Reformulation of a New Vancomycin Analog: An Example of the Importance of Buffer Species and Strength

Submitted: March 10, 2005; Accepted: December 5, 2005; Published: January 13, 2006

Jennifer L. H. Johnson[1] and Samuel H. Yalkowsky[1]

[1]Pharmaceutical Science, College of Pharmacy, University of Arizona, 1703 E Mabel St, Tucson, AZ 85721

ABSTRACT

The purpose of this research was to use our previously validated dynamic injection apparatus as a rapid method for screening pH-adjusted formulations of a new vancomycin analog, Van-An, for their potential to precipitate upon dilution. In 1 vial, Van-An was reconstituted according to the manufacturer's instructions. In a separate vial, the Van-An formulation's existing phosphate buffer species was supplemented with acetate buffer, which has a pKa in the desired range: between the pH values of the formulation (pH 3.9) and blood (pH 7.4). The formulations were injected using the dynamic injection apparatus into a flowing stream of isotonic Sorensen's phosphate buffer at rates of 0.25, 0.5, 1, and 2 mL/min. The peaks obtained with the spectrophotometer were reproducible for each injection rate/formulation combination. For the phosphate-buffered formulation, the least amount of precipitation was obtained at the 0.25 mL/min injection rate. Acetate buffer was able to substantially reduce such precipitation, even at the highest injection rate. The opacity peaks for the formulation with the acetate addition were significantly smaller ($P < .05$) than those obtained for the unaltered formulation at all 4 injection rates. The results suggest that acetate is a better buffer species than phosphate for the pH range defined. Furthermore, we present evidence to support a generally applicable approach to screening new formulations of drug products that may be clinically useful for reducing the incidence of phlebitis in humans.

KEYWORDS: Precipitation, phlebitis, pH, solubility, prediction, probability, sensitivity, specificity, in vitro model, blood surrogate, buffer species.

INTRODUCTION

With the constant development of bacterial resistance to current antibiotics, there is an ongoing need for novel antibiotics that can be used as the last line of defense in life-threatening infections. Vancomycin is a powerful antibiotic that is often used as a last resort. Unfortunately, bacteria are now becoming resistant to it.[1] For this reason, several vancomycin analogs have recently been developed. Van-An is a new proprietary second-generation vancomycin analog that is active against gram-positive bacteria.

Vancomycin is reported to have the following 6 pKa values: 7.75, 8.89 (basic), 2.18, 9.59, 10.4, and 12.0 (acidic).[2] The functional groups responsible for these values are indicated in Figure 1, and a graph of expected charge speciation is shown in Figure 2. 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.[3-9] While some of these adverse effects can be attributed to errors in the manner of preparation or administration of the injection[10] or pharmacological irritation,[11] these adverse reactions may still be formulation related. It has been previously shown that phlebitis is often related to precipitation of pH-solubilized drugs upon injection into the bloodstream.[12]

Van-An is solubilized by pH adjustment and is reported to have the following 7 pKa values: 11.68, 10.14, 9.53 (basic), 8.69, 7.32, 7.07, and 3.23 (acidic) (proprietary company information, October 2003). In short, Van-An has 1 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, approaching the neutrality of blood. The vancomycin and Van-An calculated net charges, from pH values 0 to 13, are shown in Figure 2.

According to the manufacturer, reconstitution of freeze-dried Van-An produces a solution having an approximate pH of 3.9. As seen in Figure 2, the net charge of the drug at pH 3.9 is approximately +2.1. Once the formulation is injected into the bloodstream, its pH will rise to that of blood. The pH increase from 3.9 to 7.4 will decrease the net charge of the drug to +0.7. This will produce a 25-fold decrease in drug solubility, which in turn can produce significant precipitation.

