 54].

Anticoagulation provides a striking benefit for patients whose treatment is conducted within the recommended range of the INR, 2.0 to 3.0. In this study this range for INR was considered as therapeutic range for reporting final anticoagulant response.

2.3. Pharmacostatistical Model

2.3.1. Interindividual Variability Model

Interindividual variability in PK parameters (intrinsic clearance and protein binding affinity constant for S-warfarin) and PD parameters (potency and sigmoidicity) were modeled using the proportional error model [55] as follows:

$$\theta_j = \theta_m * (1 + \eta_{\theta_j}) \quad (14)$$

Where

θ_j represents the estimate for a PK or PD parameter in the j^{th} individual ;

θ_m represents population mean of the PK or PD parameter; and

η_{θ_j} is normally distributed random variable with zero mean and variance ω^2 for variability in PK and PD parameter;

The effect of 6 levels 10, 20, 30, 40, 50, and 60% CV of interindividual variability in each parameter was investigated. The model use "sample and hold" set up in STELLA® for each PK and PD parameter as shown in Figure 1b, 1c, 1d & 1e such that the interindividual variability model cause each parameter to vary every 480 hours.

2.3.2. Intraindividual Variability Model

Intraindividual variability in dose was modeled using the proportional error model [55] as follows:

$$D_{ij} = D_i * (1 + \varepsilon_{ij}) \quad (15)$$

Where

D_{ij} is the administered dose for the j^{th} individual at time i;

D_i is the model predicted dose in j^{th} individual at time i;

ε_{ij} is normally distributed random variable with zero mean and variance σ^2; and

σ, the coefficient of variation of the variability was set to 3%, 6% and 10% CV

In this model, intraindividual variability was assumed to cause the value of dose to deviate from model predicted value by an amount that is proportional to the value of the dose. This model use sample and hold set up as shown in Figure 1g such that intraindividual variability caused the dose to vary with new dose after every 24 hours.

2.4. Simulations

The impact of variability in the dose, PK and PD parameters on response variables was studied using zero order drug input under steady-state conditions. When no variability was given to the model parameters, the dose of 7mg and 9mg resulted INR value at the lower end (2.2) and higher end (2.7) of therapeutic range, respectively. Owing to the non-linear relationship between the dose administered and observed INR, simulations studies for intraindividual variability in dose were carried out at the low and high end of the therapeutic range using daily doses of 7 mg and 9mg, respectively.

For each set of model parameters in the PK variability studies, 100 replications of response data (C_s and INR) were generated at 480 hour time point (under steady-state conditions). For each set of model parameters in PD variability studies, 100 replications of response data (INR) were generated at 480 hour time point (under steady-state conditions). For the intraindividual dose variability studies, the response (C_s and INR) was measured at every 24 hours over a 20 day period and 100 replications were

performed giving a total of 2000 responses. In all the simulation runs the response data was generated by numerical integration after every 0.01 h.

INR value within 2.0 to 3.0 was considered as therapeutic event. Any INR value < 2.0 was considered as sub-therapeutic event and any value >3.0 as event causing significant risk of hemorrhage.

2.5. Data Treatment

Response data generated from the simulations as described above were imported from STELLA® to a Microsoft Excel worksheet. The mean, standard deviation (SD), and thus % coefficient of variance (CV) in response was determined for each of the 100 data sets associated with a given experimental condition. The response values beyond 2.5*SD were excluded from the analysis.

2.6. Statistical Analysis Using ANOVA:

ANOVA design provides greater opportunity to identify the significant parameters and analyze the PK and PD interactions at different treatment combinations. In this study, 2^4 full factorial design was used to identify the individual PK parameters, PD parameters and the combination of PK & PD parameters that have significant effect on the variability in warfarin response (INR). The general linear model used for the ANOVA analysis was:

$$Y = \beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_4X_4+\beta_{12}X_1X_2+\beta_{23}X_2X_3+\beta_{34}X_3X_4+\beta_{14}X_1X_4+\beta_{13}X_1X_3+$$
$$\beta_{24}X_2X_4+\beta_{123}X_1X_2X_3+\beta_{234}X_2X_3X_4+\beta_{134}X_1X_3X_4+\beta_{1234}X_1X_2X_3X_4+\varepsilon$$

Where:

$X_1 = CL_{int\_S}$; $X_2 = K_{a\_S}$; $X_3 = IC_{50}$; $X_4 = \gamma$;

X_1, X_2, X_3 and X_4 are each of the independent variables;

X_1X_2, X_2X_3, X_3X_4, X_1X_4, X_1X_3 and X_2X_4 are the two-way interactions;

$X_1X_2X_3$, $X_2X_3X_4$ and $X_1X_3X_4$ are the three-way interactions;

$X_1X_2X_3X_4$ is the four-way interaction;

β_0 is the overall mean;

β_1, β_2, β_3, β_4 are the coefficients of factor effects;

β_{12}, β_{23}, β_{34}, β_{13}, β_{14}, β_{24} are the coefficients of two-way interaction effects;

β_{123}, β_{234}, β_{134} are the coefficients of three-way interaction effects;

β_{1234} is the coefficient of four-way interaction effect; and

ε is the error variable;

The experimental design for ANOVA studies is shown in Table 4. Table 4 lists all the 16 experiments required in a 2^4 full factorial design. The four independent variables (CL_{int_S}, K_{a_S}, IC_{50} and γ) were set at two levels each (60% CV and 30% CV). ANOVA was used to determine the significance of each of the four independent variables, two-way, three-way interactions and four-way interactions. ANOVA procedures on the response parameter (INR) were performed using Minitab® software package.

3. RESULTS

3.1. Variability in PK parameters:

3.1.1. Effect of variability in CL_{int_S}:

The effect of 6 levels of interindividual variability (10, 20, 30, 40, 50 and 60 % CV) in CL_{int_S} on variability in steady state PK response (C_S) and PD response (INR) was studied with 7 mg warfarin dose. The model was set to steady state conditions when variability was added. Table 2 and 3 show the results for variability in C_S and INR, respectively. The manner in which variability in CL_{int_S} affects C_S and INR are shown in Figure 2 and 3, respectively. There was an approximately linear relationship between the variability in CL_{int_S} and the PK & PD responses up to about 40 % CV in CL_{int_S}. Of these responses up to 30 % CV, the variability in CL_{int_S} produced approximately equal variability in C_s and slightly less in INR. At 50 % or greater variability in CL_{int_S}, the variability in C_S and INR was found to increase by greater than two fold of that considered in CL_{int_S}. From equation 2, it can be observed that the hepatic clearance becomes more sensitive as CL_{int_S} increases and therefore we see higher variability in PK and PD responses. At the maximum variability (60 % CV) in CL_{int_S}, the observed variability in INR and C_S was approximately 120 and 163 %, respectively.

3.1.2. Effect of interindividual variability in K_{a_S}/[P]:

From equation 4, it can be seen that the effect of K_{a_S} and [P] on fu are similar. In simulations, it was found that similar variability in K_{a_S} and [P] produce same variability on response values (data not shown). As a result, in this study the effect of variability in only one of these parameters was studied.

Computer simulations were carried with the fixed dose of 7 mg warfarin and dosing interval of 24 hours. The effect of interindividual variability in the K_{a_S} was translated to changes in steady state C_S and INR (at 480 hours). The results of these simulations are shown in Table 2 and 3. Figure 2 & 3 represents the effect of the variability in K_{a_S} as the function of % CV in PK and PD responses. The relationship between the variability in K_{a_S} and the variability in drug's PK and PD responses were found to be linear. Over the entire range of variability studied, the variability in C_S and INR was found to be less than that considered in K_{a_S}. As seen in Figure 2 and 3, the variability in the K_{a_S} produced small changes in steady state C_S and INR compared to that due to the variability in CL_{int_S}. For example, with 60 % CV in K_{a_S} produced 59.3 and 42.8 % CV in steady state C_S and INR, respectively whereas with 60 % CV in CL_{int_S} produced 163.6 and 153.6 % CV in steady state C_S and INR, respectively

3.1.3. Effect of interindividual variability in combined PK parameters:

After the effect of variability in individual PK parameters was studied separately, computer simulations were performed to study the combined effect of interindividual variability in CL_{int_S} and K_{a_S} on steady state C_S and INR. Interindividual variability model as shown in Figure 1c & 1d were used to facilitate the same amount of variability in both the PK parameters at the same time. Table 2 & 3 show the results for the effect of interindividual variability in combined PK on the variability in C_S and INR. Figure 2 & 3 represents the effect of variability in combined PK as the function of % CV in C_S and INR, respectively. From Table 2 & 3, and in agreement with the individual parameter

studies, both PK parameters when considered together produced less variability in INR than in C_S. Additionally, although the variability in PK parameters when combined produced higher amount of variability in C_S and INR than compared to that due to variability in individual PK parameters, the combination was less than additive(Figure 2 & 3). For example, 40% CV in combined PK parameters resulted 71.2 & 74.8 % CV in C_S and INR, respectively; 40 % CV in CL_{int_S} resulted 52.2 & 46.5 % CV in C_S and INR, respectively; and 40 % CV in K_{a_S} resulted 40.3 & 28.6 % CV in C_S and INR, respectively

3.2. Variability in PD parameters:

3.2.1. Effect of interindividual variability in IC_{50}:

The effect of interindividual variability in IC_{50} on steady state INR was studied at 6 levels of interindividual variability (10, 20, 30, 40, 50 and 60 % CV). Simulation results for variability in IC_{50} when fixed dose of 7 mg warfarin was used are shown in Table 3. Figure 3 represents the effect of variability in IC_{50} as a function of % CV in INR. From Figure 3, it can be inferred that there is an approximately linear relationship between the variability in IC_{50} and the INR up to 30 % CV. In this range, the variability in INR was found to be slightly less than that considered in IC_{50}. At 40 % or higher variability in IC_{50}, the variability in INR was found to be greater than that considered in IC_{50}. For example, 20 % CV in IC_{50} produced 14.2 % CV in INR whereas 60 % CV in IC_{50} produced approximately 151 % CV, which is 2.5 times the variability of that considered in the IC_{50}.

3.2.2. Effect of interindividual variability in Sigmoidicity:

Simulation studies were carried out with the fixed dose of 7 mg warfarin and the variability in γ was translated to variability in INR. The results of these simulations are given in Table 3. The relationship between variability in γ and resulting variability in INR is shown in Figure 3. Over the entire range of variability studied, the resulting variability in INR was found to be much less than that considered in the parameter. For example with 60 % CV in the γ, the resulting variability in INR was found to be 1/6 times of that considered in γ. However, it is unlikely that this magnitude of variability in INR is clinically significant. Comparing the variability curves for IC_{50} & γ in Figure 3, it can be concluded that the INR is less sensitive to the variability in γ than to the variability in IC_{50} or any of the PK parameters studied.

