# PRINCIPLES OF FORMULATION PHYSICAL STABILITY IN AQUEOUS MEDIA

by

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Jennifer Lynne Huff Johnson

entitled Principles of Formulation Physical Stability in Aqueous Media

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy

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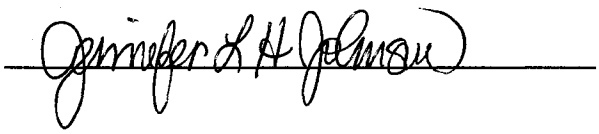
SIGNED: 

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To Jay for listening without fixing, for believing in yourself and teaching me to do the same, for not telling me to run faster, for taking me to Baskin Robbins, for making me laugh....and for your patience. I could not have gotten through it without you.

# TABLE OF CONTENTS

## TABLE OF CONTENTS-CONTINUED

# TABLE OF CONTENTS-CONTINUED

# TABLE OF CONTENTS-CONTINUED

# LIST OF FIGURES

# LIST OF TABLES

## ABSTRACT

Drugs often require formulation techniques to increase their solubility and/or increase their stability in aqueous media. In cases where solubility enhancement is necessary, ideally, the physical stability of the formulation should be maintained upon administration. Unfortunately, in some cases, drugs precipitate out of solution when the formulation is introduced into aqueous media. Intravenously, drug precipitation can cause phlebitis and orally, it can hinder absorption.

In order to avoid phlebitis, new intravenous (IV) parenterals are commonly screened by injection into animals. An *in vitro* dynamic injection model is introduced and validated. Twenty-one currently marketed IV products were used to investigate the validity of the model. Logistic regression and a receiver operator curve (ROC) indicate a value of 0.003 to best delineate phlebitic and non-phlebitic products. Measures of sensitivity (0.83), specificity (0.93), positive predictive value (0.93) and negative predictive value (0.78) indicate the model's accuracy and reliability.

The model was tested to screen different formulations of a new proprietary antibiotic, Van-An. The opacities obtained for the

formulation with acetate addition were significantly smaller than for the phosphate buffered formulation at 4 injection rates. In general the results suggest acetate as a better buffer species than phosphate for the pH range defined.

The solubilization and stabilization of an orally administered formulation of a new anti-hepatitis C drug, PG301029, is established through a novel method of complexation. Hydroxypropyl-β-cyclodextrin (HPβCD) and PG301029 are dissolved in glacial acetic acid. The acetic acid is removed by roto-evaporation such that the drug exists primarily in the complexed form. Formulated PG301029 is found to be stable upon storage and, once reconstituted, the powder dissolves rapidly and PG301029 remains stable for 21 hours. Acetic acid and HPβCD provide several formulation advantages. PG301029 and HPβCD are both soluble in acetic acid. Once prepared, the formulation can be stored in solid form. Upon reconstitution, the cyclodextrin both protects the drug from water and enhances the solubility of PG301029. This unique method results in a solid dosage form of PG301029 which is soluble, stable and highly bioavailable in mice when given orally.

# CHAPTER I: THE DYNAMIC INJECTION APPARATUS

## INTRODUCTION

In order to ensure rapid and complete bioavailability, many drugs are given by intravenous injection. This is especially true for anticancer drugs and drugs in the pre-clinical and clinical stages of development where absorption and bioavailability are being established. If the drug, at its target dose, is not soluble in the desired injection volume, it must be solubilized in order to proceed with product development. The solubilities of such drugs are frequently increased by the addition of one or more water miscible adjuvants. The most common adjuvants are cosolvents, surfactants, and buffers. Many drugs solubilized by these means precipitate when diluted or injected into the bloodstream. If a drug precipitates in the vein, the potential for venous irritation increases, due not only to mechanical irritation but also to prolonged local drug exposure at the vein wall. In fact, when injected intravenously, a number of parenterally formulated drugs produce inflammation of the vein wall (Trissel, 2001). This painful condition is

called phlebitis (Stedman, 1999). Phlebitis can result from mechanical irritation, chemical irritation or as a pharmacological response by the vein wall cells to the drug.

In the case of mechanical irritation, precipitated particles can be irregular or needle shaped and can act like sandpaper; scraping the vein walls as they are carried through the blood stream. Even a relatively small amount of particulate matter in an intravenous fluid can cause phlebitis (Turco and King, 1979; Avis *et al.,* 1986). This is why many IV fluids are passed through an in-line filter prior to injection. However, pre-injection filtration is not effective in cases where the offending particulate matter is created by dilution of the formulation in the bloodstream. Chemical irritation results from direct contact of an irritant with the vein wall cells. Acids, bases, solvents and many drugs can damage living tissue. In addition, some dissolved drugs can produce an undesirable pharmacological response by reacting with a specific receptor on the vein wall (Wu *et al.,* 1991). The undesirable effects caused chemically and pharmacologically are undoubtedly exacerbated by direct cellular contact with the highly concentrated precipitate (Li *et al.,* 1998). Thus, regardless of the cause, precipitation of the drug within

the vein upon dilution significantly compounds the irritation and is a key factor in determining the duration and severity of the irritation (Turco, 1987; Ward and Yalkowsky, 1993).

The most commonly used *in vivo* phlebitis screening method involves injecting the prototype formulation into the lateral ear vein of the rabbit (Yalkowsky *et al.,* 1998). The tail vein of the rat, the femoral vein of the dog, and the catheterized jugular vein of the monkey are also used, but less commonly (Yalkowsky *et al.,* 1983). Visual observation and temperature increase at the injection site are used to evaluate the degree of phlebitis. In the rabbit ear, the symptoms of phlebitis range from mild erythema to severe necrosis. Because the visual symptoms of phlebitis tend to develop slowly, the animals are observed over a period of time (usually 24 hours) during which they must endure these symptoms.

These *in vivo* studies offer the advantage of a live dynamic model, being relatively human-like in a variety of ways, but they are far from ideal for several reasons. In the absence of validation studies, the assumption that blood flow, in the chosen animal vein, is comparable to human blood flow may be erroneous. In addition, drug companies must

obtain animal subject approval and follow a strict protocol, which is not only time consuming but also expensive. Ultimately, the procedure inflicts pain on a large number of animals that must be sacrificed.

Muscle tissue culture lines have been developed for the assessment of chemical irritation produced by various parenteral products. These models are evaluated by measuring the depletion of creatine phosphokinase and are most applicable to assessing intramuscular irritation. Endothelial cell cultures have also been used to determine potential irritation by measuring the change in ATP levels due to fluoroquinoline exposure (Vorbach *et al.,* 1998). Although extremely useful, in both cases, they provide only a piece of the information sought. Applicability to intravenous administration would be limited to the assessment of chemical or pharmacological irritation. They cannot reflect either the effects of injection rate or the short duration of contact of the vein wall cell with a non-precipitating formulation.

A non-animal model for evaluating precipitation-induced phlebitis, which is able to assess mechanical irritation as well as the effect of varying injection rates would benefit both the animals and the pharmaceutical industry. The pharmaceutical industry would save time

and money by identifying the best of a selection of formulations prior to the necessary pre-clinical animal studies. The animals would benefit by being required in smaller numbers as well as experiencing less discomfort due to the improvements in the formulation gained by such preliminary *in vitro* testing. Such a model has previously been developed, but it has not been statistically validated. Yalkowsky et al. (1977, 1983), have developed an *in vitro* procedure for mixing a parenteral formulation with isotonic Sorensen's phosphate buffer (ISPB) at pH 7.4 in a dynamic experiment that simulates its injection into a vein and detects precipitation. The resulting precipitate, if any, creates a haziness which is quantitated turbidometrically on an ultraviolet spectrophotometer downstream from the mixing (injection) site. The dynamic *in vitro* precipitation apparatus is depicted in Figure 1.1.

Slight modifications of this technique were shown by Davio et al. (1991) to give results that correlate well with monkey data. The authors state they would not have expected Ditekiren to precipitate *in vitro* based on equilibrium solubility measurements at physiologic temperature and pH. However, significant drug emboli were observed

in the heart and lungs of the monkeys used in the *in vivo* study. When a dynamic injection *in vitro* model, similar to that currently discussed, was implemented, an accurate prediction of *in vivo* precipitation was obtained.

Greenfield et al. (1991) also evaluated Ditekiren for prediction of *in vivo* precipitation. In a static evaluation method, both human and cynomolgus monkey plasma were used to dilute the formulation in acidified dextrose. Both studies suggested the absence of significant precipitation upon dilution of Diketiren formulated at concentrations 25 times in excess of that anticipated for clinical studies. Thus, the static method failed to predict the *in vivo* precipitation in monkeys while the dynamic apparatus succeeded.

Ideally, the dynamic apparatus should indicate precipitation upon mixing of a formulated phlebitis producing drug with ISPB. In addition, it should indicate the absence of precipitation when phlebitis does not occur. To date, the generalized accuracy of this model has not been investigated. It is important to stress that opacity values are a crude measure of the precipitation. The present study evaluates the validity of the dynamic *in vitro* apparatus for predicting precipitation upon

injection of a number of marketed intravenous products and offers the possibility of becoming a standard method for determining the potential of a parenteral formulation to cause phlebitis due to precipitation.

# METHODS

## Materials

Table 1.1 lists the details of the 21 IV injectable formulations tested. Twelve of the formulations were specifically chosen due to their identification in the literature as having a history of causing phlebitis in humans. The other 9 were chosen specifically because no such reports of irritation were found in the literature. Prop1 and Prop2 are proprietary products that were donated by their manufacturers, and all other formulations were purchased through a local pharmacy or donated by their manufacturers. All formulations, except Valium®, were tested prior to their marked expiration dates. Prior to implementation of the apparatus, a wavelength scan was carried out on each formulation. This was necessary to establish an appropriate wavelength for measurement of light occlusion, rather than absorbance. Since none of the injectables absorb at 540 nm, this wavelength was selected for the evaluation of all formulations.

As is indicated in Figure 1.1, a Harvard Apparatus model 22 syringe pump was used to direct ISPB (which is used as a blood surrogate in

this experiment), at pH 7.4 and 25°C, through a 40 cm length of flexible Tygon plastic tubing, (type R-3603, inner diameter of 3mm); then through a Hellma QS quartz flow-through cell with a 1 cm path length at a 5 ml/min flow rate. This flow rate is comparable to that of human blood flow in readily accessible arm veins (Rowland and Tozer 1995). The reliability of the ISPB flow rate was confirmed between each injection by the time required to fill a 2 ml. volumetric flask. ISPB at pH 7.4 was chosen over plasma because it has a buffer capacity of 0.03. The pH and buffer capacity of stored plasma are reported to be around 6.5 (Abbott Laboratories NDA, 2003) and 0.008 (Surakitbanharn *et al.,* 1994) respectively. This pH results because stored plasma lacks the carbon dioxide that buffers "live" blood. All trials were carried out at room temperature (approximately 25°C).

A Beckman DU 640 ultraviolet spectrophotometer, holding the flow cell, was used to read occlusion of light. The 22-gauge Becton Dickinson Precision Glide sterile stainless steel needle of a 20 ml latex-free Becton Dickinson plastic syringe, containing each formulation to be tested, was introduced into the flexible tubing 10-cm upstream from the flow cell. Each formulation was injected, in triplicate, into the

flowing ISPB at rates of 1, 5 and 10 ml/min; resulting in a total of 9 opacity readings for each injectable tested. Accuracy of injection rate was confirmed for each formulation by timing a 1 ml output during the injection periods. If a formulation precipitates upon injection it produces opacity in the tubing, which passes through the flow cell and is interpreted by the spectrophotometer as reduced transmittance or increased absorbance. In actuality, the readings only represent opacity because, as previously stated, the wavelengths are specifically chosen to insure that drug concentration is not being measured.

**Analyses**

Opacity data were statistically analyzed in the following ways. Repeated measures analysis of variance (StataQuest 4 Statistical Software) was used to determine the effect of injection rate. And independent Student's t-tests (MedCalc Statistical Software) were carried out to identify the best of the 3 injection rates for clear separation of phlebitc injectables from non-phlebitc injectables. A receiver operator characteristic (ROC) curve (MedCalc Statistical Software) was used to isolate the best fit cut-off opacity value and

logistic regression (MedCalc Statistical Software) was employed to determine the probability of phlebitis corresponding to each opacity value.

# RESULTS

Sample opacity tracings produced by Valium®, Dilantin® and 0.9% NaCl solution injected into the dynamic apparatus at various rates are shown in Figure 1.2.