Each freeze-dried vial contains 100 mg of the free base, Van-An, provided in the form of the phosphate salt. Mannitol is employed as the bulking agent at 48.8 mg,

Corresponding Author: Jennifer L. H. Johnson, Pharmaceutical Science, College of Pharmacy, University of Arizona, 1703 E Mabel St, Tucson, AZ 85721. Tel: 520-770-1259 ext. 159; Fax: 520-626-4063; E-mail: jjohnson@imarx.com

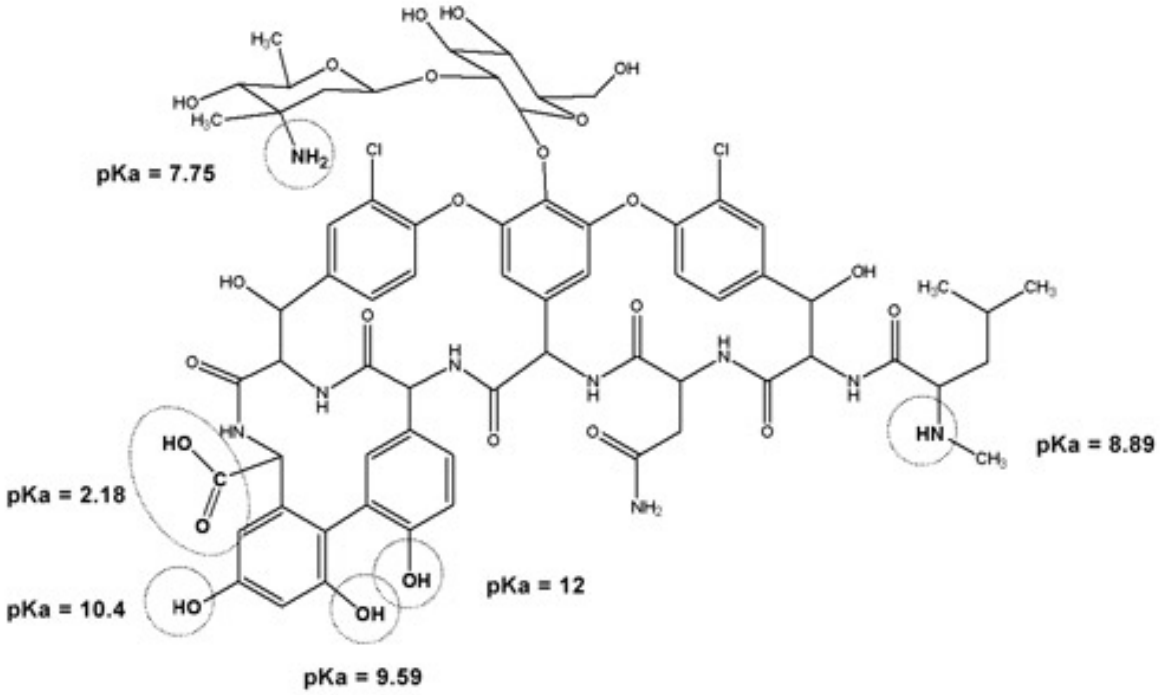

Figure 1. The structure of vancomycin.

while phosphoric acid and sodium phosphate are included so that the target reconstituted pH (3.9) can be achieved. Phosphoric acid has the following 3 pKa values: 2.15, 7.20, and 13.38. None of these are in the 3.9 range. Therefore, regardless of the phosphate concentration, the formulation is without adequate buffer ability at the formulation pH.

We used the validated dynamic injection apparatus to evaluate Van-An for its potential to cause phlebitis due to precipitation.[12] The current formulation of Van-An precipitates when diluted with a blood surrogate. Reformulation using a more appropriate buffer with a pKa within the 4 to 7 pH range can substantially reduce the precipitation. The purpose of this study was to show that the addition of 0.1M acetate (with a pKa of 4.75) to the Van-An formulation significantly reduces the precipitation of the drug upon injection and that this reduction can be clearly demonstrated using the dynamic injection apparatus.