3.2.3. Effect of interindividual variability in combined PD parameters:

After the effect of variability in individual PD parameters was studied separately, the combined effect of interindividual variability in both IC_{50} and γ on steady state INR was studied at 6 levels of variability (10, 20, 30, 40, 50 and 60 % CV). Proportional error model (shown in Figure 1d & 1e) was used to facilitate the same amount of variability in both the PD parameters at the same time. The results of these simulation studies are shown in Table 3. The relationship between the combined PD variability and the variability in INR is shown in Figure 3. Over the entire range of variability studied, the relationship between combined PD variability and INR was found to be approximately linear up to 30 % CV. In this range except with 10 % CV, the variability in drug response was found to be slightly more than that considered in the PD parameters alone. The

variability in INR due to combined PD was found to be greater than the sum of variability in INR due to individual PD parameters. At 40 % or greater variability, the drug response becomes more sensitive to the changes in PD parameters and we see steep increase in the variability curve (Figure 3). For example, with 10 % variability in combined PD, the variability in INR was found to be 6.8 % whereas with 60 % CV in combined PD, the resulting variability in INR was found to be approximately 2.8 folds of that considered in the parameters. The variability in INR was found to be higher due to combined PD than that due to combined PK.

3.3. Effect of combined PK and PD variability:

Computer simulations were carried to determine the effect of variability in combined PK and PD parameters on the drug response. The results of these simulations are given in Table 3 and Figure 3. Variability in PK & PD parameters when considered together produced higher amount of variability in INR than that produced due to variability in individual parameters or combined PK or combined PD parameters, but less than the sum of variability in INR due to individual PK & PD parameters.

There were a number of occasions in which a simulation yielded INR value that was too large. Such simulations were terminated and excluded from the study. Essentially with 60 % CV in combined PK and PD parameters could not be studied because the simulation was unsuccessfully terminated due to division by zero or a value that has become too large to represent [56].

3.4. Variability studies using ANOVA:

The simulation runs were performed as per the experimental design shown in Table 4. The distribution of INR values from the simulation runs failed to follow the normality assumption (Appendix 1). The use of square-root and cubic-root transformations on the INR values did not improved the normality. However, a natural logarithmic transformation of the INR values greatly improved the normality distribution (Appendix 2). Therefore, statistical analysis using AVOVA method was performed on the natural log transformed data to determine the significance of each parameter and their interaction(s). The results of ANOVA test are summarized in Table 5. These results indicate that PK parameter (CL_{int_S}) and PD parameter (IC_{50}) significantly affect the variability in INR at level of significance, 0.05. The two-way interaction of PK and PD parameters, CL_{int_S} & IC_{50} and CL_{int_S} & γ, were also found to significantly affect INR ($P<0.05$). None of the three-way and four-way interaction(s) of PK and PD parameters were found to be significant. Hence, these interactions were combined with error to increase the power of the test. Main effects and interaction plots further support the results of ANOVA findings. The main effect of a parameter is referred to be the change in response produced by a change in the level of the parameter. Figure 4 shows the main effects plot for Ln(INR) data. As can be seen from this plot, the level of % CV in CL_{int_S} and IC_{50} seemed to affect the INR values significantly. As the level of the CL_{int_S} changed from 30 % CV to 60 % CV, the variation in the mean response value highly increased. Similarly in the case of IC_{50}, as the level is changed from 30 % CV to 60 % CV, we see a high increase in the mean response valve. In contrast, the level of % CV in K_{a_S} and γ slightly affect the INR and this effect was minimal. Difference in response between the levels of one parameter is not the same at all levels of the other parameter(s).

These differences in the response is referred to be interaction and can be studied using interaction plot. Looking at the interaction plot shown in Figure 5, it can be concluded that there is an interaction between pairs of various parameters such as CL_{int_S} & IC_{50} , CL_{int_S} & γ and IC_{50} & K_{a_S} since the lines for these parameters in the plot are intersecting with each other.

3.5. Effect of intraindividual variability in dose:

The effect of intraindividual variability in dose on variability in C_S and INR was studied at 3 levels (3, 6 and 10 % CV) using both 7 mg and 9 mg dose of warfarin. Sample and hold set-up, as shown in Figure 1f, was used to cause the dose to change after every 24 hours. The results of dose variability studies with 7 mg and 9 mg dose of warfarin, are shown in Table 6. Figure 6 & 7 show a three-dimensional bar graph of % CV in dose Vs % CV in INR with 7 mg and 9 mg doses of warfarin, respectively. Each bar in the Figure 6 & 7 represent the magnitude of variability in PK/PD response at a given amount of variability in the dose. It can be seen in Figure 6 and 7 that variability in dose produced approximately equal variability in PK response (C_S). In contrast, the INR appears to be less sensitive to changes in the dose since the variability in the dose was associated with lower variability in INR. For example, with the maximum variability (10% CV) in 7 mg & 9 mg dose resulted 10.06 & 10.38 % CV in C_S and 6.01 & 7.40 % CV in INR, respectively. Variability in PK and PD responses were found to increase with the increase in the degree of variability in dose. Over the entire range of variability studied, the variability in dose produced approximately the same amount of variability in drug's PK response and slightly less variability in drug's PD response. For example, with 6 % CV in

7 mg warfarin essentially resulted 10.06 & 6.01 % CV in C_S and INR, respectively. Similarly, with 6 % CV in 9 mg warfarin dose essentially resulted 10.38 & 7.40 % CV in C_S and INR, respectively. These differences in PK and PD responses may be attributed to their non-linear relationship.

4. DISCUSSION

Warfarin, like all the 4-hydroxycoumarin compounds, has an asymmetric carbon atom. The clinically available warfarin preparations consists of a racemic mixture of equal amounts of two distinct S- and R-enantiomers, the former being 3 to 6 times more potent as anticoagulant [34]. Hence, variability in warfarin responses most likely arises from variability associated with S-enantiomer. Therefore in this study, we have considered only the effects of S-enantiomer on warfarin PK and PD responses.

Warfarin has well established PK-PD relationship, narrow therapeutic range and large PK/PD variability making it a ideal candidate to study the impact of various sources of variability on the dose-response relationship. In understanding complete dose-response relationship for drugs like warfarin, an integrated approach involving combined PK and PD modelling has proved tremendously helpful [5, 6, 7]. Hence, this study has attempted to describe the relationships between the PK, the PD and response to warfarin by developing an integrated PK-PD model.

The integrated PK-PD model (Figure 1a & 1b) used in this study is based on individual PK and PD models derived from plasma concentration and response data, respectively [6, 7]. This model was developed based on the idea that a thorough understanding of the impact of variability from a source needed to be based on a complete and integrated PK-PD model. The model uses additional component to report the anticoagulant response of warfarin in terms of INR (Figure 1b), which now has globally become the standard way of assessing warfarin response [36]. Previously, there have been no published studies that

have used computer simulations and an integrated PK-PD model to predict the relative impact of different sources of variability on warfarin response. Vadher et al. developed a computerized PK-PD model of the time course of warfarin action with bayesian parameter estimates and used this model to retrospectively predict the daily INR and maintenance dose during the initiation of warfarin therapy [57].

The operation of integrated PK-PD model in this study was validated by performing the run without any variability and evaluating the responses to ensure that they agreed with values calculated from basic PK and PD equations. The operation of error models were checked by collecting the parameter, which had an error model, and ensuring that the variability matched that of the model input.

Clinically, warfarin experiences a poor correlation between dose and response, primarily due to its complex pharmacology and wide inter- and intra-individual variation in dose-response relationship. Investigations have shown that variation in anticoagulant response of warfarin occur due to interindividual variability in hepatic clearance [6, 18, 49, 58], total protein concentration [29], protein binding affinity [59], potency [6] and sigmoidicity [6, 7]. In this study, a pharmacostatistical model(s) was used to provide theoretical evaluation of the relative impact of these sources of variability on warfarin response.

In the first part of the simulation study, variability was considered in each of the PK and PD parameters separately and their relative impact on variability in warfarin response was determined. In the second part of the simulation study, variability in combined PK

parameters, combined PD parameters, combined PK and PD parameters were considered and their impact on variability in warfarin response was investigated. Finally, the relative impact of intraindividual variability in the dose on the variability in the warfarin response was studied.

The simulation studies were conducted using a dose of 7 mg daily since in the PK-PD model this dose resulted an INR of 2.2, which is at the lower end of therapeutic range of warfarin. This dose is comparitively higher than that used clinically and this is probably because the PK and PD parameter values used in this study were derived from healthy, young volunteers. And the dose of 9 mg daily gave an INR of 2.7, which is at the upper end of the therapeutic range, and was used in the dose variability study because of the non-linear PD model.

PK variability was studied by varying CL_{int_S} and K_{a_S}. The value of volume of distribution was kept constant throughout the simulation studies because the variability in this parameter was reported to be less important [6].

CL_{int_S} was studied because it is important parameter that affects clearance and several studies have demonstrated clinically significant outcomes when this parameter changes [6, 18]. As seen in equation 5, clearance which in itself is critical in determining the steady state plasma concentration.

The variability in CL_{int_S} occurs due to differences in the activity of S-warfarin metabolizing enzyme system. There are several well-established PK drug interactions with warfarin [60]. Many of these drug interactions involve the induction or inhibition of the cytochrome P450 enzymes with associated reduced or increased anticoagulant effects, respectively. Interaction of warfarin with phenylbutazone and metronidazole results in potentiation of anticoagulant effect mainly due to inhibition of the cytochrome P450 isoform [60]. The interaction of warfarin with carbamazepine, phenobarbital, phenytoin and rifampin results in decrease of anticoagulant effect because of the enzyme induction. William et al. reported marked interindividual differences in the rate of metabolism of oral anticoagulants in man [27]. Bowles [58] and Gurwitz et al. [61] conducted studies to determine the effect of age on the anticoagulant response of warfarin and reported that elderly people show an exaggerated anticoagulant response to warfarin, possibly because of the decrease in clearance with age.

Chan et al. reported 31 % CV in hepatic clearance of S-warfarin [6]. Routledge et al. concluded 40 % CV in total clearance of warfarin [18]. Based on this information, the effect of interindividual variability in CL_{int_S} was studied from 10 to 60 % CV (Figure 3 & 4). The statistical analysis of computer-simulated data using ANOVA concluded that the variability in CL_{int_S} alone or in combination with IC_{50}/γ are significant ($P<0.05$) and need to be carefully considered to perform dose optimization in warfarin therapy and use the drug safely.

Warfarin is highly protein bound (in excess of 99%) and the anticoagulant effect is caused by the very small fraction of the drug that is free. Albumin acts as the storage depot for warfarin. As indicated in section 1.5.1 of this thesis, the variation in P and K_{a_S} can occur due to various physiologic and pathologic conditions. Under these circumstances clinically significant changes in the anticoagulant response of warfarin have been observed [29]. Drugs that can displace the albumin will also in theory increase the action of warfarin. However, this effect may be counteracted by more rapid elimination of the drug [59]. Interestingly, although variability in protein binding produced almost equivalent variability in C_S (Table 2), this source of variability had little impact on INR, especially in comparison to variability in CL_{int_S} (Figure 3). The statistical analysis of the computer-simulated data using ANOVA indicated that K_{a_S} do not significantly affect the warfarin response ($P<0.05$).