## The effect of injection rate

Table 1.2 lists average opacity readings from phlebitic and non-phlebitic formulations at all three injection rates. Repeated measures analysis of variance indicates there was no significant effect of injection rate consistent across all injectables studied over the 1 to 10 ml/min range ($p>0.5$) (StataQuest Statistical Software). For some formulations, such as Valium, Cordarone®, Nafcillin and Phytonadione, the 1 ml/min injection rate produced less precipitation than did the 5 ml/min injection with the 10 ml/min injection rate having produced the most precipitation. For others, such as Dobutamine and Dilantin®, the opposite trend was observed.

Kay and Rolly (1977), Turco (1987), Myrdal et al. (1995) and Sweetana and Akers (1996) have all stated that drug concentration and

therefore, rate of administration are important factors in determining the presence and extent of venous irritation. In fact, it has been generally accepted, and demonstrated in Figure 1.2a, that the slower the rate of intravenous administration, the less precipitation can be expected. Contrarily, Ward and Yalkowsky (1993) showed that it is possible to obtain reduced precipitation by injecting at a faster rate than that of surrogate blood flow. They were able to demonstrate an effect they termed "plug flow" when the injection rate of amiodarone was 6 times that of the ISPB.

In plug flow, the entire injected dose travels through the vein in a plug-like fashion. This results in dilution of the formulation and resultant precipitation mostly at the edges of the plug. In contrast, the center of the plug remains relatively precipitation-free as it is protected from mixing with the blood. More specifically, Ward and Yalkowsky (1993) explain that an opacity tracing of an i.v. injectable experiencing plug flow dynamics shows two opacity peaks corresponding in time with the beginning and ending of the injection period. An example of this can be seen in Figure 1.2b. As the injection rate of Dilantin® increased from 5 to 10 ml/min the two peaks begin to resolve

themselves suggesting that precipitation is highest at the edges of the plug as it enters and then exits the flow cell.

Various other formulations tested in the present experiment, such as Prop1, Prop2 and Ciprofloxacin support the plug-flow concept. When these formulations were injected at twice the rate of the blood surrogate, a reduction in precipitation (as compared to the trials when injection rate was equal to blood surrogate flow rate) was observed.

**Evaluation of model accuracy**

As previously stated, there is often a definite effect of varying injection rate on opacity but the direction of that effect is not consistent across all IV injectables. In cases, such as phytonadione, where the 1 ml/min injection rate showed a 100x reduction in opacity from the other two rates it is reasonable to question whether it would still be accurate to classify phytonadione at the lowest injection rate as phlebitic. In addition, some parenteral i.v.s, such as ciprofloxacin, showed a reduction in opacity at the 10 ml/min injection rate as compared to the other two rates. Again, it seems possible that such a low opacity value may indicate a lack of precipitation at the higher rate of administration.

In order to determine which set of data (1ml/min, 5ml/min, 10ml/min or all three administration rates combined) best predicts true phlebitic outcome, independent Student's t-tests were carried out for all sets. The best fit data set was determined to be at 5ml/min (p=0.0002) (MedCalc Statistical Software) as compared to the 1ml/min (p=0.001) (MedCalc Statistical Software) and 10 ml/min (p=0.001) (MedCalc Statistical Software) injection rates. Although using opacity data from all injection rates allowed for a statistically significant separation of phlebitic and non-phlebitic distributions at p=0.0001 (MedCalc Statistical Software), it was not considered sufficiently superior to data from only the 5 ml/min injection rate because of the relative increase, from 0.006 to 0.01, in overlapping borderline opacity values. Thus, at the 5 ml/min rate, the model was found to allow superior separation of phlebitic from non-phlebitic drugs as well as provide the least amount of overlap in borderline opacity values.

Using the 5 ml/min injection rate values, a receiver/operator curve (ROC), in Figure 1.3, was created (MedCalc Statistical Software). This method, which is commonly used in evaluating diagnostic tools (Arndt, 1988), determines the most appropriate cut-off value to distinguish

between significant precipitation and insignificant noise. It facilitates isolation of the opacity value allowing the highest combination of model sensitivity (probability of detecting precipitation when phlebitis has been reported) and specificity (probability of not detecting precipitation when phlebitis has not been reported). Maximization of both parameters results in minimization of the chance of obtaining false negatives (predicting no irritation when phlebitis will occur) or false positives (predicting phlebitis when it will not occur).

The curve was generated by plotting the calculated sensitivity (y-axis) and 1- the specificity (x-axis) of the model should each obtained opacity value be used as the delineating value for recognizing precipitation. As can be seen from the circled point in the plot, the maximum combination of model sensitivity (p=0.83) (MedCalc Statistical Software) and specificity (p=0.93) (MedCalc Statistical Software) is achieved using a delineating opacity value of >0.003 (choosing a point to the right of the circle increases sensitivity at the expense of substantially reducing specificity).

Having established the delineating value of >0.003 for recognizing precipitation, positive predictive value and negative predictive value of

the model can be calculated as p = 0.93 (MedCalc Statistical Software) and p = 0.78 (MedCalc Statistical Software), respectively. Additional strength of the model can be seen through the calculated area under the curve (AUC) of 0.95 (MedCalc Statistical Software). Theoretically, the AUC of a ROC plot shows increasing accuracy of the model as the AUC approaches 1.00. The AUC can be considered roughly analogous to the coefficient of determination ($r^2$ value) of a regression line in that it also evaluates how well the tested model works.

The choice of the best cut-off opacity value (>0.003, indicated by the circled point in Figure 1.3) is supported by logistic regression (MedCalc Statistical Software). Logistic regression was chosen because it is customarily used to identify the effect of a continuous independent variable on a dependent variable when that dependent variable is dichotomous (Pagano and Gauvreau, 1993). Figure 1.4 shows the predicted regression line for determining the probability of phlebitis from opacity. It also shows experimental opacity data points for drugs reported to be phlebitic (at p=1.0) and non-phlebitic (at p=0). It can be easily seen that there is an obvious trend for phlebitic formulations to be consistently higher in opacity readings than non-phlebitic formulations.

The vertical dotted line marks the delineating log opacity value of $10^{-2.5}$ (opacity of 0.003) described above.

## DISCUSSION

Although in opposite directions, the magnitudes of precipitation observed in Figures 1.2a and 1.2b for Valium® and Dilantin® are a direct function of the injection rate. Figure 1.2a shows that when Valium® is injected very slowly there is much less precipitation. This corresponds well with the following statement, which is taken from the package insert, "The solution should be injected slowly taking at least one minute for each 5 mg (1 ml) given." On the other hand, Figure 1.2b shows that the amount of precipitation is reduced when Dilantin® is injected relatively quickly. The very small peaks observed in Figure 1.2c for the 0.9% sodium chloride solution are not due to precipitation. These peaks are the result of Schleiren patterns produced by the mixing of the liquids with different refractive indicies.

One plausible explanation for why the 5 ml/min injection rate better predicts reported phlebitic outcome over the 1 ml/min and 10 ml/min injection rates focuses on the administrator of the injection. An injection rate equal to that of the ISPB flow rate may provide the dilutional micro-environment which is most representative of that which develops most often in a human vein. Since phlebitic reports

herein are clinically based, it is possible that professionals administering these parenterals may have a tendency to inject at a rate close to that of blood flow. This natural tendency may result from a desire to minimize the amount of time in which the needle is inserted in the patient. As a consequence, that administrator may aim to inject as quickly as possible without causing patient discomfort. Theoretically, as the injection rate surpasses the blood flow rate, increased resistance and subsequent patient discomfort might be expected. Therefore, it may be most common for medical professionals to inject at a rate equal to blood flow.

Using an opacity cut-off of >0.003, sensitivity, specificity, positive predictive value and negative predictive value are found to be 0.83, 0.93, 0.93 and 0.78 respectively. These are probability measurements indicating the usefulness of the model. These measures suggest the model is more specific than sensitive and has higher positive predictive value than negative predictive value. This means that using >0.003 to delineate, the model is specific to identifying phlebitic precipitation over background noise 93% percent of the time, yet is still sensitive enough to identify precipitation when it does occur at an 83% margin.

Furthermore, the high positive predictive value indicates that when an opacity reading >0.003 is obtained, one can be 93% confident that the probability of phlebitis corresponding to that opacity reading is accurate. Obviously, the higher an opacity value is above 0.003, the more likely phlebitis is to occur. As a result, the model is found to be predictive of phlebitis due to precipitation and is consequently useful as a standard pre-*in-vivo* screening technique; it provides accurate prediction 86% of the time. The dynamic apparatus can reliably indicate whether or not a formulation is likely to precipitate upon intravenous injection, resulting in phlebitis. This is the same information that would be obtained from *in vivo* studies but requires less time and money. It also minimizes the unnecessary use of animals during early formulation development.

The value of the current model is that it should identify precipitation over background noise on a "yes" or "no" basis. The goal of the present study was to test the model and identify the best fit opacity value allowing the user confidence in identifying precipitation when it occurs. In addition, a user should be able to make such "extent of opacity/ extent of phlebitis" correlations when comparing various formulations

of the same drug. Such is the expected use of the dynamic apparatus. It should be noted that the present model does not account for possible effects of protein binding. We are assuming that protein binding is not likely to be a major factor given the small amount of blood that comes in contact with the formulation at the injection site.

Should a new parenteral be tested in the dynamic apparatus and indicate significant opacity of >>0.003, before administration to animals, the IV injectable should be reformulated. In turn, if a new parenteral does not indicate significant opacity (<<0.003) in the apparatus; phlebitis can be considered unlikely. This can be a very powerful tool for selecting the best in a group of alternative formulations.

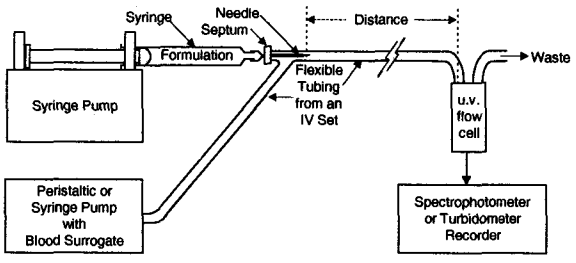



**Figure 1.1.** Dynamic *in vitro* apparatus for evaluating precipitation upon injection

**Table 1.1.** Details of IV injectables[a] tested. All injectables were prepared for testing according to the manufacturers' instructions.

| Drug | Maufacturer | Lot # |
|---|---|---|
| Prop2[b] | -------- | N/A[c] |
| Prop1[b] | -------- | N/A[c] |
| Valium®[d] | Roche | 0368 |
| Dilantin® | Parke-Davis | 03661P |
| Cordarone® | Wyeth-Ayerst | 031200 |
| Nafcillin | Apothecon | 1H38074 |
| Phytonadione | Abbott | 85353DK |
| Lidocaine | Amer. Regent | 9603 |
| Ciprofloxacin | Bayer | OCAP |
| Acyclovir | Bedford | 259700 |
| Ofloxacin | McNeil | 7GAB5 |
| Dobutamine | Abbott | 86513DK |
| Dopamine | Amer.Regent | N/A[c] |
| Epinephrine | IMS Ltd. | DT0005G1 |
| Atropine sulfate | Abbott | 83274DK |
| Bretylium tosylate | Amer. Regent | 2146 |
| Diltiazem | Bedford | 206433 |
| Normal saline | Baxter | C468272 |
| Dextrose, 5% | Baxter | G975565 |
| Furosemide | Amer. Regent | 0282 |
| Sterile water | Travenol | 6G658X0A |

[a] All formulations were tested prior to their expiration dates except Valium®.
[b] Proprietary formulations.
[c] Information not available.
[d] Product was tested after expiration date.