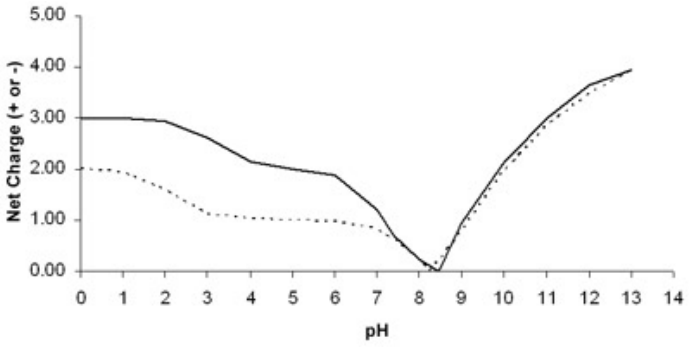

Figure 2. Calculated net charges of vancomycin (dotted line) and Van-An (solid line) across the 0 to 13 pH range. Calculations were made according to the following equations: net charge = $10^{-pH}/(10^{-pKa} + 10^{-pH})$ for bases and $-10^{-pKa}/(10^{-pKa} + 10^{-pH})$ for acids.

Materials and Methods

Model

Figure 3 shows a schematic of the dynamic injection apparatus. A full description of the setup has been previously published.[12] The surrogate blood flow rate used is comparable to that of human blood flow in readily accessible arm veins.[13] Isotonic Sorensen's phosphate buffer (ISPB) at pH 7.4 was chosen as a blood surrogate rather than plasma because this ISPB 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[14] and 0.008,[15] respectively, because stored plasma lacks the carbon

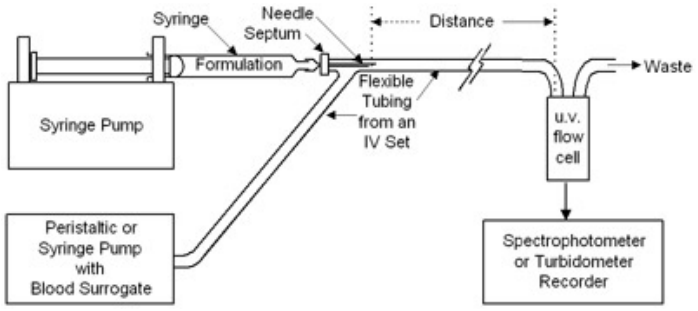

Figure 3. The dynamic in vitro apparatus for evaluating precipitation upon injection.

dioxide that buffers blood in a living organism. All trials were performed at room temperature (~25°C).

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.

Standardization of the Model

Because the spectrophotometer used in the current study was different from the one used in the preliminary study, [12] the model was standardized as follows: phenytoin and amiodarone (positive controls), and diltiazem and furosemide (negative controls), all of which were used in the preliminary study, were injected, in triplicate, into the model at a rate of 5 mL/min. Through use of *t* tests, their resulting opacity values were compared with those from the preliminary study.

Vial Preparation

Original Formulation

One vial of Van-An was reconstituted to a final concentration of 1.6 mg/mL according to manufacturer's instructions as follows: 10 mL of sterile water for injection (Baxter Healthcare, Deerfield, IL) was added to the freeze-dried vial. The rubber stopper was replaced, and dissolution was facilitated by gentle inversion of the vial for ~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 mL of 5% dextrose, and the resulting pH was measured. The final phosphate concentration was 2 mM. Once again, clarity of solution was confirmed by the lack of a Tyndall effect.

Acetate-Supplemented Formulation

One vial of Van-An was reconstituted according to manufacturer's instructions as described above, with 1 alteration. A 0.37-g amount of glacial acetic acid (formula weight [FW] = 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 1M KOH. Once again, clarity of solution was confirmed by the lack of a Tyndall effect. The final concentrations of Van-An and acetate were 1.6 mg/mL and 0.1M, respectively.

Data Collection

The dynamic injection apparatus depicted in Figure 3 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 0.1M acetate addition were compared with those of the same lot of unaltered formulation. Next, *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 $P < .05$ level of confidence.