The relationship between C_S and PCA is given by (Pitsiu et al 1993)

$$PCA = [\ 100 / (1 + (C_s^{\gamma} / IC_{50}^{\gamma}))\ ] \qquad (16)$$

The relationship between the PD parameters and the INR can be derived by subtituting the equation (12) and (16) in equation (13):

$$INR = [1.001 + (0.355 * C_s^{\gamma} / IC_{50}^{\gamma})\ ]^{ISI} \qquad (17)$$

Thus, the warfarin response in terms of INR is dependent on PD parameters such as IC_{50} and γ. IC_{50} and γ was reported to vary from individual to individual [6, 7]. Therefore, the impact of variability in these PD parameters on INR was studied.

Potency (IC_{50}) represents the concentration of drug that produces 50% of the maximum effect. It explains the differences in sensitivity of the drug to the receptors. As the IC_{50} increases, the drug gets less potent and a smaller response is achieved from a given dose. This condition is referred to as warfarin resistance. In contrast as the IC_{50} decreases, the drug gets more potent and a higher response is achieved from a given dose. Such condition is usually termed as warfarin sensitivity. In the literature several studies have reported about the warfarin resistance and sensitivity. In most cases the exact mechanism for these changes has not been identified. However, possible explanations for such changes in IC_{50} of warfarin include altered receptor affinity, noncompliance, exogenous consumption of vitamin K, hereditary reasons, laboratory error and concurrent ingestion of warfarin with nutritional supplements containing vitamin K are known to decrease warfarin's effects. In early 1985, Alving et al reported that 57-year-old black women and her family developed warfarin resistance due to hereditary reasons that altered PDs of warfarin [(43)]. Warfarin resistance associated with infusion of high doses of lipids such as propofol containing 10 % soybean oil as an emulsified preparation was investigated by MacLaren et al. and concluded that lipid emulsions may interfere pharmacodynamically with warfarin activity by enhancing the production of clotting factors, facilitating platelet aggregation or may facilitate warfarin binding to albumin [(62)].

The amount of interindividual variability in IC_{50} was chosen based on the values reported in the literature. The population analysis performed by Chan et al. using NONMEM approach showed that IC_{50} of unbound fraction of S-warfarin varied by 58 % CV [(6)]. In this study, the effect of interindividual variability in IC_{50} on warfarin response was

demonstrated from 10 % to 60 % CV (Figure 3). Analysis of the computer-simulated data suggests that warfarin response is highly sensitive to variability in IC_{50} alone or in combination with CL_{int_S} (Table 5).

Sigmoidicity refers to the slope value for the relationship between the drug concentration and pharmacological effects. Interindividual variability in γ for warfarin is reported in the literature [6, 7]. It is difficult to explain the reason for the variation in this slope parameter, as this parameter has no clear physiological interpretation. However, the variability in γ is of substantial clinical significance. Individuals having low γ value (steeper slope) for the relationship between drug concentration and the anticoagulant effect will be very sensitive to small changes in drug concentration and becomes difficult to maintain their INR values in the therapeutic range. On the contrary, individuals with higher γ value for drug concentration-effect relationship will not be much sensitive to small changes in drug concentration. Chan et al. [6] reported 25 % CV and Pitsiu et al. [7] reported 42 % CV in γ. However, this study found that variability in γ, when varied at various levels from 10 % to 60 % CV (Figure 3), had little impact on warfarin response.

Variability in dose results due to weight variation, assay error, flow properties of the tablet blend and various formulation processes. The United States Pharmacopoeia allows up to 5 % variability in the dose of warfarin [63]. Based on this information, the intraindividual variability in the dose was studied at three levels (3, 6 and 10 % CV). Variability in the dose resulted in almost the same amount of variability in C_S and slightly more than half in INR. Thus, the PK and PD responses appear to be less sensitive

to the changes in dose. As seen from Figure 8, it can be concluded that the dose of warfarin when used at the lower end and higher end of the therapeutic range does not produce significant difference in the PK & PD responses.

In conclusion, the computer simulations in this study have demonstrated that CL_{int_S} and IC_{50} are most influencing parameters that affect the dose-response relationship than the other parameters studied. Therefore, it is important to consider the influence of variability in these parameters in optimizing the dose and thereby achieving the desired therapeutic effect in patients who have the history of hepatic impairment, warfarin resistance and sensitivity.

CONCLUSIONS

- An integrated PK-PD model was successfully developed for warfarin using STELLA®.

- This study demonstrated the importance of considering PD model along with PK model to understand the dose-response relationship for warfarin.

- Computer simulations were performed using a pharmacostatistical PK-PD model to understand the manner in which various PK and PD parameters affect the dose-concentration-effect relationship for warfarin. Analysis of simulation data demonstrated that interindividual variability in PK parameter (CL_{int_S}) and PD parameter (IC_{50}) most significantly affect the variability in warfarin response. The variability in these parameters should be carefully considered in order to use the drug safely and effectively.

- Findings from the statistical analysis strongly suggest that CL_{int_S}, IC_{50} and the combination of PK & PD parameters such as CL_{int_S} & IC_{50} and CL_{int_S} & γ significantly affect the variability in INR ($P < 0.05$).

- The drug response was found to be less sensitive to the intraindividual variability in dose and interindividual variability in K_{a_S} and γ.

Thus, simulation studies using STELLA produced sensible results that helps in understanding the manner in which the variability in various PK and PD parameters affect the overall dose-response relationship. Such information is important and useful in optimizing the doses in specific population under risk and uses the drug effectively and safely. It is to be noted that the conclusions of this study are obviously limited to the theoretical assumptions considered in the model.

Table 1: Population average values of the PK and PD parameters used in the model

Parameter	Mean Value (Input to Model) *
PK parameters	
V_{d_S}	11.8 L
V_{d_R}	10.5 L
CL_{int_S}	59.21 L/h
CL_{int_R}	32.85 L/h
K_{a_S}	300000 h^{-1}
K_{a_R}	246600.49 h^{-1}
PD parameters	
IC_{50}	0.394
$CA_{\_normal}$	100
γ	1.0
ISI	2.2
K_d	0.094 h^{-1}

* For sources of these values see the text.

Table 2: Effect of interindividual variability in PK parameters on C_S when 7 mg dose of warfarin was used

% CV in PK parameter	**% CV in C_S due to variability in [n=100]**		
	CL_{int_S}	**K_{a_S}**	**Combined PK**
10	10.1	10.3	14.6
20	20.5	20.6	31.3
30	32.9	30.5	50.2
40	52.2	40.3	71.2
50	120.6	49.8	130.5
60	163.6	59.3	200.5

Abbreviations: Cl_{int_S} = Intrinsic clearance of S-enantiomer of warfarin
K_{a_S} = Protein binding affinity constant of S-enantiomer of warfarin
Cp = Overall warfarin plasma concentration
C_S = Plasma concentration of S-enantiomer of warfarin
n = Number of study individuals
Combined PK = Variability in Cl_{int_S} and K_{a_S}

Table 3: Effect of interindividual variability in PK and PD parameters on INR when 7 mg dose of warfarin was used

% CV in PK/ PD parameter	**% CV in INR due to variability in [n=100]**						
	Cl_{int_S}	**K_{a_S}**	**Combined PK**	**IC_{50}**	**γ**	**Combined PD**	**Combined PK & PD**
10	6.8	7.1	9.0	6.8	1.6	6.2	12.9
20	14.1	14.3	23.5	14.2	3.2	28.5	31.6
30	24.4	21.4	42.5	24.5	4.8	48.7	63.7
40	46.5	28.6	74.8	46.7	6.5	87.5	121.7
50	119.1	35.7	122.6	117.9	8.2	143.2	157.8
60	153.6	42.8	161.2	151.5	10.1	166.1	N/A

Abbreviations: Cl_{int_S} = Intrinsic clearance of S-enantiomer of warfarin
K_{a_S} = Protein binding affinity constant of S-enantiomer of warfarin
IC_{50} = Potency of S-enantiomer of warfarin
γ = Sigmoidicity
INR = International Normalized Ratio
n = Number of study individuals
N/A = Not Applicable
Combined PK = Variability in Cl_{int_S} and K_{a_S}
Combined PD = Variability in IC_{50} and γ
Combined PK & PD = Variability in Cl_{int_S} , K_{a_S}, IC_{50} and γ

Table 4: Experimental design for ANOVA test

Run Number	% CV Parameter			
	CL_{int_S}	K_{a_S}	IC_{50}	γ
1	-	-	-	-
2	-	+	-	-
3	-	-	+	-
4	-	+	+	-
5	+	-	-	-
6	+	+	-	-
7	+	-	+	-
8	+	+	+	-
9	-	-	-	+
10	-	+	-	+
11	-	-	+	+
12	-	+	+	+
13	+	-	-	+
14	+	+	-	+
15	+	-	+	+
16	+	+	+	+

Abbreviations: (-) means 30% CV and (+) means 60% CV
Cl_{int_S} = Intrinsic clearance of S-enantiomer of warfarin
K_{a_S} = Protein binding affinity constant of S-enantiomer of warfarin
IC_{50} = Plasma concentration of S-warfarin that produces 50% inhibition of clotting factor synthesis
γ = Sigmoidicity

Table 5: Analysis of variance of effect of variability in PK and PD parameters on Ln(INR)

Source	DF▲	Seq SS✣	Adj SS✣	Adj MS†	F	P*
CL_{int_S}	1	3.2115	2.8096	2.8096	3.41	**0.060**
K_{a_S}	1	0.7613	0.8196	0.8196	1.00	0.316
IC_{50}	1	3.7037	3.4390	3.4390	4.18	**0.041**
γ	1	0.1913	0.2329	0.2329	0.28	0.590
$CL_{int\_S}*K_{a\_S}$	1	0.3629	0.5025	0.5025	0.61	0.480
$CL_{int\_S}*IC_{50}$	1	3.1134	3.2090	3.2090	3.90	**0.049**
$CL_{int\_S}*\gamma$	1	3.2399	3.2149	3.2149	3.91	**0.047**
$K_{a\_S}*IC_{50}$	1	0.5449	0.5997	0.5997	0.73	0.418
$K_{a\_S}*\gamma$	1	0.0358	0.0417	0.0417	0.05	0.832
$IC_{50}*\gamma$	1	0.0516	0.0517	0.0517	0.06	0.802
Error	1495	1231.2517	1231.2517	0.8236		
Total	1505	1246.4681				

▲ DF = Degrees of Freedom
✣ Seq SS = Sequential Sum of Squares
✣Adj SS = Adjusted Sum of Squares
† Adj MS = Adjusted Mean Square
* $P < 0.06$

Table 6: Effect of intraindividual variability in dose on variability in C_S and INR.