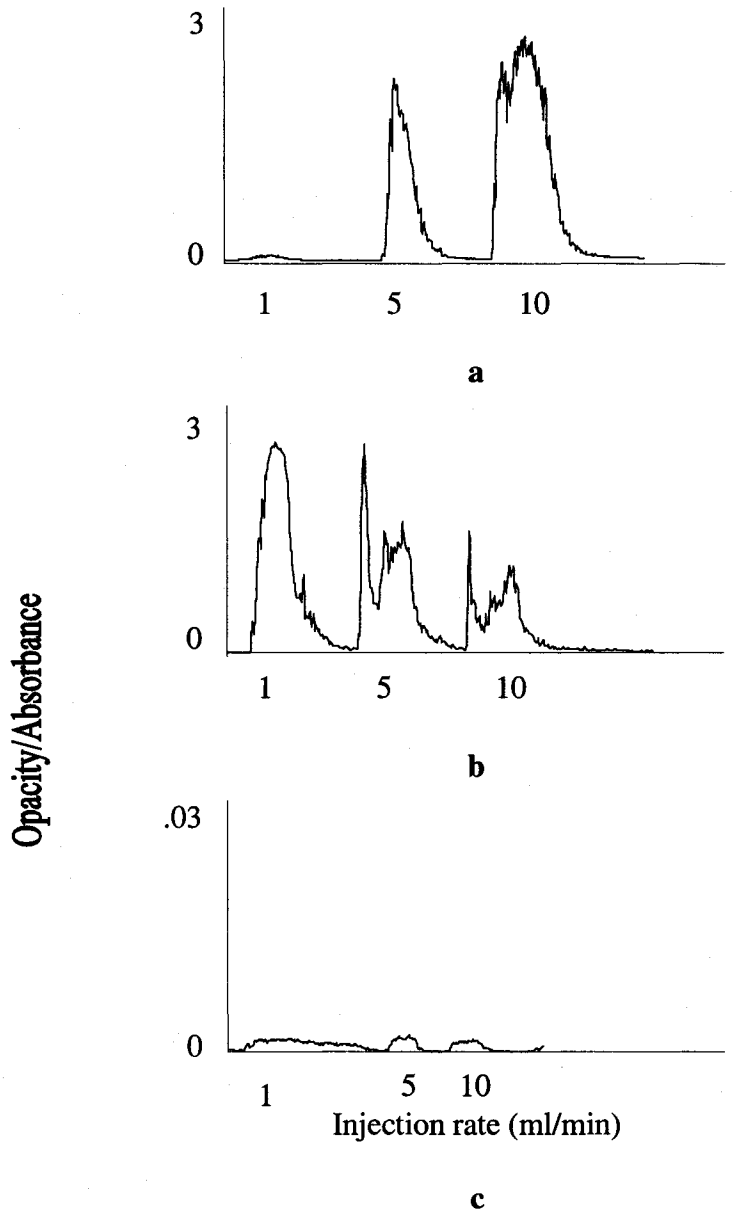



**Figure 1.2.** Opacity produced by **a.** Valium®, **b.** Dilantin® and **c.** 0.9% NaCl solution injected into ISPB flowing at a rate of 5 ml/min. The x-axes represent the varying injection rates of 1, 5 and 10 ml/min from left to right respectively. The y-axes represent opacity produced in the dynamic apparatus. Note the scale difference on the y-axis in **c** as compared to **a** and **b.**

**Table 1.2.** List of IV injectables tested, whether they are reported to cause phlebitis[a] , their averaged ranked opacity values for all injection rates and their individual opacity readings for each injection rate. (All injectables were prepared for testing according to the manufacturers' instructions.)

| Drug | Phlebitis Reported | Avg.Opacity Ranked | Opacity by Injection rate[b] 1 ml/min | 5 ml/min | 10 ml/min |
|---|---|---|---|---|---|
| Prop2[c] | yes[d] | 4.02 | 4.26 | 4.31 | 3.48 |
| Prop1[c] | yes[d] | 2.67 | 4.43 | 1.67 | 1.91 |
| Valium® | yes | 1.70 | 0.67 | 2.10 | 2.35 |
| Dilantin® | yes | 1.196 | 1.87 | 1.05 | 0.67 |
| Cordarone® | yes | 0.315 | 0.24 | 0.32 | 0.39 |
| Nafcillin | yes | 0.186 | 0.020 | 0.22 | 0.32 |
| Phytonadione | yes | 0.027 | 0.000 | 0.030 | 0.060 |
| Lidocaine | yes | 0.019 | 0.010 | 0.020 | 0.030 |
| Ciprofloxacin | yes | 0.006 | 0.0090 | 0.0067 | 0.0023 |
| Acyclovir | yes[e] | 0.005 | 0.0035 | 0.0050 | 0.0056 |
| Ofloxacin | yes | 0.003[f] | 0.0025 | 0.0035 | 0.0020 |
| Dobutamine | yes | 0.003[f] | 0.0044 | 0.0013 | 0.0019 |
| Dopamine | no | 0.003[f] | 0.0 | 0.0027 | 0.0054 |
| Epinephrine | no | 0.003[f] | 0.0020 | 0.0029 | 0.0042 |
| Atropine sulfate | no | 0.001 | 0.0019 | 0.0010 | 0.0014 |
| Bretylium tosylate | no | 0.001 | 0.0019 | 0.0010 | 0.0014 |
| Diltiazem | no | 0.001 | 0.0 | 0.0023 | 0.0010 |
| Normal saline | no | 0.0008 | 0.00040 | 0.00082 | 0.0013 |
| Dextrose, 5% | no | 0 | 0.0 | 0.0 | 0.0 |
| Furosemide | no | 0 | 0.0 | 0.0 | 0.0 |
| Sterile water | no | 0 | 0.0 | 0.0 | 0.0 |

[a] All reports of phlebitis were obtained from the PDR, except proprietary formulations and Acyclovir.

[b] The ISPB flow rate was 5 ml/min for all injections.

[c] Proprietary formulations.

[d] Company report

[e] Arndt, 1988.

[f] Designates delineating opacity value for determining phlebitic potential

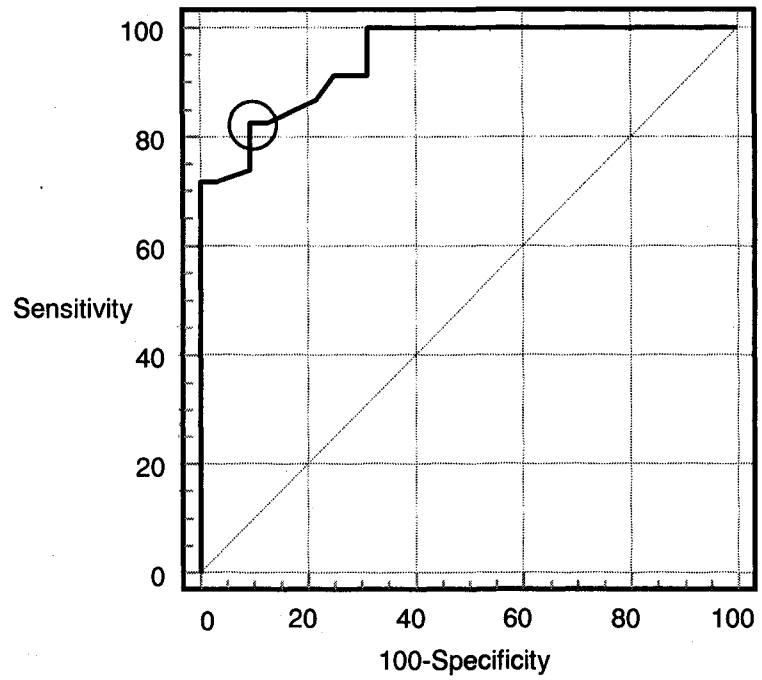



**Figure 1.3.** Receiver/Operator (ROC) plot (MedCalc Statistical Software). Maximum model predictive efficiency is obtained where sensitivity and specificity are simultaneously maximized. This is achieved when sensitivity = 0.83 and specificity = 0.93 at the chosen delineating value (circled) of >0.003 au/opacity. The calculated area under the curve (AUC) is 0.95. The diagonal line from top right to bottom left is shown for comparison. It describes an AUC of 0.50 or a model that is poorly predictive.

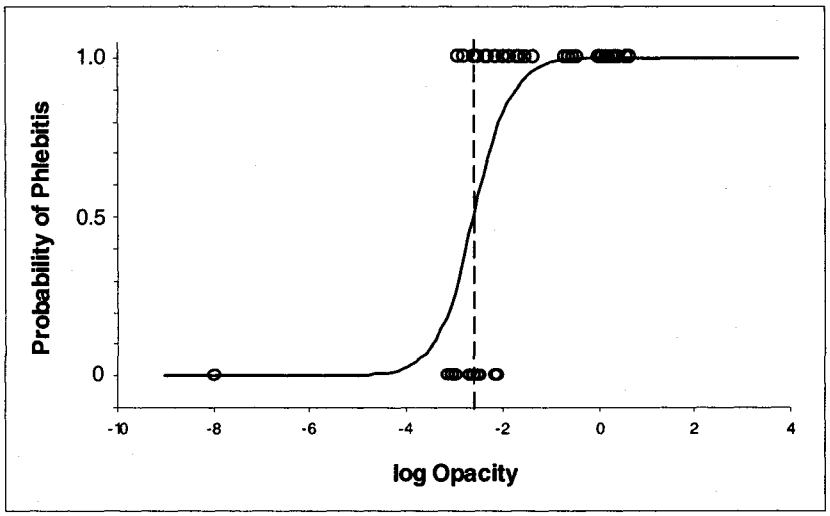
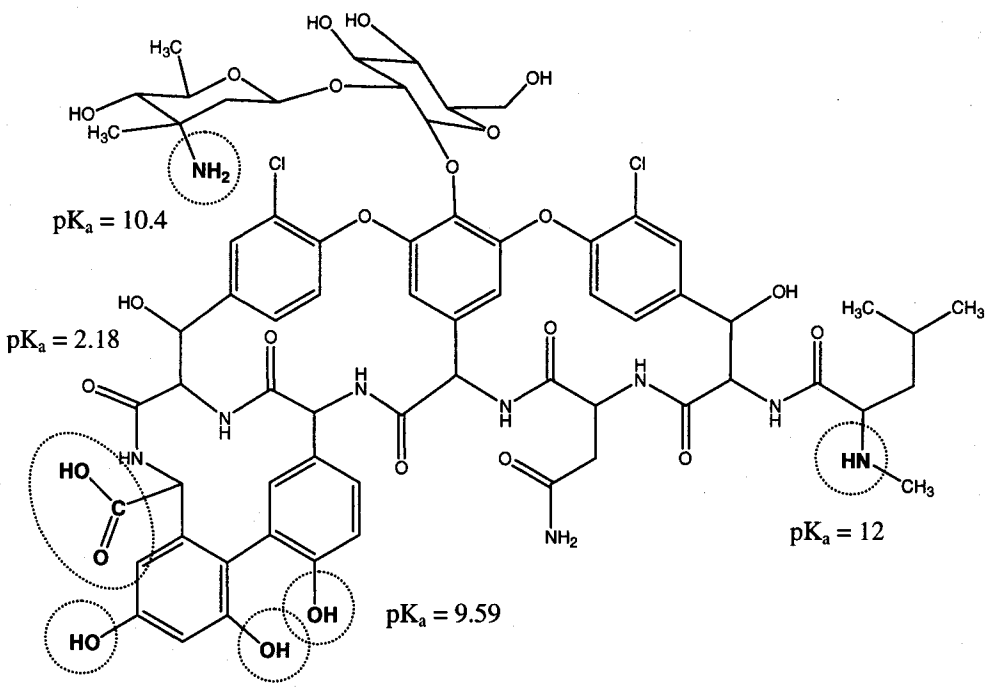



**Figure 1.4.** Logistic regression (MedCalc Statistical Software), of the probability of phlebitis given opacity. Each data point represents one 5 ml/min injection. Points below the 0.5 probability indicate formulations known to be irritation-free and points above 0.5 probability indicate those known to cause phlebitis. The solid black line, obtained through logistic regression, represents the predicted probability of phlebitis given the corresponding opacity value. The x-axis represents log opacity. The log of the opacity value at which probability of phlebitis is 0.5 is determined to be -2.5 (opacity = 0.003) as is indicated by the dotted line. Thus, as opacity readings increase above 0.003 the probability of phlebitis occurring also increases until there is a 0.95 probability of phlebitis at an opacity value of 0.03 (log opacity = -1.5). In the opposite direction, as an opacity value decreases below 0.003 the probability of phlebitis diminishes until there is only a 0.05 probability of phlebitis at an opacity value of 0.0002 (log opacity = -3.7). Note that opacities of 0 were assigned log values of -8; thus, the circle located at log opacity = -8 represents multiple data points.

# CHAPTER II: INTRAVENOUS FORMULATION EVALUATION

## INTRODUCTION

With the constant development of bacterial resistance to current antibiotics, there is an ongoing need for novel antibiotics which can be used as the last line of defense in life threatening infections. Vancomycin is a powerful antibiotic which is often used as a last resort. Unfortunately, bacteria are now becoming resistant (Hadaway and Chamallas, 2003).




**Vancomycin**

Vancomycin is reported to have the following 6 $pK_a$ values: 12.0, 10.40 (basic), 9.59, 8.89, 7.75 and 2.18 (acidic) (Takács-Novák *et al.*, 1993). The

functional groups responsible for these values are indicated in the structure above. In general, glycoside antibiotics are poorly soluble at neutral pH but they are ionizable. Therefore, pH adjustment is often the best formulation strategy to achieve desired drug concentrations.