Results and Discussion

Model

Figure 4 shows the wavelength scan of Van-An, which indicates that a wavelength of 540 nm can be used for reading blockage of light as opposed to absorbance.

Standardization of Model

No significant differences (all *P* values > .10) were found between the new values and the old values[12] for the 4 intravenous injectables screened. Therefore, the new

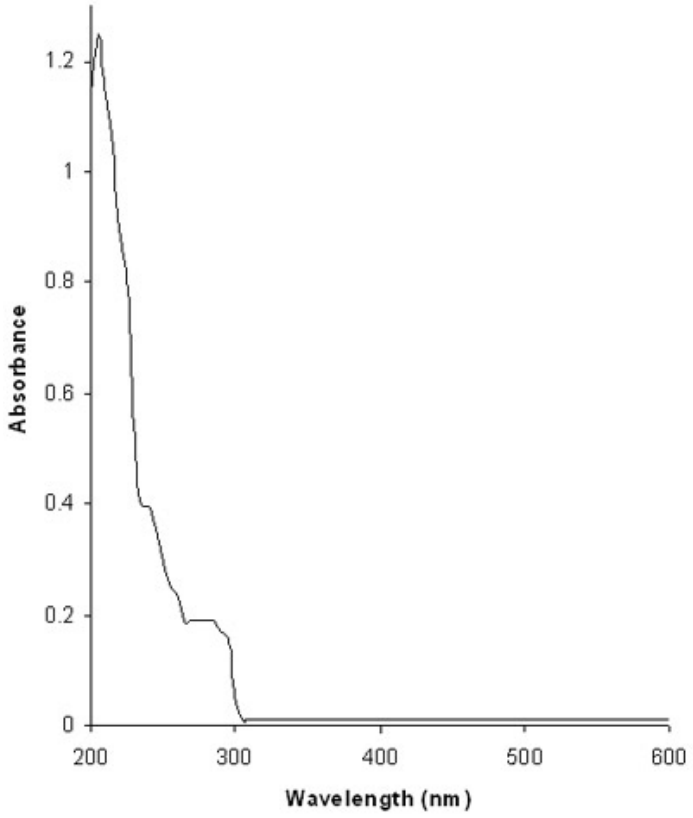

Figure 4. Van-An wavelength scan. Note the minimal absorbance at 540 nm.

spectrophotometer is determined to be reliable and consistent with the originally validated model. Furthermore, because the original validation study was performed a more than a year before the present study, interday reliability was established.

The dynamic injection apparatus is a model. Like all models, it does not absolutely represent an in vivo system. Having albumin in the blood surrogate and running the experiments at body temperature, 37°C—although physiologically accurate—were not considered necessary for the intended purpose. In developing the model, we intended to achieve 1 central goal: offering simplicity without sacrificing predictive power. The preliminary validation study of Johnson et al[12] showed that running the analyses without albumin and at room temperature allowed this goal to be achieved. Furthermore, 2 other published studies[16,17] that used a similar dynamic prediction method also suggest that raising the temperature to 37°C does not improve accuracy.

Spectrophotometric Data

Figure 5 shows the opacity data obtained for the solution with the phosphate buffer system. Each x-axis represents the time taken to collect 1 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 numbers in the upper right corners of each graph are the injection rates tested.

The opacity tracings for the 0.1M acetate-buffered formulation are not shown, because they were flat line tracings that never rose above a value of 0.015 for any of the injection rates tested. The absence of peaks reflects the relative absence of precipitation in the acetate-buffered formulation as compared with the phosphate-buffered system.