% CV in Dose	**% CV in [n=2000]**	
	C_S	**INR**
With Dose 7mg		
3	3.10	1.77
6	5.93	3.55
10	10.06	6.01
With Dose 9mg		
3	2.93	2.21
6	5.87	4.34
10	10.38	7.40

Abbreviations: CV = Coefficient of Variation
INR = International Normalized Ratio
n = Number of study individuals

Figure 1: Schematic diagram showing various models in STELLA®

(a) Pharmacokinetic model

(b) Pharmacodynamic model

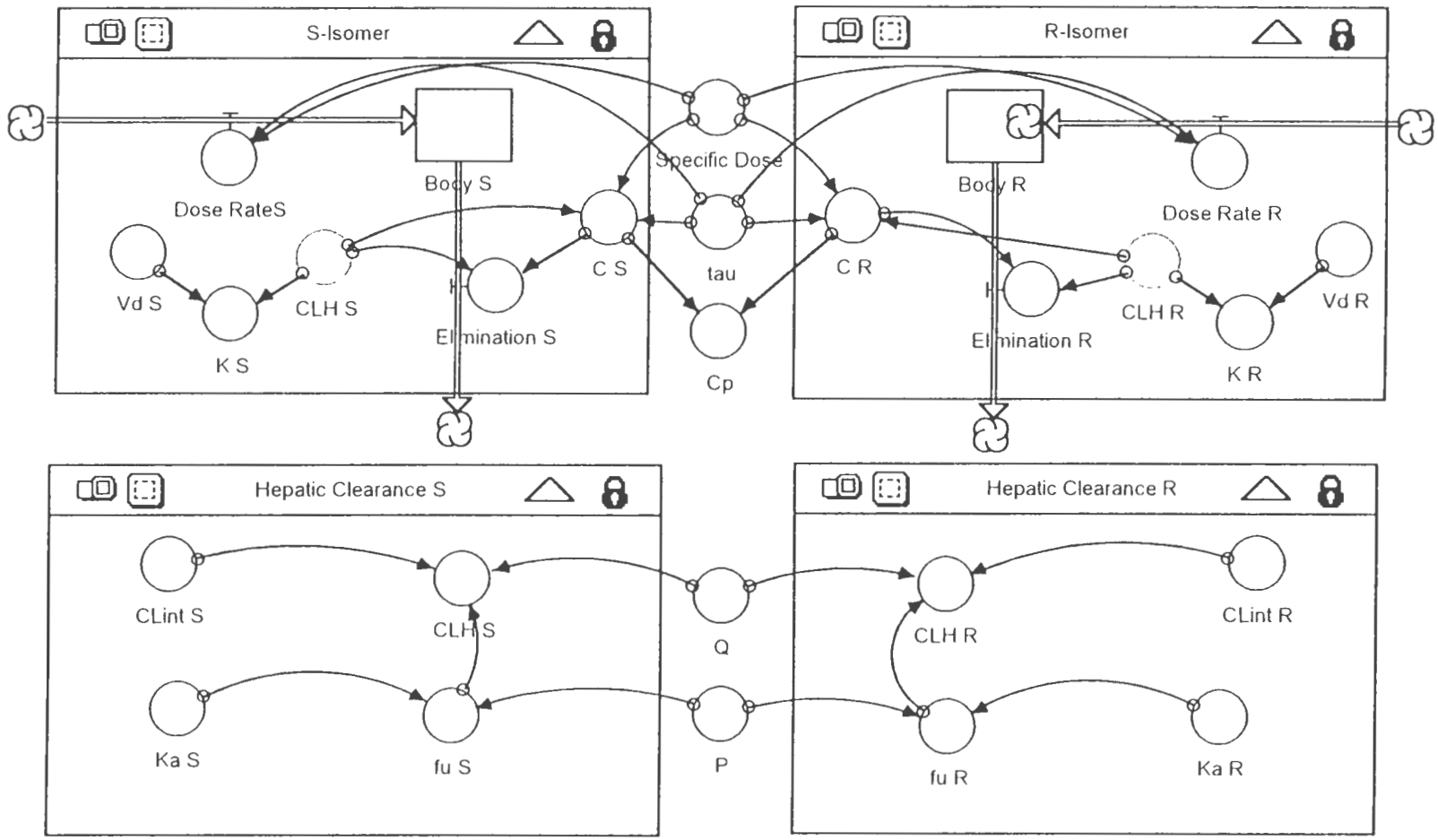
(c) Interindividual variability in CL_{int_S}

(d) Interindividual variability in K_{a_S}

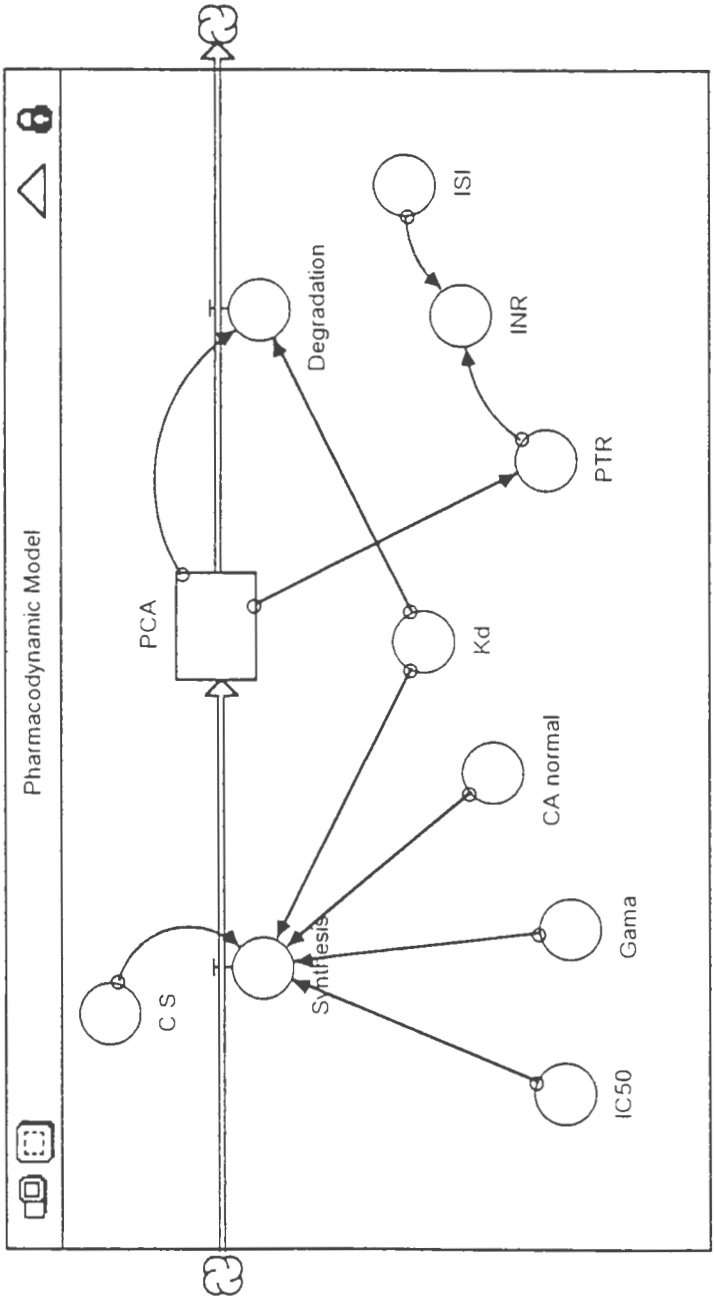
(e) Interindividual Variability in IC_{50}

(f) Interindividual variability in γ

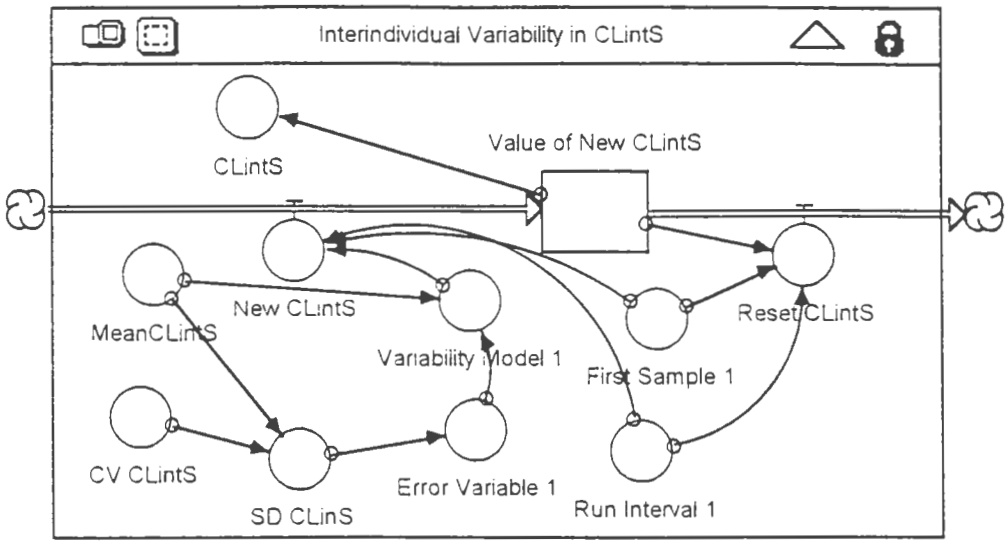
(g) Intraindividual variability in dose


S-Isomer
R-Isomer
Specific Dose
Body S
Body R
Dose RateS
Dose Rate R
Vd S
CLH S
C S
tau
C R
CLH R
Vd R
K S
Cp
K R
Hepatic Clearance S
Hepatic Clearance R
CLint S
CLH S
Q
CLH R
CLint R
Ka S
fu S
P
fu R
Ka R

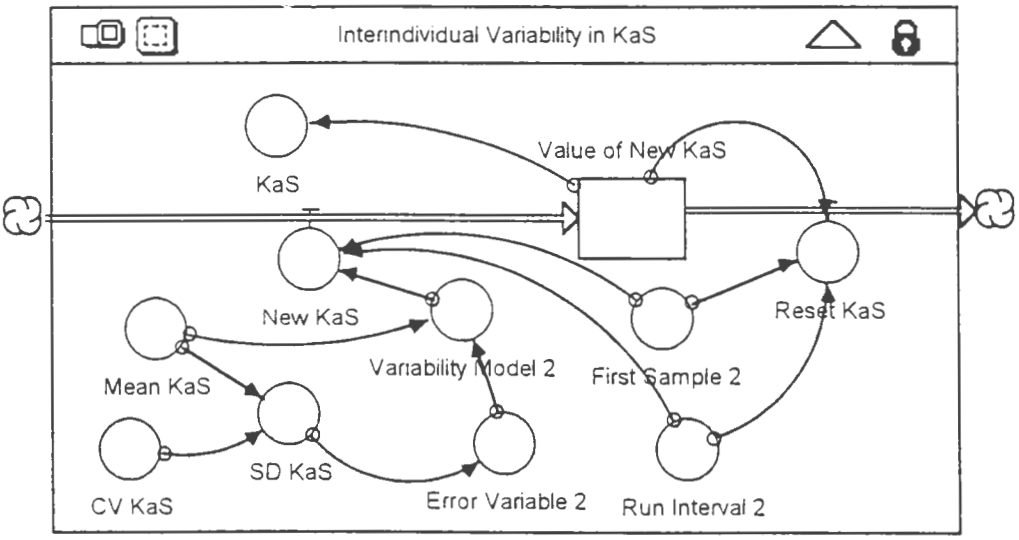
(a)