Vancomycin and its analogs have been associated with injection site irritation in humans (Vam Bambeke *et al.,* 2004; Targocid package insert, 2004; Salaria, 2001; Malabarba and Ciabatti 2001; Biospace Beat Press Release, 2004; Douchin *et al.,* 2004a; Douchin *et al.,* 2004b). Evidence exists suggesting that some of these adverse effects can be attributed to errors in the manner of preparation or administration of the injection (Taxis and Barber, 2003) or pharmacological irritation (Lanbeck *et al.,* 2002). Nevertheless, the possibility of these adverse reactions being formulation related should not be overlooked. We have previously shown that phlebitis is often related to precipitation of pH-solubilized drugs upon injection into the bloodstream (Johnson *et al.,* 2003).

Van-An is a new proprietary second generation vancomycin analogue that is active against gram positive bacteria. It is solubilized by pH adjustment and is reported to have the following 7 $pK_a$ values: 11.68, 10.14, 9.53 (basic), 8.69, 7.32, 7.07 and 3.23 (acidic) (proprietary company

information). In short, Van-An has one more ionizable amine group than Vancomycin. That additional basic moiety serves to increase the solubility of the drug at low pH but it can also place Van-An at a higher degree of supersaturation when the pH increases toward the neutrality of blood. The calculated charge speciations for both molecules are shown in Figure 2.1.

Reconstitution of freeze dried Van-An produces a solution having an approximate pH of 3.9. According to Figure 2.1, the net charge of the compound at pH 3.9 would be approximately +2.1. Once injected into the bloodstream, the formulation pH will rise to that of blood. The pH increase from 3.9 to 7.4 will decrease the net charge of the drug from +2.1 to +0.7. An overall reduction in net charge of (2.1 – 0.7)/2.1 = 2/3 can be expected. When the net charge of a drug decreases, so does its solubility. Depending upon the intrinsic aqueous solubility of Van-An and how far above that concentration it is formulated, such a change in pH can result in significant precipitation.

As is shown in Table 2.1, the pH of the Van-An formulation is controlled by phosphate, some of which is released from the salt form of the drug in solution (proprietary company information). Phosphoric acid has the following 3 $pK_a$s: 2.15, 7.20 and 13.38. Note that none are in the 3.9 range.

Therefore, regardless of the phosphate concentration, the formulation is without adequate buffer ability at pH 3.9.

Van-An is evaluated for its potential to cause phlebitis due to precipitation employing the validated dynamic injection apparatus (Johnson *et al.,* 2003). We show that the current formulation of Van-An does precipitate when diluted with a blood surrogate. Reformulation using a more concentrated buffer with a $pK_a$ within the 4 to 7 pH range can substantially reduce the precipitation. Therefore, this study was designed to determine whether the addition of 0.1 M acetate, with a $pK_a$ of 4.75, will sufficiently reduce the precipitation upon injection.

# METHODS

## The Model

Prior to implementation of the apparatus, an ultraviolet-visible scan was obtained to determine a wavelength acceptable for detection of precipitation and not absorbance of Van-An. The scan shown in Figure 2.2 indicates that a wavelength of 540 nm can be used.

As is indicated in Figure 1.1, a variable flow mini-pump (Control Company) was used to direct a blood surrogate, at pH 7.4, through the 3 mm inner diameter of flexible plastic tubing (Tygon, type R-3603) of an i.v. set; then through a quartz flow-through cell (Hellma QS) with a 1 cm path length at a 5 ml/min flow rate. This flow rate is comparable to that of human blood flow in readily accessible arm veins (Rowland and Tozer 1995) Isotonic Sorensen's phosphate buffer (ISPB) at pH 7.4 was chosen as a blood surrogate over plasma because it has a buffer capacity of 0.03 which is close to that of blood. The pH and buffer capacity of stored plasma are reported to be around 6.5 (Abbott Laboratories NDA, 2004) and 0.008 (Surakitbanharn *et al.,* 1994) respectively. This pH results because stored plasma lacks the carbon dioxide that buffers blood in a living organism. All trials were carried out at room

temperature (approximately 25°C).

A 22-gauge stainless steel needle (Becton Dickinson Precision Glide) of a 20 ml latex-free plastic syringe (Becton Dickinson), containing each formulation to be tested, was introduced into the flexible tubing 10-cm upstream from the flow cell at the septum of the i.v. set. A syringe pump (Harvard Apparatus Precision, model 22) was used to inject the formulations into the flowing ISPB. A Varian model 50 ultraviolet spectrophotometer, holding the flow cell, was used to read occlusion of light. If a formulation precipitates upon injection it produces opacity in the tubing, which passes through the flow cell and is interpreted by the spectrophotometer as reduced transmittance or increased absorbance. In actuality, the readings only represent opacity because, as previously stated, the wavelength was specifically chosen to insure that the concentrations of dissolved drug and formulation components do not affect the absorbance measurements.

**Standardization of the Model**

Because the spectrophotometer used in the current study was different from the one used in the preliminary study (Johnson *et al.,* 2003), the

model was standardized as follows. Dilantin®, Cordarone®, (positive controls), Diltiazem and Furosemide (negative controls), which were used in the preliminary study, were injected, in triplicate, into the model at a rate of 5 ml/min. Using Student t-tests, their resulting opacity values were compared with those from the preliminary study. No significant differences (all p-values > 0.10) were found between the new and old values. Therefore, the new spectrophotometer is determined to be reliable and consistent with the originally validated model.

## Vial Preparation

### The Manufacturer's Formulation

One vial of Van-An was reconstituted to a final concentration of 1.6 mg/ml according to manufacturer's instructions as follows: Ten milliliters of sterile water for injection (Baxter) were added to the freeze-dryed vial. The rubber stopper was replaced and dissolution was facilitated by gentle inversion of the vial for approximately 2 minutes. Dissolution was confirmed, by the lack of the Tyndall effect by laser pointer. The contents of

the reconstituted vial were added to 52.5 mls of 5% dextrose and the resulting pH was measured. The final phosphate concentration was 2 mM. Once again, clarity of solution was confirmed using the lack of a Tyndall effect.

### The Acetate-Supplemented Formulation

One vial of Van-An was reconstituted according to manufacturer's instructions as described above with one alteration. A 0.37 g. amount of glacial acetic acid (F.W. = 60.05 g/mol) was added to the 5% dextrose prior to mixing with the contents of the reconstituted vial. The pH was adjusted up to 3.5 using 1 M KOH. Once again, clarity of solution was confirmed using the lack of a Tyndall effect. The final concentrations of Van-An and acetate were 1.6 mg/ml and 0.1 M, respectively.

## Data Collection

The dynamic injection apparatus depicted in Figure 1.1 was used to assess precipitation. The reconstitution according to manufacturer's instructions and the acetate supplemented solution were each injected, in triplicate, into the flowing ISPB at rates of 0.25, 0.5, 1 and 2 ml/min. Each

injection was monitored for 30 seconds using a digital stopwatch.

**Analysis**

Opacity data collected with the 0.1 M acetate addition, were compared to those of the same lot of unaltered formulation. Student t-tests were used to compare the peak opacities between the manufacturer's formulation and the acetate supplemented formulation at each injection rate. Significant differences were determined at the $p<0.05$ level of confidence.

## RESULTS

Figures 2.3 and 2.4 show all of the opacity data obtained for the solution with the added acetate buffer and the one without, respectively. Each x-axis represents the duration of time taken to collect one triplicate set of injections. The y-axes show absorbance, which in this case represents blockage of light due to opacity in the flow cell. The 3 numbers above each trace line along the x-axes are the peak absorbance values obtained during each of the triplicate 30 second injection periods.

Figure 2.5 shows a graphical comparison of the 2 formulations. The error bars represent the 95% confidence interval of the opacity measurement at each injection rate. It is clear that the addition of the acetate buffer reduces the precipitation produced at all 4 injection rates.

## DISCUSSION

The dynamic injection apparatus is a model system. Thus, as is true of all *in vitro* models, it lacks absolute representation of an *in vivo* system. The presence of albumin in the blood surrogate and running the experiments at body temperature, 37°C, were not considered necessary for the intended purpose. In developing the model, the goal was to offer simplicity without sacrificing prediction. The preliminary validation study (Johnson *et al.*, 2003) showed that running the analyses without albumin and at room temperature allowed both goals to be achieved. Furthermore, two other published studies (Davio *et al.*, 1991; Greenfield *et al.*, 1991) which use a similar dynamic prediction method, also suggest that raising the temperature to 37°C does not improve accuracy.

Using the dynamic injection apparatus, the addition of 0.1 M acetate buffer provides the Van-An formulation with a buffering species possessing a $pK_a$ in the target range between 4 and 7. In addition, the 0.1 M concentration has a buffer capacity sufficient to hold the pH of the formulation low enough to dramatically reduce precipitation upon mixing with the blood surrogate. The proposed formulation adjustment combined with injection of the formulation into larger veins, with larger volumes of

blood flow (Faulding Pharmaceuticals, 1999; Kastango and Hadaway 2002; Hadaway and Chamallas 2003) may lead to a lower incidence of clinical phlebitis due to precipitation. This formulation approach may be generally applicable to other drugs with similar ionization profiles to that of Van-An.

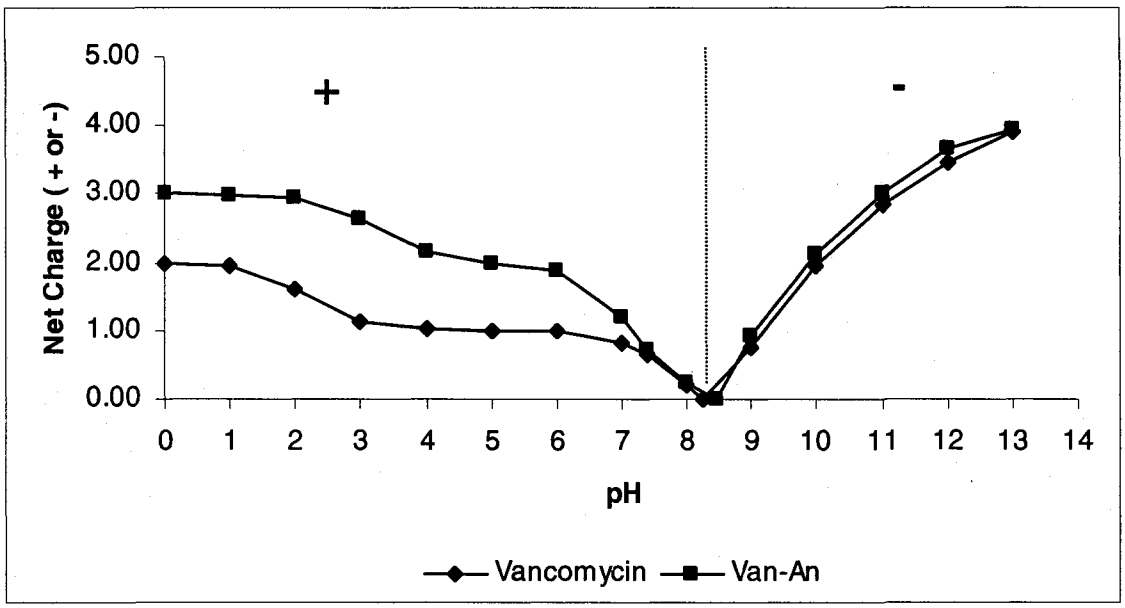



**Figure 2.1.** Calculated net charges of Vancomycin and Van-An across the 0 to 14 pH range. Calculations were made according to the following equations: Net charge $=10^{-pH}/(10^{-pKa}+10^{-pH})$ for bases and $=10^{-pKa}/(10^{-pKa}+10^{-pH})$ for acids.

**Table 2.1. Van-An Composition / Formulation**

| **Ingredients** | **Function** | **Amount (Overfill)[2]** |
|---|---|---|
| Van-An (as the phosphate salt)[1] | Drug Substance | 100.0 mg (2.5 mg) |
| Mannitol | Bulking agent, stabilizer | 48.8 mg (0.2 mg) |
| Phosphoric Acid[3] | pH adjustment | q.s. for pH 3.6-3.9 |
| Monohydrate Sodium Phosphate[4] | pH adjustment | q.s for pH 3.6-3.9 |

[1] The amount of the phosphate salt used in formulating the product is adjusted to achieve the target potency.

[2] A 2.5% overfill is used to ensure 100 mg may be delivered from the vial.