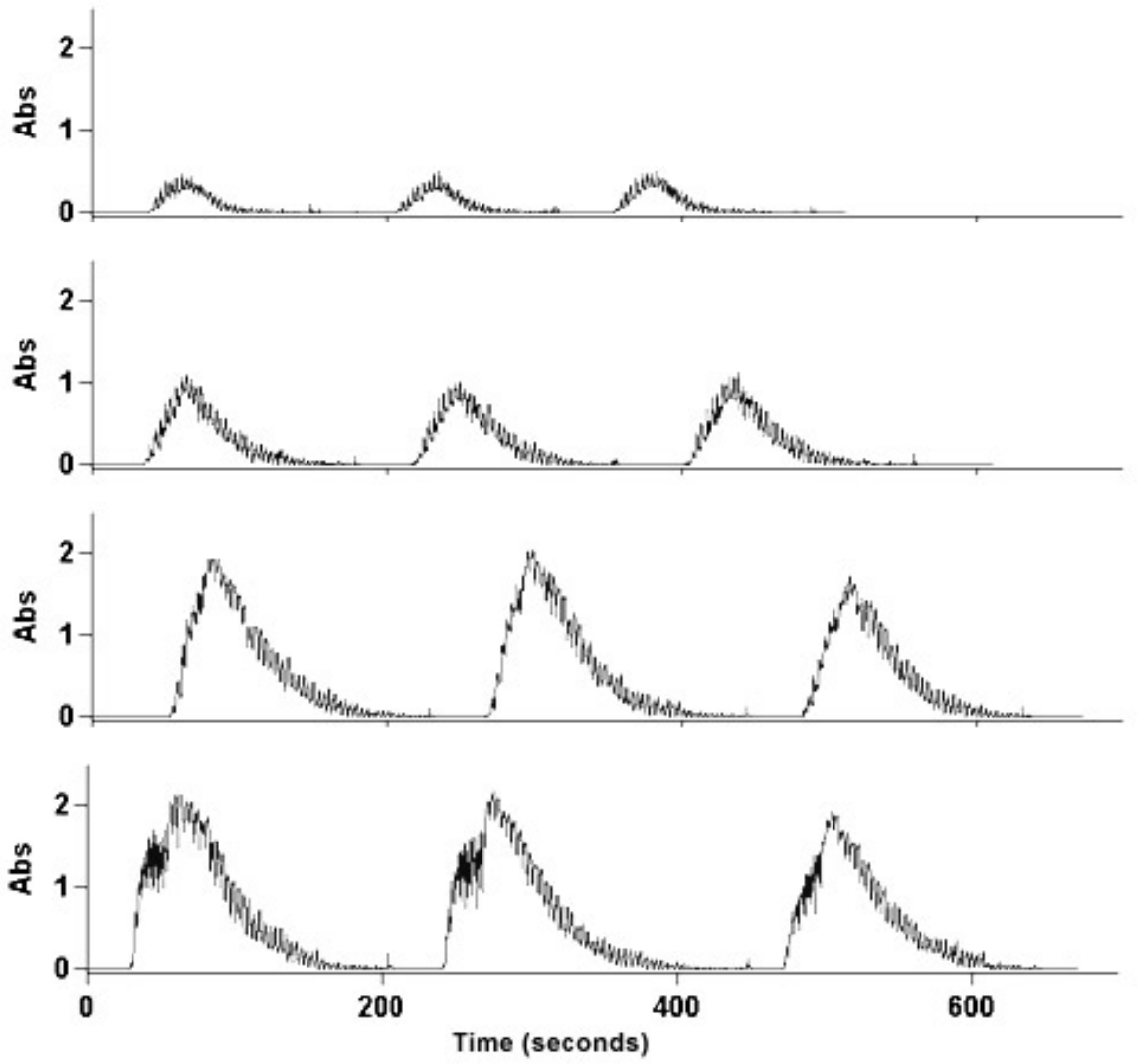

Figure 5. Van-An reconstituted as directed by the manufacturer. From top to bottom, opacity data for injection rates of 0.25, 0.5, 1, and 2 mL/min are shown.