Pharmacodynamic Model
C S
Synthesis
IC50
Gama
CA normal
PCA
Kd
Degradation
ISI
INR
PTR

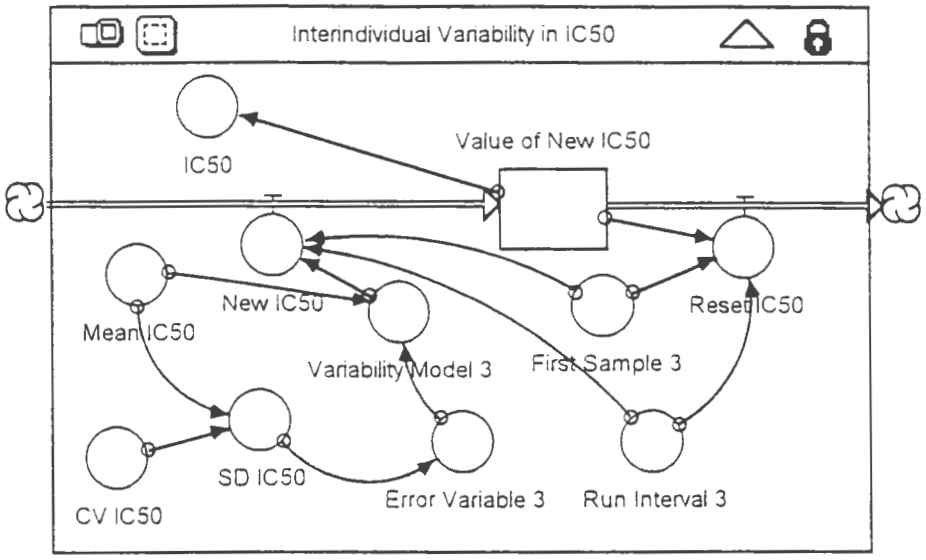
(b)


Interindividual Variability in CLintS
CLintS
Value of New CLintS
New CLintS
MeanCLintS
Variability Model 1
First Sample 1
Reset CLintS
CV CLintS
SD CLinS
Error Variable 1
Run Interval 1

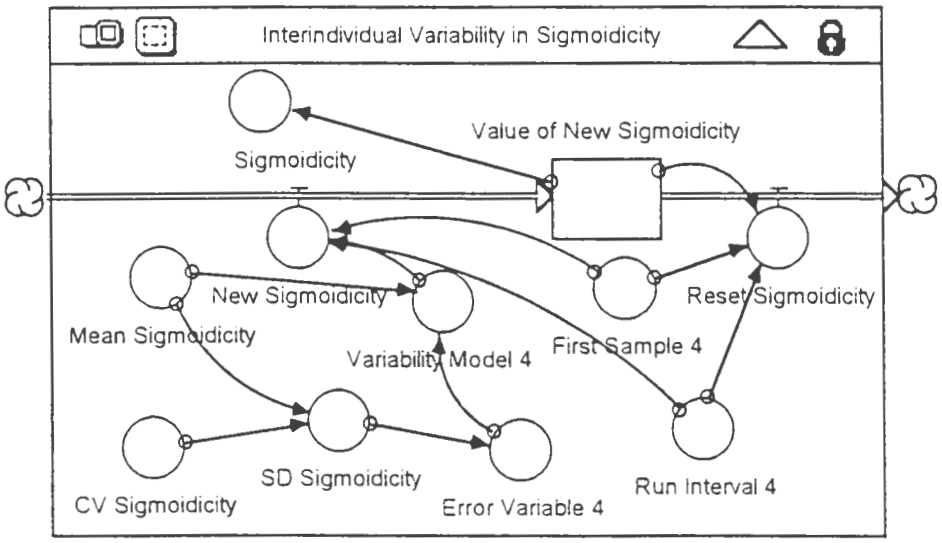
(c)


Interindividual Variability in KaS
KaS
Value of New KaS
New KaS
Mean KaS
Variability Model 2
First Sample 2
Reset KaS
CV KaS
SD KaS
Error Variable 2
Run Interval 2

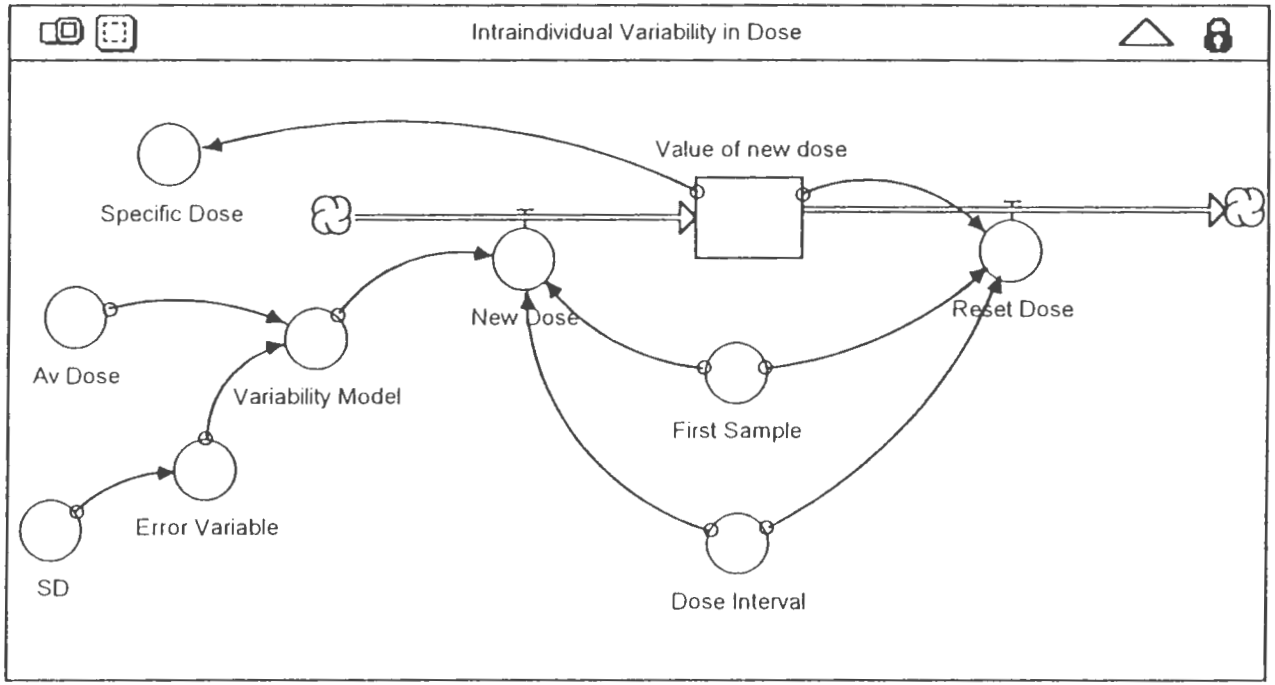
(d)


Interindividual Variability in IC50
IC50
Value of New IC50
New IC50
Mean IC50
Reset IC50
Variability Model 3
First Sample 3
SD IC50
CV IC50
Error Variable 3
Run Interval 3

(e)


Interindividual Variability in Sigmoidicity
Sigmoidicity
Value of New Sigmoidicity
New Sigmoidicity
Mean Sigmoidicity
Reset Sigmoidicity
Variability Model 4
First Sample 4
SD Sigmoidicity
CV Sigmoidicity
Error Variable 4
Run Interval 4

(f)


Intraindividual Variability in Dose
Value of new dose
Specific Dose
New Dose
Reset Dose
Av Dose
Variability Model
First Sample
Error Variable
SD
Dose Interval

(g)

Figure 2: Effect of interindividual variability in PK parameters as the function of coefficient of variation (CV) in C_S when 7 mg dose of warfarin was used.

250
200
150
100
50
0
% CV in Cs
0
10
20
30
40
50
60
70
% CV in parameter
Clint_S
Ka_S
Combined PK

Figure 3: Effect of interindividual variability in PK and PD parameters as the function of coefficient of variation (CV) in INR when 7 mg dose of warfarin was used

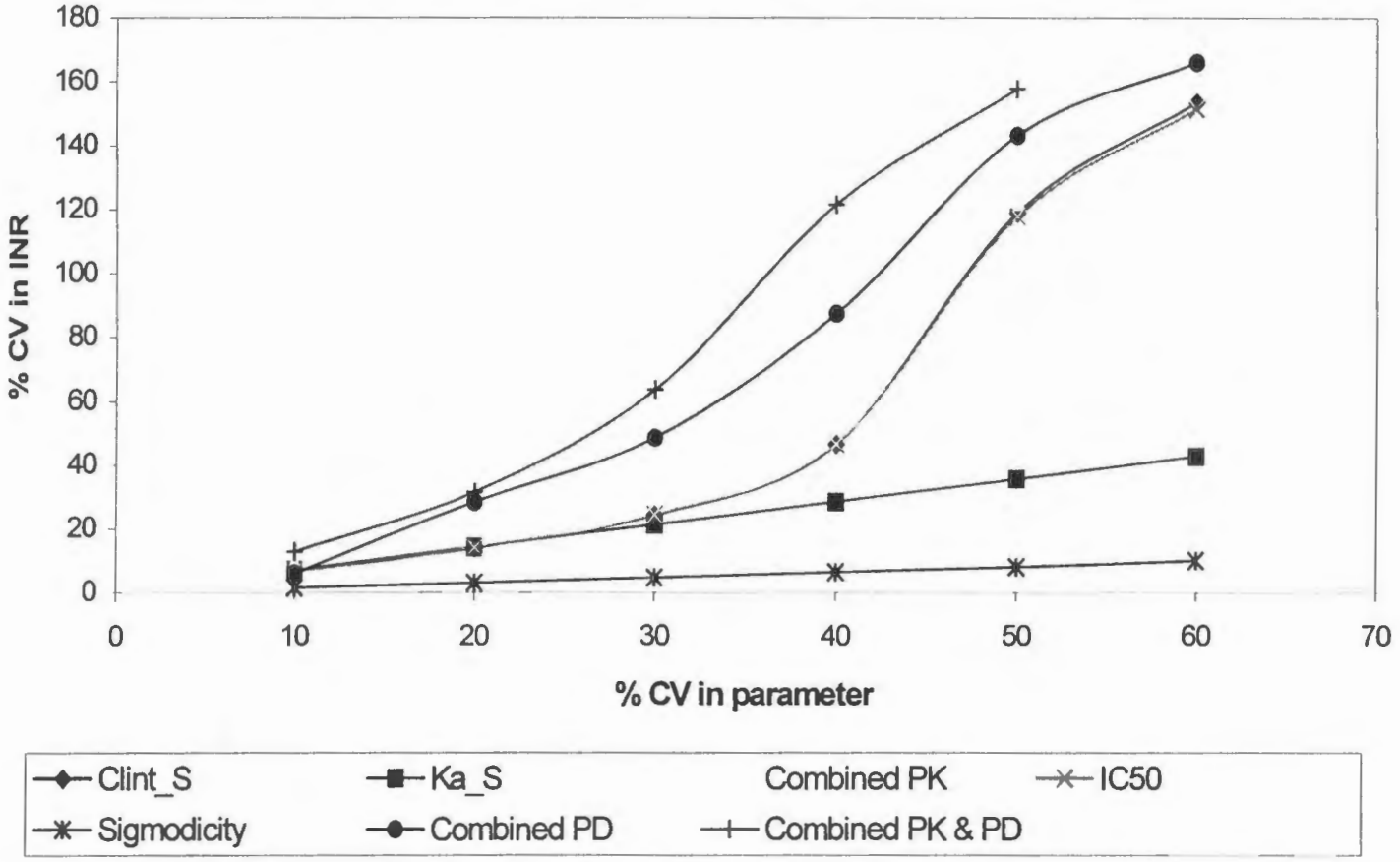

180
160
140
120
100
80
60
40
20
0
% CV in INR
0
10
20
30
40
50
60
70
% CV in parameter
Clint_S
Ka_S
Combined PK
IC50
Sigmodicity
Combined PD
Combined PK & PD

Figure 4: Main effects plot of Ln(INR) data.