[3] Used as a 10% solution in Water for Injection

[4] Used as a 40% solution in Water for Injection

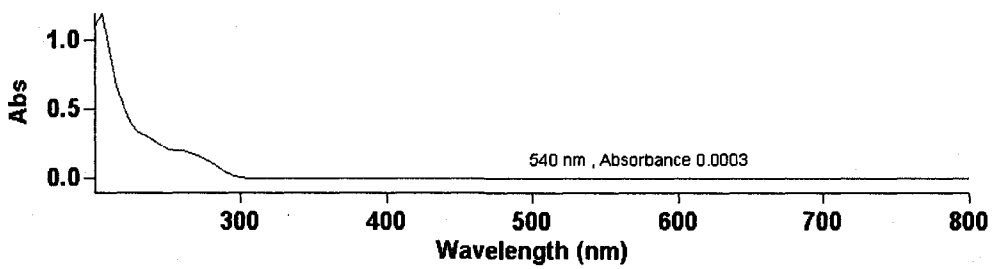



**Figure 2.2.** Van-An wavelength scan.

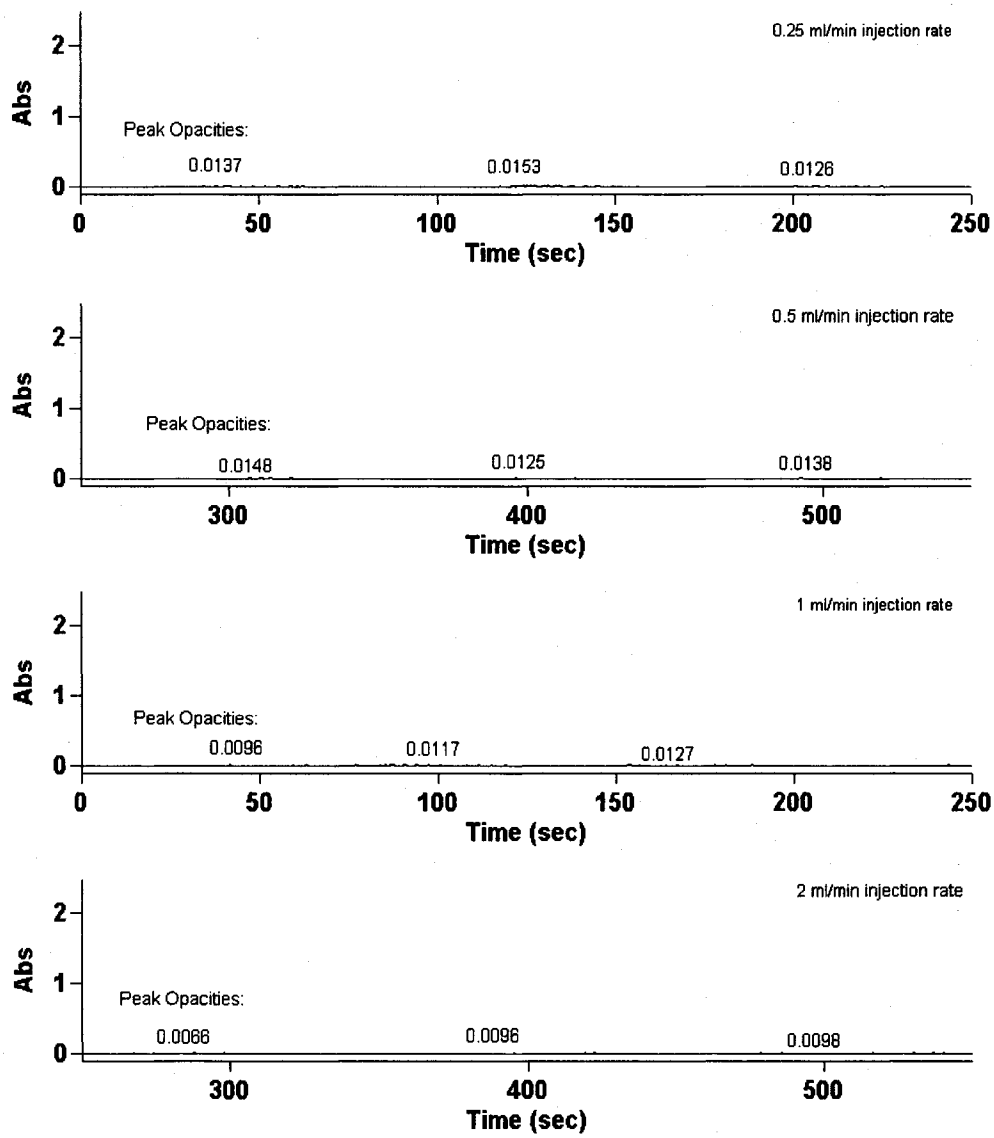



**Figure 2.3.** Van-An reconstituted as directed by the manufacturer with 0.1 M acetate buffer added. From top to bottom, opacity data for injection rates of 0.25, 0.5, 1 and 2 ml/min. are shown.

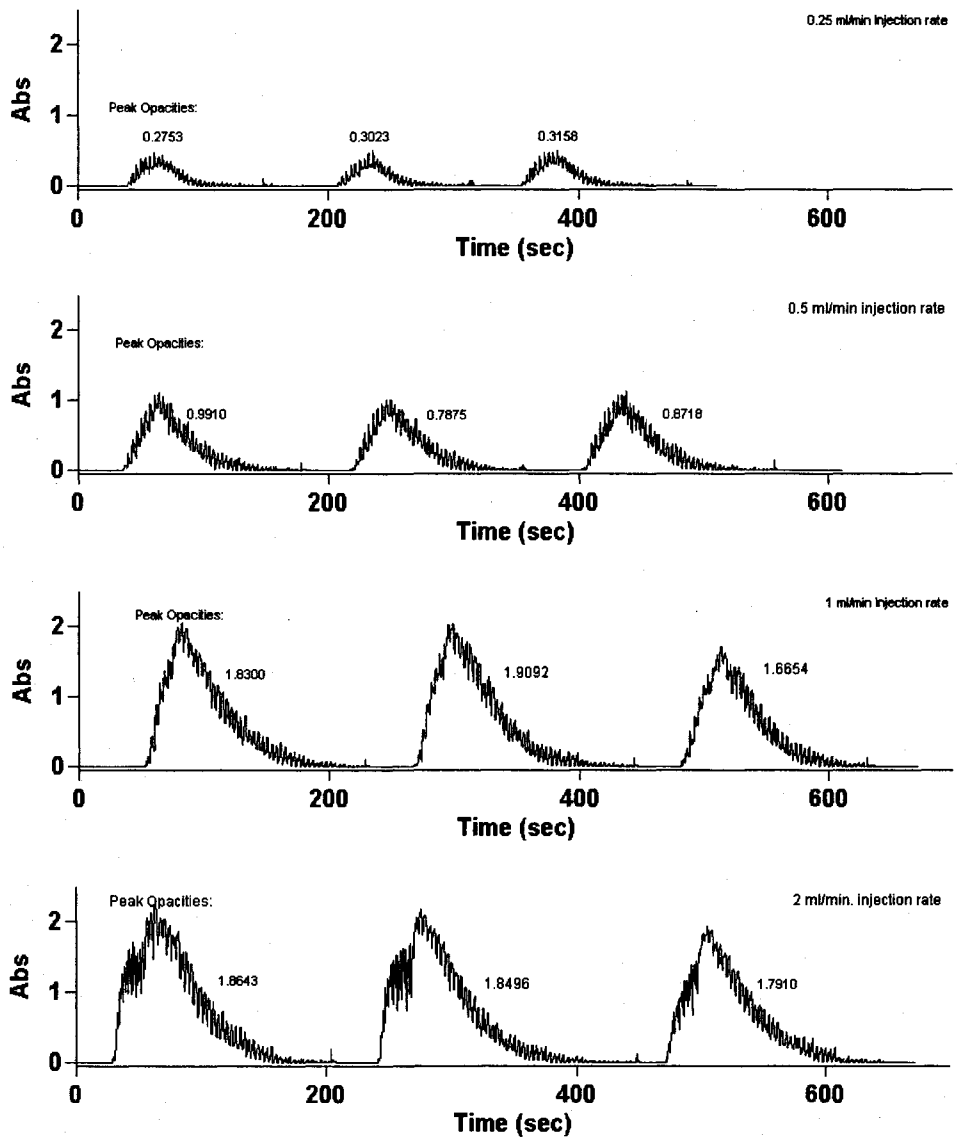



**Figure 2.4.** Van-An reconstituted as directed by the manufacturer. From top to bottom, opacity data for injection rates of 0.25, 0.5, 1 and 2 ml/min. are shown.

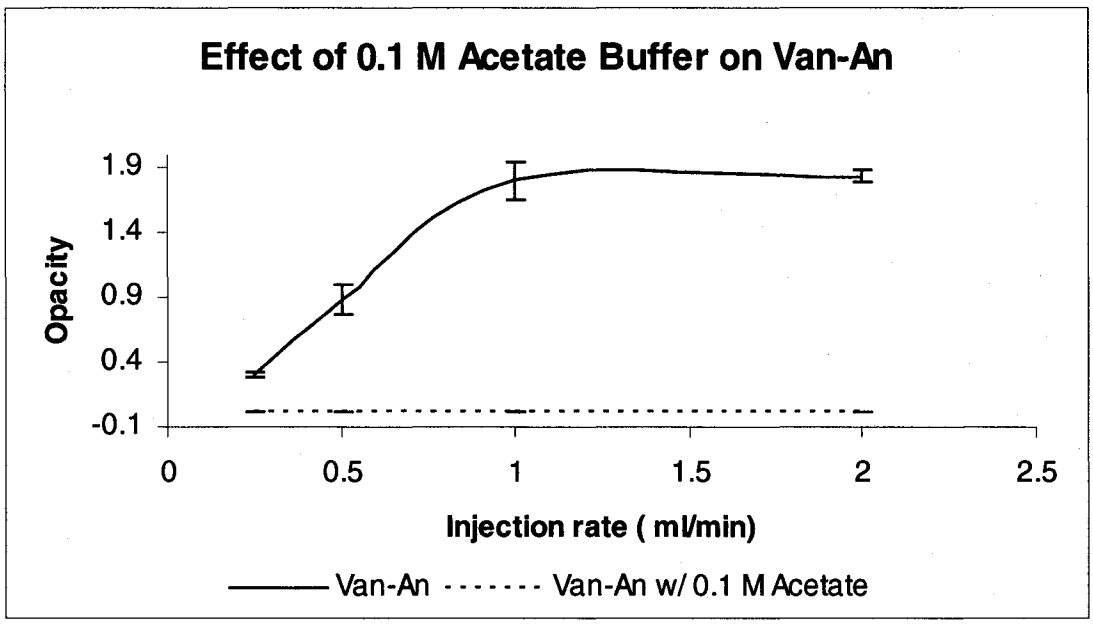



**Figure 2.5.** Opacity differences obtained when comparing 2 newly reconstituted vials of Van-An. One with 0.1 M Acetate buffer added (dotted line) and the other without (solid line). The error bars represent the 95% confidence interval of the triplicate measurement at each injection rate. Note that the x-axis crosses the y-axis below zero in order to show the acetate buffered data line. These values, as can be seen in Figure 2.3, are all very close to zero.

# CHAPTER III: ORAL FORMULATION EVALUATION

## INTRODUCTION

Hepatitis C virus infects approximately 4 million people in the United States and more than 500 million worldwide. In the United States, approximately 10,000 people die annually from Hepatitis C-related illnesses (He *et al.*, 2005).

Interferon and Ribavirin are currently used to treat Hepatitis C. A treatment program combining both of these drugs is considered to be most effective in establishing a sustained virological response. According to Rossi and Wright (2003), the advent of pegylated interferons has increased effectiveness compared to standard interferon both for monotherapeutic administration as well as for combination therapy with oral Ribavirin. Nevertheless, these approaches are reported to be unsuccessful in more than 50% of the patients treated (Thuluvath *et al.,* 2004).

In addition to its relatively low success rate, Interferon is known to cause a multitude of side effects. Some of these side effects, such as depression, suicidal ideation, compromised kidney function and thyroid disease (CDC Website, 2004; Physician's Desk Reference, 2002), are

serious enough to dramatically decrease patient compliance. Another challenge to patient compliance is that Interferon must be administered parenterally, as it is not well absorbed orally (Physician's Desk Reference, 2002).