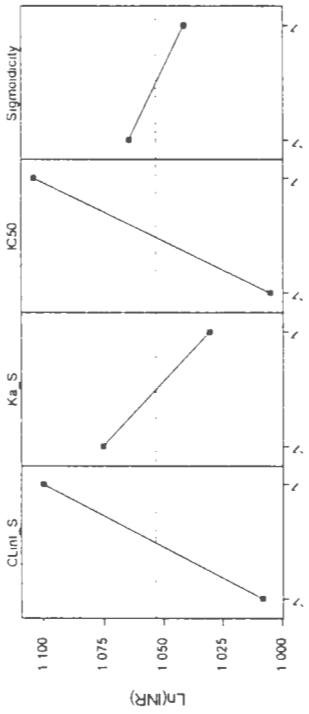

CLint_S
Ka_S
IC50
Sigmoidicity
1 100
1 075
1 050
1 025
1 000
Ln(NR)

Figure 5: Interaction plot of Ln(INR) data.

CLint_S
.1
.1
Ka_S
.1
.1
IC50
.1
.1
Sigmoidicity
1.1
1.0
0.9

Figure 6: Effect of intraindividual variability in dose (7 mg) as the function of coefficient of variation (CV) in C_S and INR.

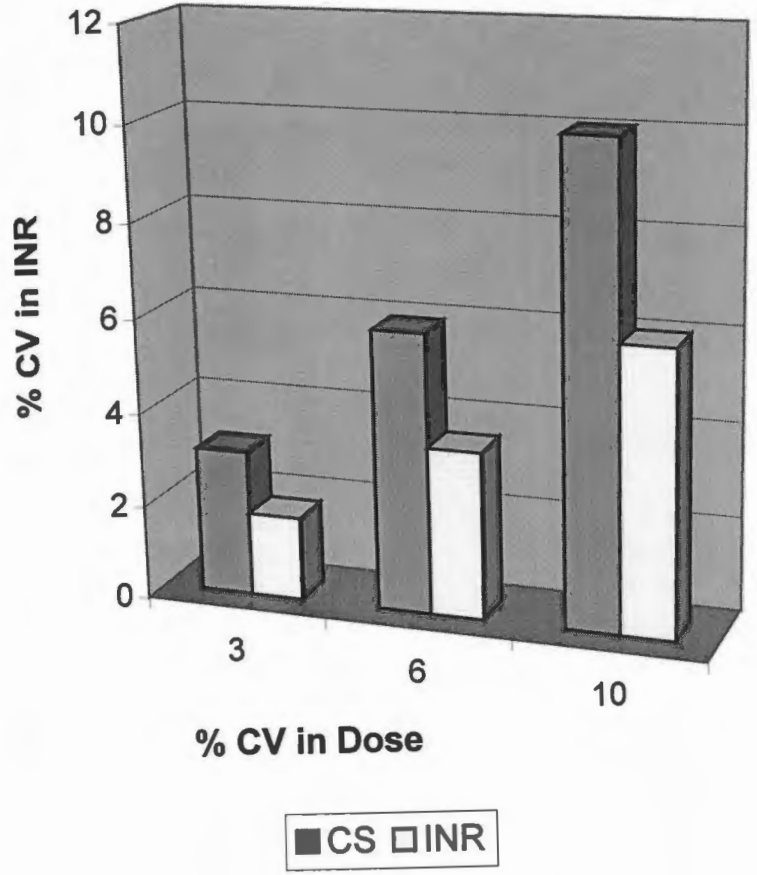

12
10
8
6
4
2
0
% CV in INR
3
6
10
% CV in Dose
CS
INR

Figure 7: Effect of intraindividual variability in dose (9 mg) as the function of coefficient of variation (CV) in C_S and INR.

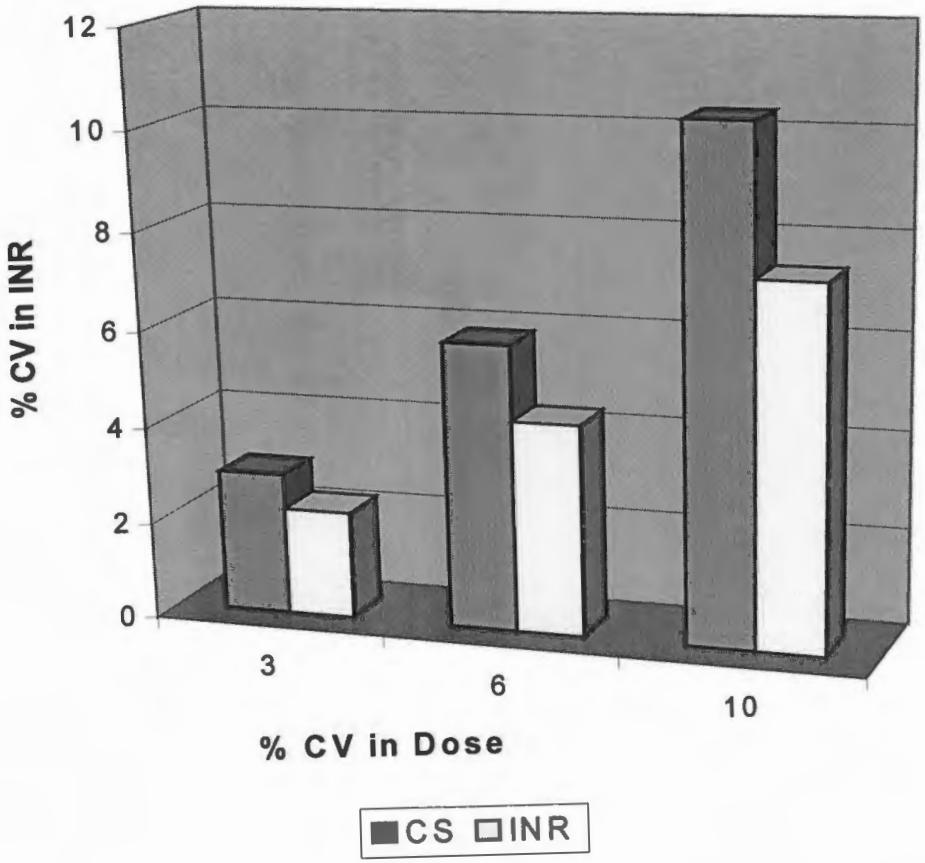
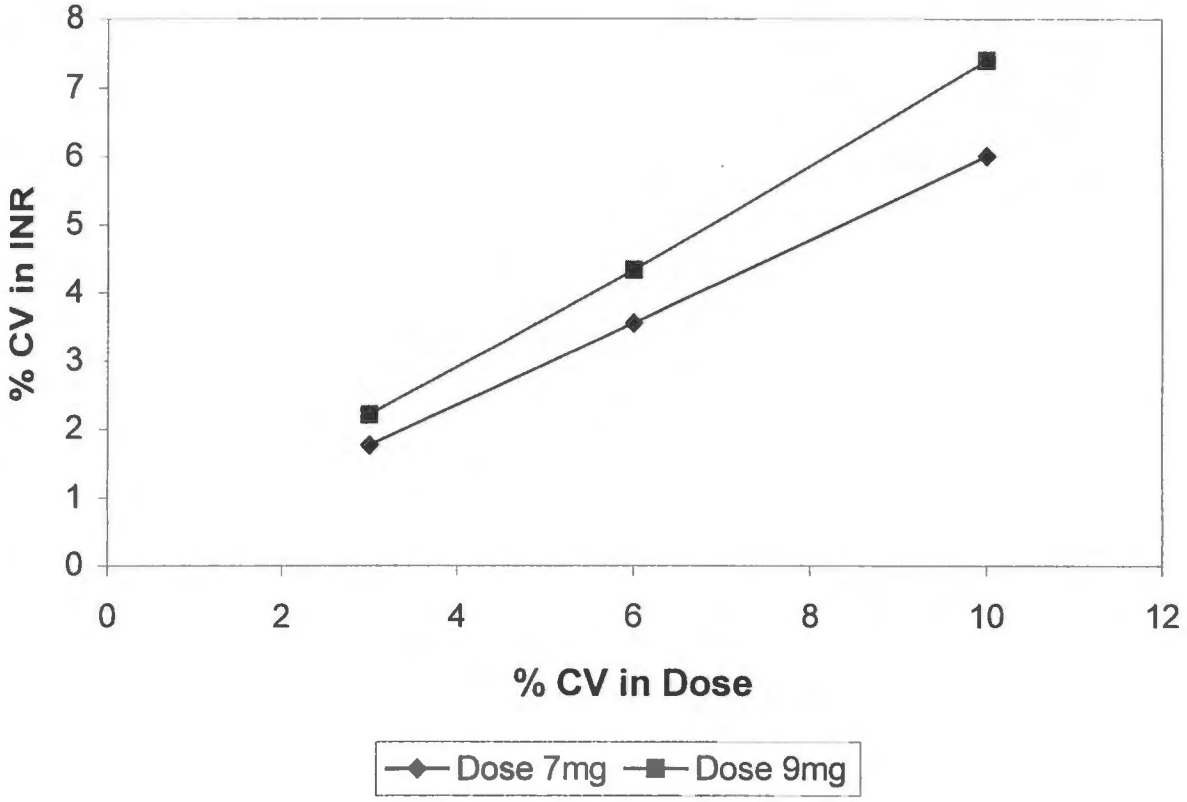

12
10
8
6
4
2
0
% CV in INR
3
6
10
% CV in Dose
CS
INR

Figure 8: Effect of intraindividual variability in dose as the function of coefficient of variation (CV) in INR with dose of 7 mg and 9 mg warfarin.