In cell-based assays, PG301029 has been found to show three times the potency (Procter & Gamble unpublished company report), 100 times the therapeutic index and far fewer side effects in comparison to Interferon and Ribavirin (Jia *et al.,* 2002). In addition, PG301029 reduces toxicity by 100 to 200 times when given in conjunction with Interferon and Ribavirin (Procter & Gamble unpublished company report). Furthermore, this non-polar molecule is not likely to present gastrointestinal permeability problems. Provided the existing solubility and stability challenges can be overcome, the drug can be given orally. Thus, there is a great need for this drug to proceed to market.

PG301029 (5-phenyl-3-thioureido-1,2,4-thiadiazole), shown in Figure 3.1, has a molecular weight of 236.32 and it melts and decomposes at approximately 245°C. The molecule has a calculated logP of 2.7 (Scifinder Scholar, 2004), and four calculated $pK_a$s. The two acidic $pK_a$s are greater

than 10 and the two basic $pK_a$s are below 2 (Scifinder Scholar, 2004; ACD©/$Pk_a$ Software, 2004).

Unfortunately, PG301029 is presently found to have a very low aqueous solubility of 0.7 μg/mL and to degrade in water. Inspite of the drug's high partition coefficient, these two obstacles have prevented useful blood levels from being achieved. In addition, the drug's lack of a utilizable $pK_a$ makes it difficult to formulate. If PG301029 can be formulated for oral administration to solve the aqueous stability and solubility issues, that formulation may become a valuable aid to treat hepatitis C.

Many publications have provided evidence that inclusion complexation can increase drug solubility and stability alike (Connors, 1997, Tommasini *et al.,* 2004). The benzene moiety of PG301029 makes it an ideal candidate for inclusion complexation with hydroxylpropyl-β-cyclodextrin (HPβCD) (Ran *et al.,* 2001; Nalluri *et al.,* 2003). Loftsson *et al.* (2004) have shown that the formation of complexes can be inceased between HPβCD and various acidic and basic drugs by ionizing the drug in aqueous media. In fact, Kruss et al. (2004), Loftsson (2003) and Loftsson and Masson (2004) have filed patent applications proposing variations on this ionization approach to enhancing complexation efficiency. Because PG301029 is not

ionizable and not stable in aqueous media, an alternative approach is presented which dissolves the drug and forces complexation without ever exposing PG301029 to water. Neat acetic acid is employed as the ideal solvent to dissolve HPBCD and PG301029 together and solvent evaporation induces complexation as the solution becomes increasingly condensed. The final product is a dry powder consisting of HPBCD, PG301029 and acetic acid where the drug is presumed to predominantly exist in the complexed form.

# METHODS

## Materials

The PG301029 used in this study was GLP grade and donated by Procter & Gamble. All other materials used were at least reagent grade and all of the studies were done at room temperature: 25 ± 2°C .

## PG301029 Melting Point Determination

The melting point and subsequent decomposition of PG301029 were measured by differential scanning calorimetry (DSC) (TA Instruments 910, model 910001-901) at a heating rate of 5°C /minute.

## Determination of PG301029 Intrinsic Solubility

The apparent intrinsic aqueous solubility of the drug was determined, in triplicate, by over saturating Millipore® (reverse osmosis) water with the raw drug, rotating for 2 hours and filtering prior to analyzing the clear solution by HPLC.

**Determination of Solubility Dependence on pH**

The solubility of PG301029 was measured in 0.1 M HCl, unbuffered water, 0.1 M Sodium phosphate and 0.1 Glycine/NaOH buffer solutions at pH values of 1, 6.1, 7.5 and 10 respectively. Each sample was prepared by over saturating the solution with drug followed by sonication and agitation by vortex. The triplicate samples of each solution were filtered prior to being aanlyzed by HPLC according to the assay described below.

**Preparation of PG301029 /Acetic Acid/ HPβCD Formulation**

HPβCD (Cavasol® W7 HP, Wacker Biochem Corp, Adrian, MI., USA) and PG301029 are initially dissolved together in glacial acetic acid (A.C.S. Reagent, Spectrum Quality Products, Inc.) at a 1/0.0022/10 weight ratio, respectively. This rather high (454.5 to 1) ratio of cyclodextrin to drug was found to best facilitate complexation of the drug. Dissolution is facilitated by alternation of vortexing and sonicating until the solution is clear as evidenced by the absence of a Tyndall effect when illuminated by a laser pointer. The acetic acid is then removed by roto-evaporation for approximately 30 minutes. This allows the solution to become gradually more concentrated; thereby, encouraging complexation to occur. Once all of

the liquid is removed, the final result is a solid white powder consisting mostly of HPβCD and PG301029. A residual amount of acetic acid is also present.

**Preparation of PG301029 Physical Mixtures**

Two physical mixtures of equal component ratios to the final formulation were prepared. One mixture consisted of HPβCD/PG301029 (454.5/1) (w/w) or (100/1) molar ratio and the other of HPβCD/acetic acid/PG301029 (454.5/50/1) (w/w/w) or (100/200/1) molar ratio. Both were prepared by trituration for 5 minutes using a mortar and pestle.

**HPLC Assay**

PG301029 concentrations were assayed by HPLC using an Agilent 1100 series photo diode array detector. A Restek, Pinnacle Octyl Amine, 5μm, 150 x 4.6 mm column was used to separate eluents while running a mobile phase of acetonitrile and water in a 32:68 concentration ratio. The mobile phase flow rate was set to 1 ml/min and the column temperature was controlled at 37°C. Each injection volume was 20 μl. The wavelength of

detection was 254 nm, and the run and PG301029 retention times were 10 and 6 minutes, respectively.

## Characterization of the HPBCD/Acetic Acid/ PG301029 Formulation

### Formulated PG301029 concentration

To determine the drug concentration present in the proposed solid formulation, approximately 10 mg aliquots of the solid powder were weighed and then dissolved in 2 mLs of the HPLC mobile phase. All samples were analyzed, in quadruplicate, according to the HPLC assay detailed above.

### Determination of residual acetic acid

To determine the acetic acid present in the proposed solid formulation, a standard curve was established by diluting acetic acid with mobile phase into 3 different known concentrations. Then approximately 10 mg aliquots of the solid powder were weighed and then dissolved, in quadruplicate, in 2 mLs of the HPLC mobile phase. All samples were analyzed according to the HPLC assay detailed above.

**Characterization of the residual acetic acid**

Approximately 20 mg. aliquots of the formulation were weighed and heated in attempt to remove the residual acetic acid. Two samples were heated at 60°C for 4 hours and 2 others were heated at 120°C for 12 hours. All samples were subsequently reconstituted and analyzed by HPLC for acetic acid and drug concentration.

**DSC formulation characterization**

The solid HPβCD/acetic acid/PG301029 formulation, pure HPβCD and the HPβCD/PG301029 physical mixture were each separately analyzed by differential scanning calorimetry (TA Instruments 910, model 910001-901). Each sample was heated from room temperature to 300°C at a rate of 5°C /minute.

**Physical stability upon reconstitution**

The physical stability of the HPBCD/Acetic Acid formulated PG301029 upon reconstitution was determined in Millipore® (reverse osmosis) water at pH 6.1. The necessity of the acetic acid step to establish

the HPβCD/PG301029 complex was investigated. The two physical mixtures and the HPBCD/Acetic Acid/PG301029 formulation were reconstituted as follows: approximately 10 mg aliquots of each powder were weighed and then dissolved in 2 mLs of Millipore® water. After vortexing for 30 seconds, the HPβCD/PG301029 and the HPBCD/Acetic Acid/PG301029 solutions were filtered such that the soluble drug concentrations were obtained in triplicate, for each powder, using the HPLC assay described above. The HPβCD/acetic acid/PG301029 physical mixture did not require HPLC analysis because the mixture did not dissolve when reconstituted.

**Determination of the aqueous chemical stability of PG301029**

The aqueous stability of both the unformulated raw drug and the HPBCD formulated PG301029 was determined for 21 hours in 3 systems: unbuffered Millipore® water at pH 6.1, simulated gastric fluid (SGF) at pH 1.2 and simulated intestinal fluid (SIF) at pH 7.5. The SGF and SIF were prepared according to USP 28 instructions but without the pepsin and pancreatin. All of the drug concentration determinations were obtained in triplicate using the HPLC assay described above.

### *Unformulated PG301029*

The initial unformulated drug concentrations were prepared in triplicate by adding an excess of PG301029 to a 4 ml volume of the aqueous solution. Each vial was rotated for 2 hours, filtered by a 0.45 μm Acrodisc® syringe filter, and assayed both immediately and approximately every 2 hours thereafter, for a 21 hour period.

### *Formulated PG301029*

The acetic acid/HPβCD formulated PG301029 solutions were prepared by reconstituting approximate 200 mg. aliquots of the solid formulation in 2 ml of aqueous solution. These solutions were also assayed for drug concentration immediately upon reconstitution as well as every 2 hours for the following 21 hours.

## Chemical stability of PG301029 in the dry powder formulation

The stability of the drug in the proposed solid HPβCD formulation, upon storage, was determined by HPLC assay of the drug concentration immediately after reconstitution in water. This was done in quadruplicate

immediately after initial formulation preparation and at 4, 5, 6, 8, 10 and 13 months of storage at room temperature. All stability studies were carried out at room temperature.

## Pharmacokinetic Evaluation of the HPβCD/Acetic Acid Formulation

### Dosing the mice

The solid formulation was reconstituted in water for injection at a ratio of 1/10 (w/w). Four female BALB/c mice were dosed by oral gavage at a PG301029 concentration of 5 mg/kg at time zero. Plasma levels of the drug were assayed at 0.5, 1, 2, 4 and 8 hours. The resulting PG301029 concentration vs. time profile was evaluated for the area under the curve (AUC) from 0 to 8 hours.

### Analysis of PG301029 in mouse plasma

Plasma PG301029 concentrations were determined using a modification of the LC-MS assay previously reported (Wong *et al.*, 2001). Briefly, 100 µl of plasma sample or plasma standard was mixed with 100 µl of acetonitrile containing the internal standard (2-benzimidazolyl-

acetonitrile) at 510 ng/ml. The sample was vortexed and centrifuged for 10 minutes at 10,000 g. An aliquot of the supernatant was injected onto the LC-MS system. The LC-MS system consisted of a ThermoFinniganTSQ Quantum triple quadruple mass spectrometer in tandem with a Surveyor LS system (San Jose, CA). Chromatographic separation was achieved on a Pinnacle Octyl Amine column (4.6 x 150 mm) with a mobile phase of acetonitrile and water in the volume ratio of 32 and 68, at a flow rate of 800 µl/min. The mass spectrometric analysis was performed by atmosphere pressure chemical ionization mode with positive ion detection. Nitrogen was used as the collision gas. The ion pairs of m/z 237/220 and 158/131 were used to monitor PG301029 and internal standard, respectively. An eight point linear calibration curve was established over the concentration range of 0-2500 ng/ml for quantification.

**Pharmacokinetic analysis**

Plasma PG301029 concentrations from four mice for each time point were averaged. The averaged concentration-time data were analyzed by the non-compartment approach using the WinNonLin program (v4.0.1). The following pharmacokinetic parameters were obtained: area under the plasma

concentration-time profile (AUC), terminal half life ($T_{1/2}$), oral clearance ($CL_S/F$) and apparent volume of distribution ($V_\beta/F$).

# RESULTS

## Unformulated PG301029 Characterization

The melting point of the compound is 245°C, above which it decomposes. The apparent intrinsic aqueous solubility of PG301029 is 0.7 μg/mL. The solubility of PG301029 is also 0.7 ug/ml at pH values of 1, 6.1, 7.5 and 10.

## Formulated PG301029 Solubility and Stability

Once introduced into water, the reconstituted pH is approximately 3 and the proposed vehicle increases the soluble drug concentration to 0.2 mg/mL. As indicated in Figure 3.2, raw PG301029 is stable in SIF at pH 7.5, relatively unstable in water and the degradation is most pronounced in SGF at pH 1.2. In contrast, the drug is stable under all 3 aqueous conditions in the HPβCD formulation. Figure 3.3 portrays the 13 month stability of the drug upon dry storage in the solid formulation.

## Final Formulation Component Concentration

The final solid powder consists of HPβCD, acetic acid and PG301029 in a molar ratio of 100/200/1, respectively. The residual acetic acid content

in the solid formulation is 10% by weight. Once reconstituted, the final concentration is only 1%.

**Characterization of the Residual Acetic Acid**

At 60°C for 4 hours and 120°C for 12 hours, approximately 20% of the remaining acetic acid was removed; resulting in a final average residual concentration of 8%. Analyses of the PG301029 concentrations showed that under both temperatures the drug degraded by 10% or more.

**DSC Characterization**

Because the PG301029 concentration is so low and the DSC pan size can only accommodate 5 mg in a sample, neither the solid HPβCD/acetic acid/PG301029 formulation nor the HPβCD/PG301029 physical mixture produced a drug endotherm. Both scans were comparable to the pure HPβCD reference scan.

**The Physical Stability of PG301029 Upon Reconstitution**

The use of acetic acid is shown to facilitate the complex as the soluble PG301029 concentration upon reconstitution is only 0.05 mg/ml for the

HPBCD/PG301029 physical mixture. This is compared to a soluble concentration of approximately 0.2 mg/ml when acetic acid is used to form the complex. In addition, the method in which the acetic acid is utilized and then evaporated is important as evidenced by the fact that the ternary physical mixture of HPBCD, acetic acid and PG301029, in equivalent ratios, does not dissolve upon reconstitution.

**Pharmacokinetic Results**

Figure 3.4 displays the plasma concentration vs. time curve obtained in mice, following oral administration of PG301029 formulated in acetic acid/HPβCD. For comparison, the additional curve represents a previous oral administration of unformulated PG301029 also done in mice (Wong *et al.*, 2001).

The 0 to 8 hour AUC for the HPβCD formulation was 663.3 (ng/ml)(h) and the $CL_S/F$ and $V_\beta/F$ were 6.1 L/h/kg and 28.3 L/kg, respectively. According to Wong et al. (2001) unformulated drug produced a maximum plasma concentration of 1790 ng/ml and a 0 to 8 hour AUC of 5900 (ng/ml)(h) for a 5000 mg/kg dose. Assuming linear pharmacokinetics, the maximum plasma concentration and 0 to 8 hour AUC would be 1.79

ng/ml and 5.9 (ng/ml)(h), respectively, for a 5 mg/kg dose. The 0 to 8 hour AUC of 663.3 (ng/ml)(h) obtained with the HPβCD formulation is approximately 110 times that of the unformulated drug of 5.9 (ng/ml)(h) for a 5 mg/kg dose.

**DISCUSSION**

Previously, Wong *et al.* (2001) reported the PG301029 solubility to be 50 μg/mL. This discrepancy with our measured 0.7 ug/mL is believed to be due to degradation and the difference in the 2 methods of analysis. Wong et al. (2001) used spectrophotometry to determine the drug concentration, which is unable to separate the drug from its degradation products. HPLC enables the differentiation of drug from degradation products.

The presence of the benzene ring in PG301029 was the guiding factor in formulating with cyclodextrin. A benzene ring is the ideal moiety to snugly form an inclusion complex with HPβCD (Ran *et al.,* 2001; Nalluri *et al.,* 2003). A relatively large amount of cyclodextrin is used in the current formulation. This ensures that the PG301029 is primarily in the complexed form (Loftsson *et al.,* 2004). The unique nature of the proposed formulation is in the use of acetic acid to induce complexation. Acetic acid is ideal for this purpose because it is GRAS-listed and both the drug and the

cyclodextrin are soluble enough in acetic acid to obtain a solution at the desired concentration. In addition, PG301029 is stable in acetic acid.

Although differential scanning calorimetry was not directly able to support the assumption of complex formation, it was able to suggest that the lack of a drug endotherm is not due to amorphic drug but rather due to the low concentration of PG301029 present in the sample pan. The same result is observed with the binary physical mixture of HPBCD/PG301029 as it also showed no drug endotherm. Evidence, to support the existence of the complex between PG301029 and HPBCD, lies in the prolonged stability of the drug upon reconstitution; which, is most dramatically observed under acidic conditions.

The mechanism of increasing the stability of PG301029 is unknown. It is possible that the presence of the cyclodextrin simply provides steric hindrance or that the cyclodextrin hydroxyl groups interact with the sulfur group. In any case, degradation is prevented.

Aree *et al.* (2003) have reported that acetic acid forms an inclusion complex with cyclodextrin when HPβCD is dissolved in 10% acetic acid and allowed to re-crystallize through slow solvent evaporation. It is unknown whether the residual acetic acid present in the proposed dry

powder formulation is part of the complex. However, the fact that the remaining acetic acid was not easily removed combined with the final stoichiometric ratio of 2 acetic acid molecules per cyclodextrin molecule, suggests that it is, to some degree, involved in the complex.

Using only HPβCD and glacial acetic acid, an oral dosage form is presented offering no possibility for aqueous degradation until it is administered. In the proposed formulation, the solubility as well as the chemical stability of PG301029 are substantially increased in aqueous media. The HPβCD provides a shield for the drug from the hydrophobic effect and from aqueous degradation. The acetic acid is shown to facilitate the complex as by comparison, the HPβCD/PG301029 physical mixture only increased the PG301029 solubility by a factor of 71. Specifically, once dissolved in water, the vehicle increases the soluble concentration by a factor of 285 (from 0.7 μg/mL to 0.2 mg/mL) and degradation is prevented for 21 hours after reconstitution in water and simulated gastro-intestinal fluids. Furthermore, the proposed formulation increases PG301029 bioavailability in mice over 100 times above that of the unformulated drug (Wong *et al.,* 2001). The formulation can be given orally in solid form or

reconstituted with water at a 1/10 (w/w) ratio immediately before administration.

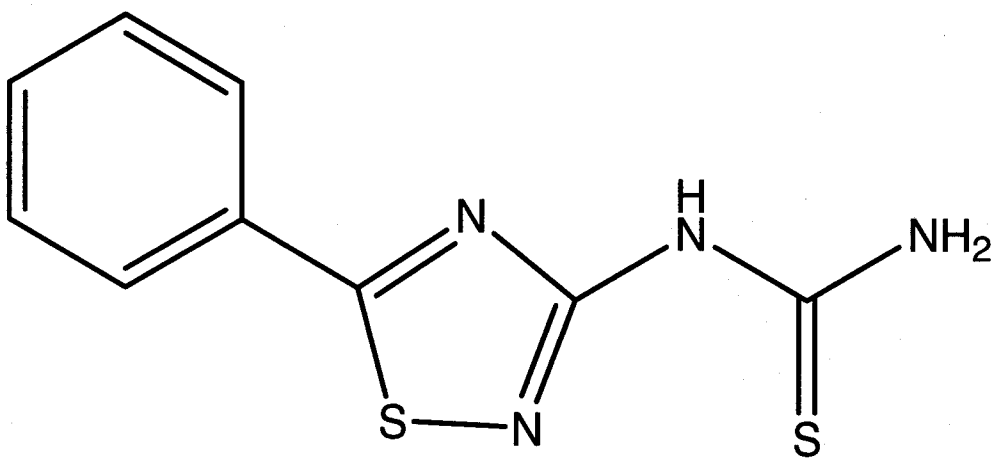



**Figure 3.1**. The structure of PG301029.

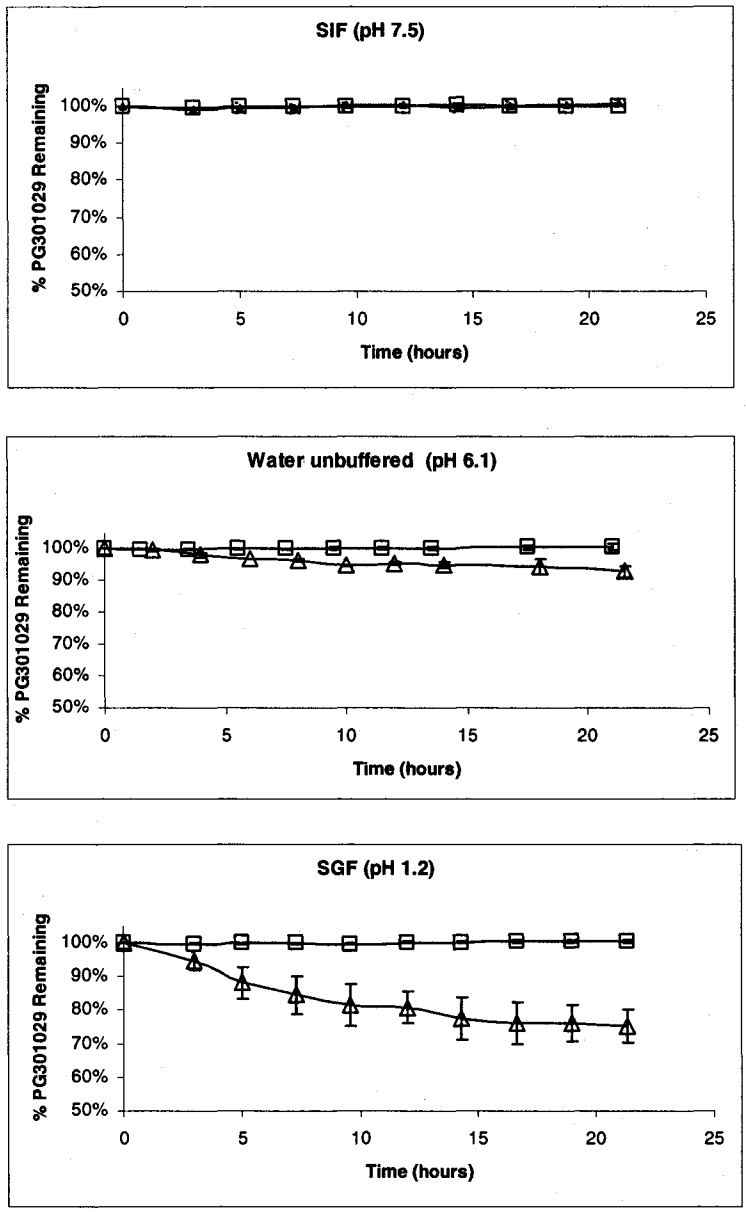



**Figure 3.2.** A comparison of the stability of unformulated PG301029 (triangles) at an initial concentration of 0.7 μg/ml and acetic acid/HPβCD formulated PG301029 (squares) at an initial concentration of 190 μg/ml. The graphs, represent PG301029 concentrations with time in simulated intestinal fluid at pH 7.5, water at pH 6.1 and simulated gastric fluid at pH 1.2, respectively, from top to bottom. Error bars represent one standard deviation of the mean determined concentrations. Note that the two lines in the SIF graph are not significantly different at any time point. In some cases the error bar lines are not distinguishable from the data points because the standard deviation was less than 2%.

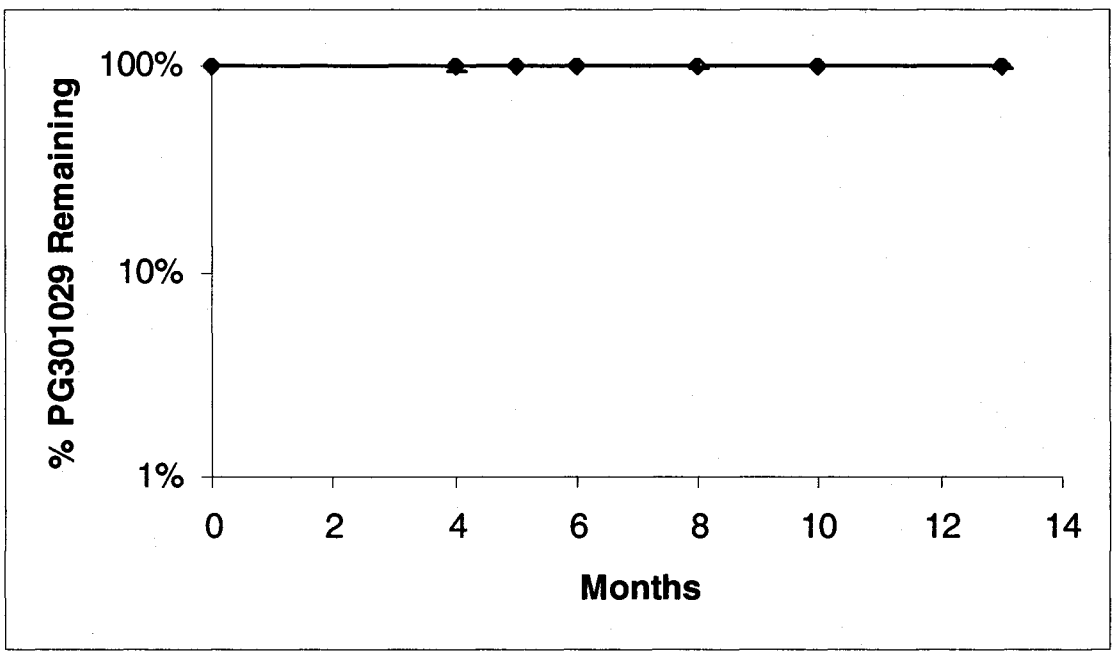



**Figure 3.3.** Stability of PG301029 upon storage in the acetic acid/HPβCD solid dosage form. The error bars represent one standard deviation of the quadruplicate measurement. Most of the error bars are not easily visualized because the standard deviations of the mean determined concentrations were always less than 5%. Thus, in some cases, the error bar lines are not easily distinguished from the data points.