% CV in INR
8
7
6
5
4
3
2
1
0
0
2
4
6
8
10
12
% CV in Dose
Dose 7mg
Dose 9mg

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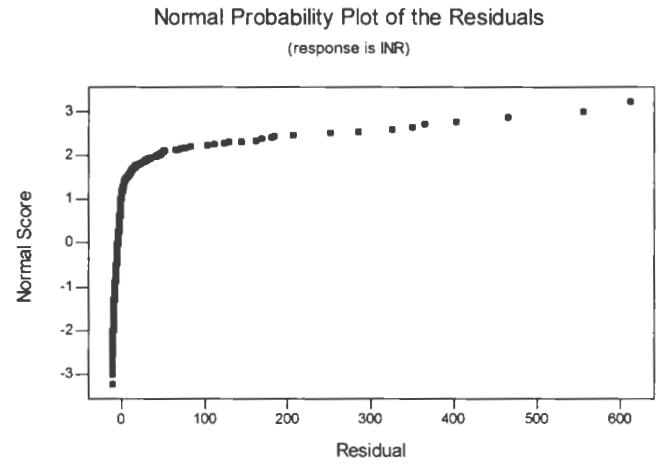
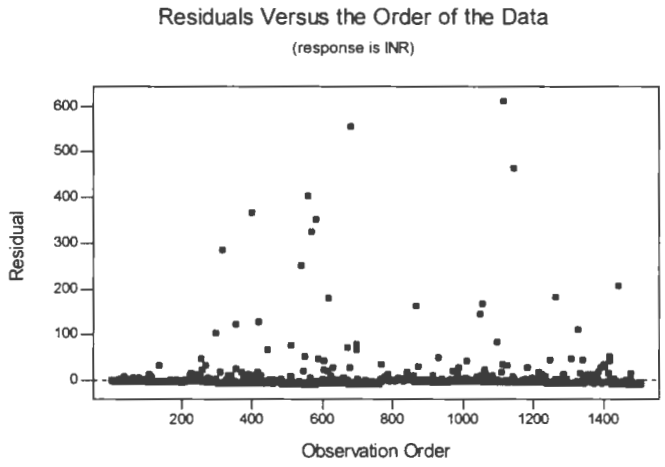
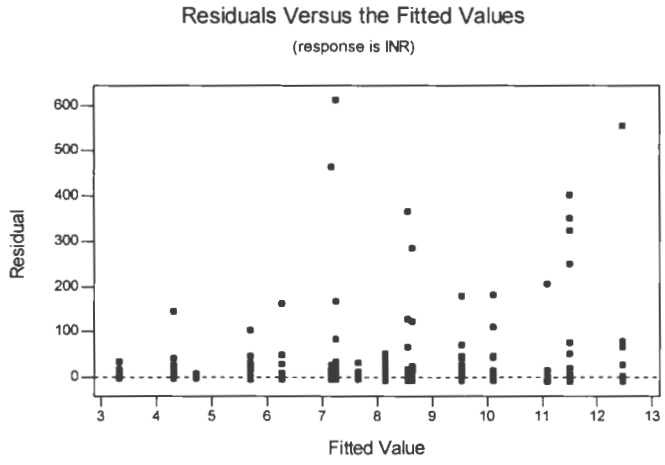
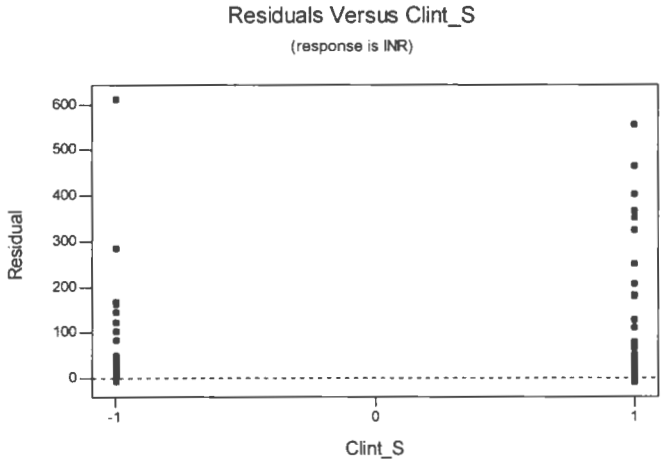
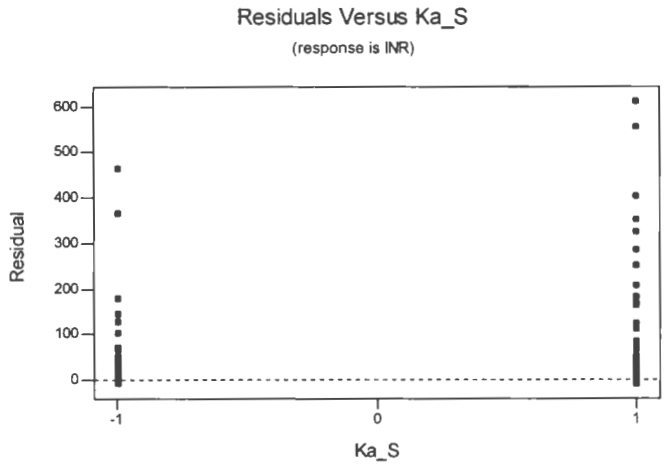
Appendix 1: Model adequacy check for INR data using ANOVA test.

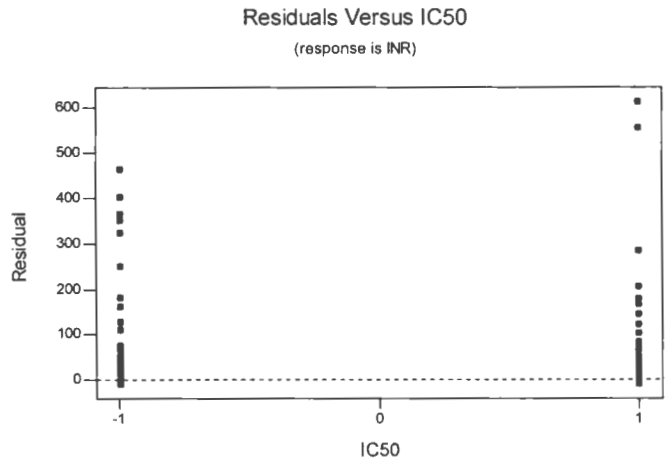

Normal Probability Plot of the Residuals
(response is INR)
Normal Score
3
2
1
0
-1
-2
-3
0
100
200
300
400
500
600
Residual

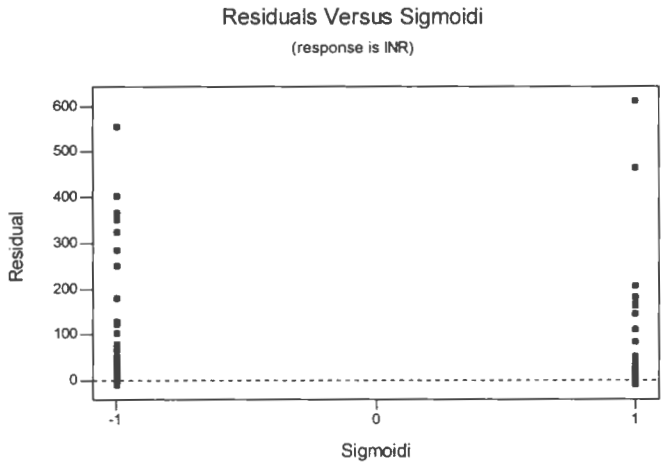

Residuals Versus the Order of the Data
(response is INR)
Residual
600
500
400
300
200
100
0
200
400
600
800
1000
1200
1400
Observation Order


Residuals Versus the Fitted Values
(response is INR)
Residual
600
500
400
300
200
100
0
3
4
5
6
7
8
9
10
11
12
13
Fitted Value

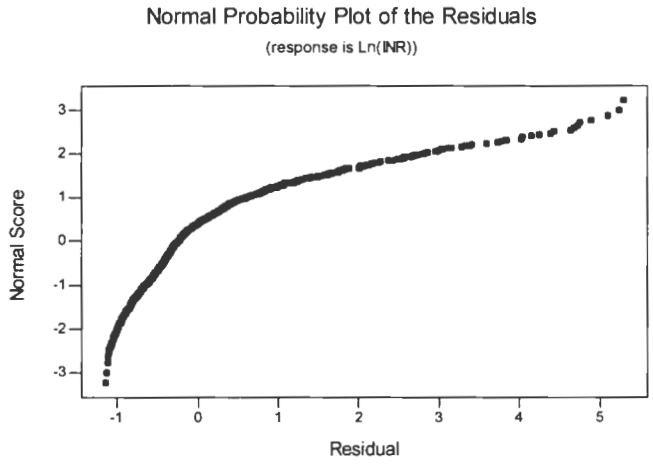

Residuals Versus Clint_S
(response is INR)
Residual
600
500
400
300
200
100
0
-1
0
1
Clint_S

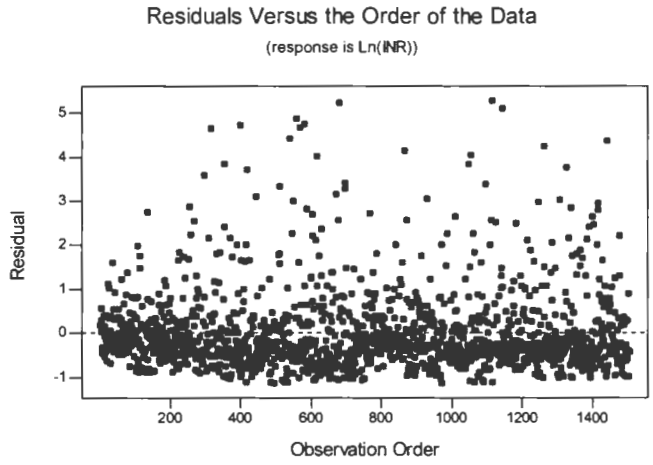

Residuals Versus Ka_S
(response is INR)
Residual
600
500
400
300
200
100
0
-1
0
1
Ka_S

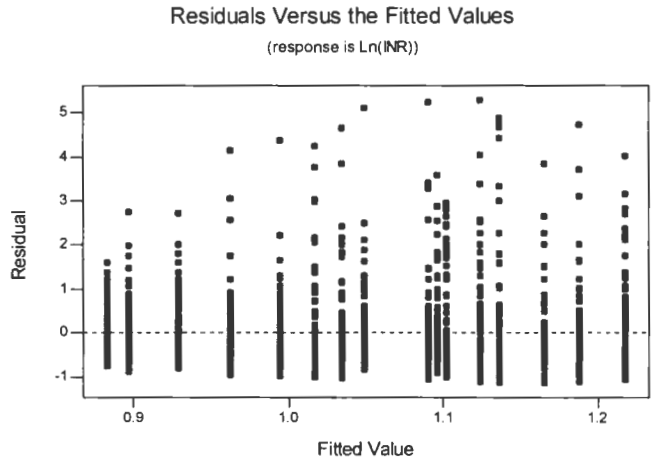

Residuals Versus IC50
(response is INR)
Residual
600
500
400
300
200
100
0
-1
0
1
IC50