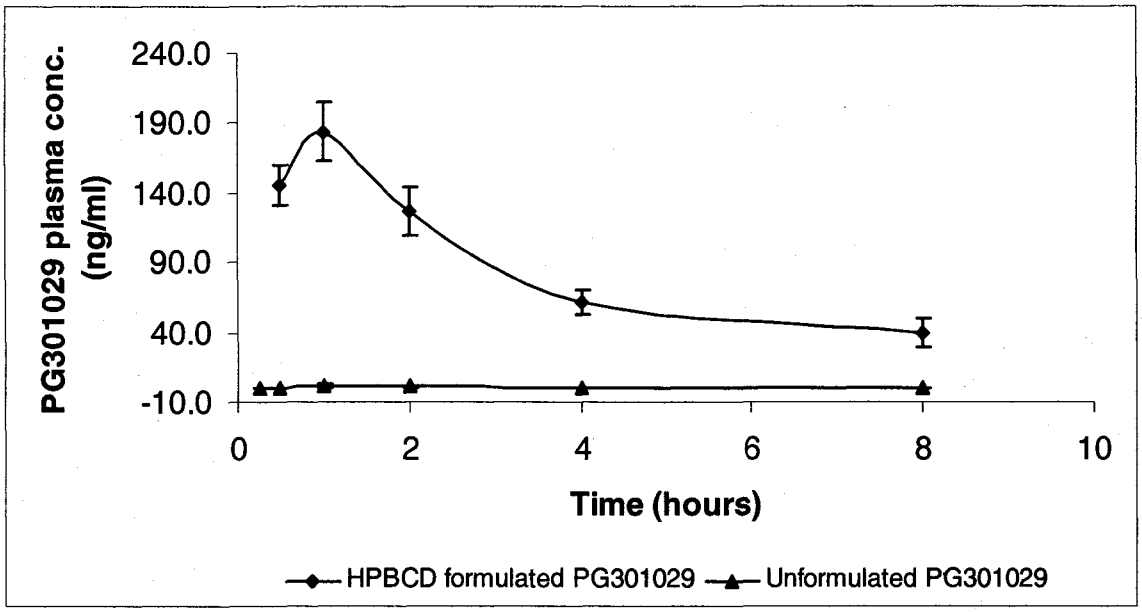



**Figure 3.4.** Plasma concentration-time profiles for PG301029 in the acetic acid/HPβCD formulation, ◆, and unformulated (Wong *et al.*, 2001),▲. Assuming linear pharmacokinetics, the unformulated PG301029 data were dose normalized from 5000 mg/kg to 5 mg/kg in order to make the current comparison. Error bars represent one standard deviation above and below the mean concentration of PG301029 measured for each time point. Note that the x-axis intercepts the y-axis at -10. This was done in order to display the unformulated drug profile separate from the x-axis.

# REFERENCES

Abbott Laboratories NDA no. 13-025/S-035 submission. Internet Address: www.fda.gov/cder/ogd/rld/13025S35.pdf (accessed 11/18/2004).

ACD©/$Pk_a$ software. Version 7 for Microsoft Windows. Advanced Chemistry Development Inc., 90 Adelaide St. West, Suite 600, Toronto, Ontario. M5H 3V9. Canada.

Avis KE, Lachman L, Lieberman HA (1986) Pharmaceutical Dosage Forms: Parenteral Medications, Volume 2, p. 217-222.

Aree T, Schulz B, Reck G. (2003) Crystal Structures of β-Cyclodextrin Complexes with Formic Acid and Acetic Acid. *Journal of Inclusion Phenomena and Macrocylic Chemistry*. 47:39-45.

Arndt KA, 1988. Adverse reactions to acyclovir: Topical, oral, and intravenous. *J Am Acad Dermatol* 18:188-190.

CDC Website. http://www.cdc.gov/ncidod/diseases/hepatitis. (accessed 10/28/2004).

ClogP© for Windows, Version 4 Software. BioByte Corporation, 201 W. 4th Street, Suite 204, Clairemont, CA. 91711.

Connors KA (1997) The Stability of Cyclodextrin Complexes in Solution. *Chem. Rev.* 97:1325-1357.

Davio SR, McShane MM, Kaluk TJ, Zaya RM, Cole SL (1991) Precipitation of the rennin inhibitor diketirin upon i.v. infusion; in vitro studies and their relationship to in vivo precipitation in the cynomolgus monkey. *Pharm. Res.* 8:80-83.

Douchin K, Shaw J, Spencer E, Seroogy J, Barriere S, Wilbraham D (2004) Single dose pharmacokinetics (PK) of telavancin (TLV) in healthy elderly subjects. *Clinical Microbiology and Infection*. 10(3): 275.

Douchin K, Shaw J, Spencer E, Seroogy J, Barriere S, Wilbraham D (2004) Single dose pharmacokinetics (PK) of telavancin (TLV) in subjects with renal dysfunction. *Clinical Microbiology and Infection.* 10(3): 276.

Faulding Pharmaceuticals. (1999) Editorial: Vancomycin Injection. *The Pharmaceutical Journal.* 263(7073):880.

Greenfield JC, Loux SJ, Sood VK, Jenkins KM, Davio SR (1991) In Vitro Evaluation of the Plasma and Blood Compatibility of a Parenteral Formulation for Ditekiren, a Novel Renin Inhibitor Pseudopeptide. *Pharm. Res.* 8:475-479.

Hadaway L, Chamallas SN. (2003) Vancomycin. *Journal of Infusion Nursing.* 26(5):278-284.

He Y, Johnson JLH, Yalkowsky SH. (2005) Oral Formulation of a Novel Antiviral Agent, PG301029, in a Mixture of Gelucire 44/14 and DMA (2:1, wt/wt). *AAPS Pharm. Sci. Tech.*6 (1) Article 1.

Internet Press Release from Biospace Beat: Biotechnology and Pharmaceutical News. Website address: http://www.biospace.com/news_story.cfm?StoryID=14456320&full=1. (Accessed 12/28/2004)\

Jia L,Wong H, Cerna C, Weitman SD (2002) Effect of Nanonization on Absorption of 301029: *Ex Vivo* and *In Vivo* Pharmacokinetic Correlations Determined by Liquid Chromatography/Mass Spectrometry. *Pharm. Res.* 19: 1091-1096.

Johnson JLH, He Y, Yalkowsky SH (2003) Prediction of Precipitation-Induced Phlebitis: A Statistical Validation of an In Vitro Model. *J Pharm Sci.* 92:1574-1581.

Kastango ES, Hadaway L (2002) New Perspectives on Vancomycin Use in Home Care, Part 2: Delivery Systems. *Int. Jour. Pharm. Comp.* 6(1):55-57.

Kay B, Rolly G (1977) The effect of a change of formulation on the incidence of pain after intravenous injection. *Acta Anaest Belg* 4:317-322.

Kruss B, Mauz A, Ruehr K, Stassen J, Veit C, Wagner K.. (2004) Formulation for the parenteral application of a sodium channel blocker. *US PCT Int. Appl.* 18 pp.

Lanbeck P, Odenholt I, Paulsen O (2002) Antibiotics differ in their tendency to cause infusion phlebitis: a prospective observational study. *Scand. J. Infect. Dis.* 34:512-519.

Li P, Vishnuvajjala R, Tabibi SE, Yalkowsky SH (1998) Evaluation of in vitro Precipitation Methods. *J. Pharm. Sci.* 87:196-199.

Loftsson T. (2003) Non-inclusion cyclodextrin complexes. *US PCT Int. Appl.* 36 pp.

Loftsson T, Masson M. (2004) Method to improve complexation efficacy and produce high-energy cyclodextrin complexes. *US PCT Int. Appl.* 36 pp.

Loftsson T, Sigurdsson HH, Masson M, Schipper N (2004) Preparation of Solid Drug/Cyclodextrin Complexes of Acidic and Basic Drugs. *Pharmazie.* 59: 25-29.

Malabarba A, Ciabatti R (2001) Glycopeptide Derivatives. *Current Medicinal Chemistry.* 8: 1759-1773.

MedCalc Statistical Software, version 6.16

Myrdal PB, Simamora P, Surakitbanharn Y, Yalkowsky SH (1995) Studies in Phlebitis. VII: In Vitro and in Vivo Evaluation of pH-Solubilized Levomopamil. *J. Pharm. Sci.* 84:849-852.

Nalluri BN, Chowdary KPR, Murthy KVR, Hayman AR, Becket G (2003) Physicochemical Characterization and Dissolution Properties of Nimesulide-Cyclodextrin Binary Systems. *AAPS Pharm.Sci.Tech.* 4(1): article 2.

Nozawa, SR, Rigoli, IC, Thedei Jr., G, Rossi, A. Mind the Buffering Capacity of Citric Acid. On-line: www.fgsc.net/fgn42/nozawa.htm (accessed 11/18/2004).

Pagano M, Gauvreau K (1993) Principles of Biostatistics. Duxbury Press, Belmont, California, p. 473-475

*Physician's Desk Reference*, Edition 56, 2002.

Pop E, Loftsson T, Bodor, N. (1991) Solubilization and Stabilization of a Benzylpenicillin Chemical Delivery System by 2-Hydroxypropyl-β-cyclodextrin. *Pharm. Res.* 8:1044-1049.

Ran Y, Zhao L, Xu Q, Yalkowsky SH (2001) Solubilization of Cyclosporin A. *AAPS Pharm.Sci.Tech.* 2(1): article 2.

Rossi SJ, Wright TL (2003) New developments in the treatment of hepatitis C. *Gut.* 52: 756-757.

Rowland M, Tozer TM. (1995) Clinical pharmacokinetcs concepts and applications, 3rd Edition, Baltimore: Lippincott Williams & Wilkins, p. 138.

Salaria, M (2001) Teicoplanin. *Indian Pediatrics.* 38:372-375.

Scifinder Scholar for Windows, 2004 Edition.
www.cas.org/SCIFINDER/SCHOLAR2004

Stedman's Medical Dictionary, 1999.

StataQuest 4 Statistical Software, Windows 95 Version.

Surakibanharn Y, Simamora P, Ward GH, Yalkowsky SH (1994) Precipitation of pH solubilized phenytoin. *Int. J. Pharm.* 109:27-33.

Sweetana S, Akers MJ (1996) Solubility Principles and Practices for Parenteral Drug Dosage Form Development. *PDA J Pharm Sci & Tech* 50:330-342.

Takács-Novák K, Noszál B, Tokes-Kovesdi M, Szasz G (1993) Acid Base Properties and Proton-Speciation of Vancomycin. *Int. J. Pharm.* 89:261-263.

Targocid for injection package insert. Website address: http://home.intekom.com/pharm/roussel/targocid.html.(Accessed 12/28/2004)

Taxis K, Barber N (2003) Ethnographic study of incidence and severity of intravenous drug errors. *British Medical Journal.* 326:1-4.

Thuluvath PJ, Maheshwari A, Mehdi J, Faribanks KD, Wu LLW, Gelrud LG, Ryan MJ, Anania FA, Lobis IF, Black M (2004) Randomised, double blind, placebo controlled trial of interferon, ribavirin, and amantadine versus interferon, ribavirin, and placebo in treatment naïve patients with chronic hepatitis C. *Gut.* 53: 130-135.

Tomasini S, Raneri D, FIcarra R, Calabro ML, Stancanelli R, FIcarra P. (2004) Improvement in solubility and dissolution rate of flavinoids by complexation with β-cyclodextrin. *J.Pharm.Bio.Anal.* 35:379-387.

Trissel LA. (2001) Handbook on Injectable Drugs, 11th ed., American Society of Health System Pharmacists, p. XIV.

Turco SJ, King RE (1979) Sterile Dosage Forms, 2nd ed., Lea & Febiger Pub. Co., p.407.

Turco SJ (1987) Infusion phlebitis: A review of the Literature. *Parenterals* 5:1-8.

Vam Bambeke F, Van Laethem Y, Courvalin P, Tulkens PM (2004) Glycopeptide Antibiotics: from Conventional Molecules to New Derivatives. *Drugs.* 64(9): 913-936.