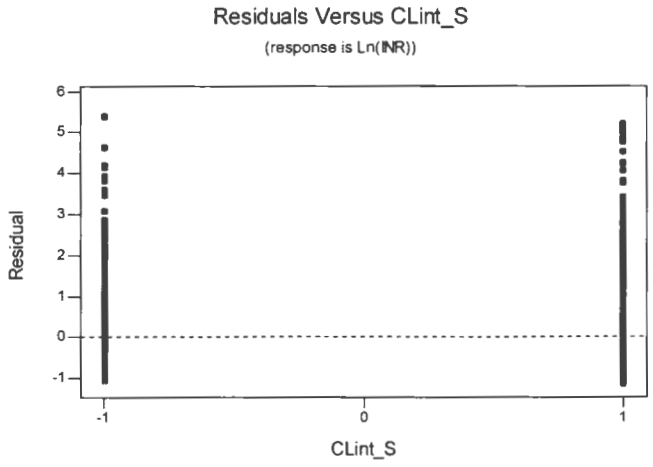

Residuals Versus Sigmoidi
(response is INR)
Residual
600
500
400
300
200
100
0
-1
0
1
Sigmoidi

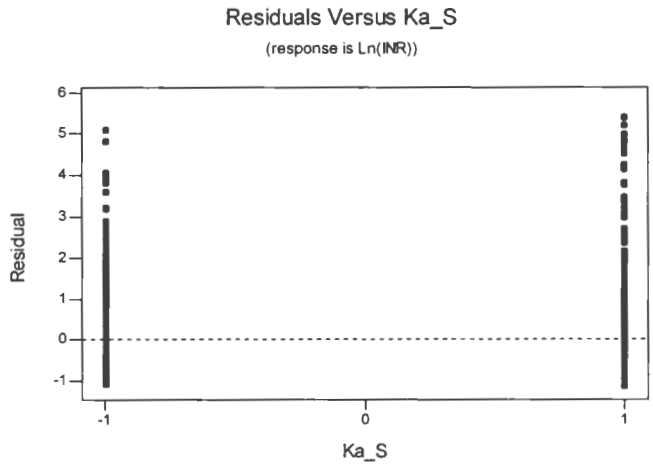
Appendix 2: Model adequacy check for Ln(INR) data using ANOVA test.

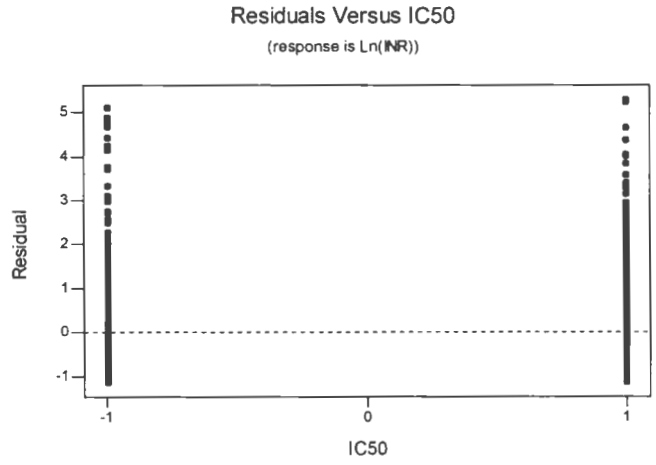

Normal Probability Plot of the Residuals
(response is Ln(INR))
Normal Score
Residual

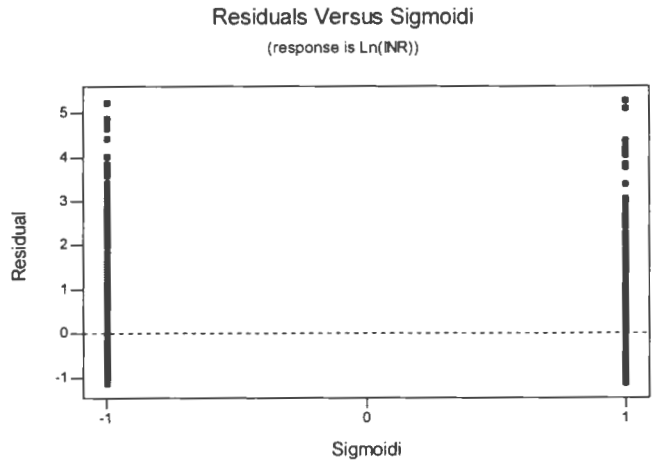

Residuals Versus the Order of the Data
(response is Ln(INR))
Residual
Observation Order

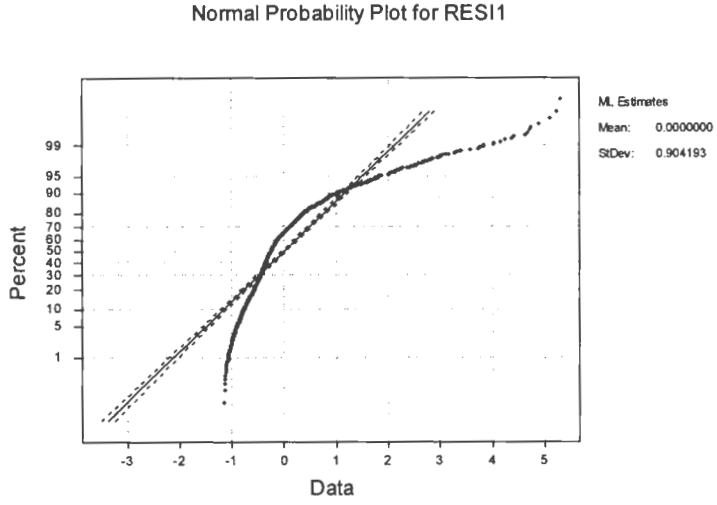

Residuals Versus the Fitted Values
(response is Ln(INR))
Residual
Fitted Value


Residuals Versus CLint_S
(response is Ln(INR))
Residual
6
5
4
3
2
1
0
-1
-1
0
1
CLint_S

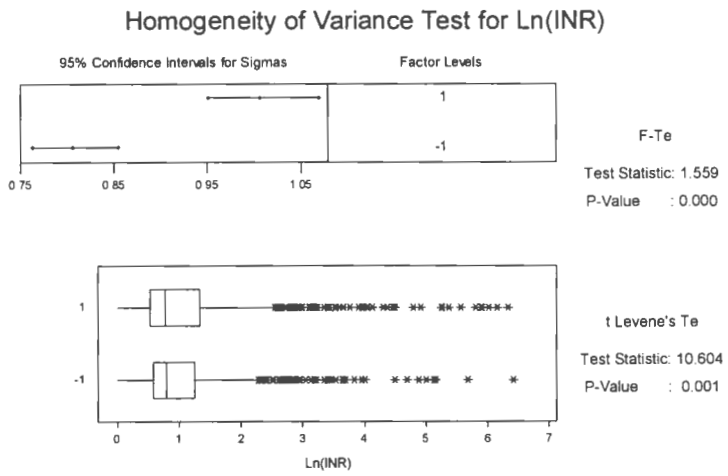

Residuals Versus Ka_S
(response is Ln(INR))
Residual
6
5
4
3
2
1
0
-1
-1
0
1
Ka_S


Residuals Versus IC50
(response is Ln(INR))
Residual
5
4
3
2
1
0
-1
-1
0
1
IC50

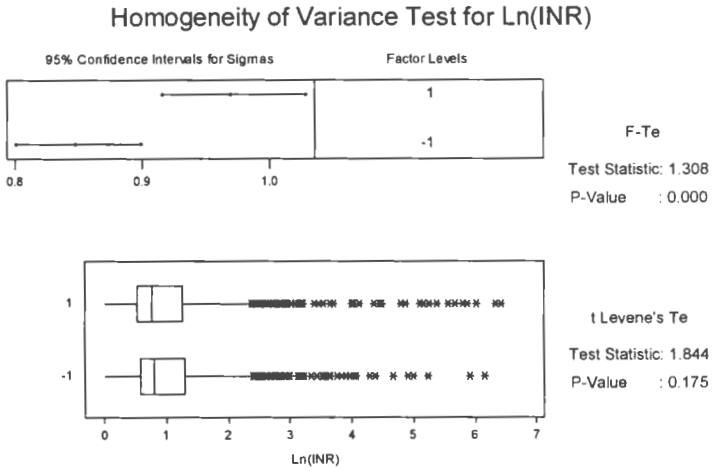

Residuals Versus Sigmoidi
(response is Ln(INR))
Residual
5
4
3
2
1
0
-1
-1
0
1
Sigmoidi


Normal Probability Plot for RESI1
Percent
99
95
90
80
70
60
50
40
30
20
10
5
1
-3
-2
-1
0
1
2
3
4
5
Data
ML Estimates
Mean: 0.0000000
StDev: 0.904193

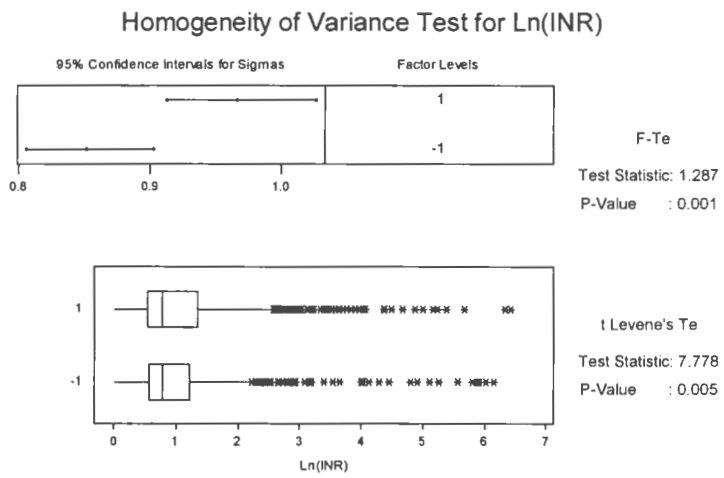
Barlett's test for CL_{int_S}:



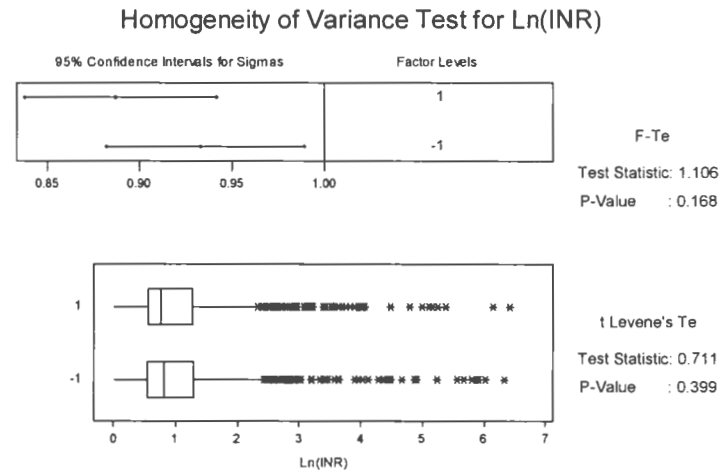
Barlett's test for K_{a_S}:



Barlett's test forIC_{50}:



Barlett's test for γ:



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