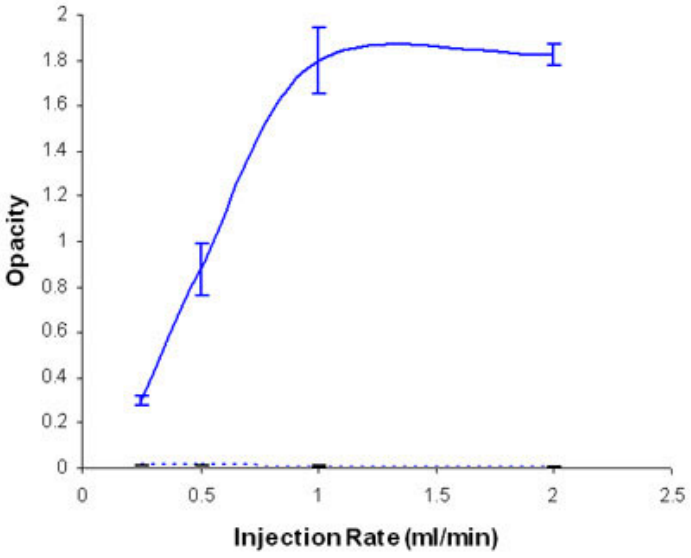

Figure 6. Opacity differences obtained when comparing 2 newly reconstituted vials of Van-An: one with 0.1M acetate buffer added (dotted line close to the x-axis) and the other without (solid line). The error bars represent the 95% confidence interval of the triplicate measurement at each injection rate.

Data Analysis

Figure 6 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 at a statistically significant level ($P < .05$). Thus, the acetate buffer addition is advantageous from a physical stability perspective, and the improved formulation would be expected to be less irritating when administered intravenously.

It should be noted that the dynamic injection apparatus assesses the potential for mechanical phlebitis due to precipitation upon injection, which is a matter of physical stability. The model does not address the chemical stability of a formulation. Therefore, additional experiments, in conjunction with those described herein, are important to determine the viability of a formulation from a full stability perspective.

CONCLUSION

The addition of 0.1M acetate buffer provides the Van-An formulation with a buffering species possessing a pKa in the target range between 4 and 7. In addition, the 0.1M concentration establishes 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,[1,18,19] may lead to a lower incidence of clinical phlebitis due to precipitation. This formulation approach may be generally applicable to other drugs with ionization profiles similar to that of Van-An.

REFERENCES

1. Hadaway L, Chamallas SN. Vancomycin. *J Infus Nurs.* 2003;26: 278–284.

2. Takács-Novák K, Noszál B, Tokes-Kovesdi M, Szasz G. Acid base properties and proton-speciation of vancomycin. *Int J Pharm.* 1993;89:261–263.

3. Malabarba A, Ciabatti R. Glycopeptide derivatives. *Curr Med Chem.* 2001;8:1759–1773.

4. Salaria M. Teicoplanin. *Indian Pediatr.* 2001;38:372–375.

5. Douchin K, Shaw J, Spencer E, Seroogy J, Barriere S, Wilbraham D. Single dose pharmacokinetics (PK) of telavancin (TLV) in healthy elderly subjects. *Clin Microbiol Infect.* 2004;10:275.

6. Douchin K, Shaw J, Spencer E, Seroogy J, Barriere S, Wilbraham D. Single dose pharmacokinetics (PK) of telavancin (TLV) in subjects with renal dysfunction. *Clin Microbiol Infect.* 2004;10:276.

7. Internet press release from Biospace Beat. Biotechnology and Pharmaceutical News. Available at: http://www.biospace.com/news_ story.cfm?StoryID=14456320&full=1. Accessed: December 28, 2004.

8. Targocid for Injection package insert. Available at: http://home. intekom.com/pharm/roussel/targocid.html. Accessed: December 28, 2004.

9. Van Bambeke F, Van Laethem Y, Courvalin P, Tulkens PM. Glycopeptide antibiotics: from conventional molecules to new derivatives. *Drugs.* 2004;64:913–936.

10. Taxis K, Barber N. Ethnographic study of incidence and severity of intravenous drug errors. *BMJ.* 2003;326:1–4.

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

12. Johnson JLH, He Y, Yalkowsky SH. Prediction of precipitation-induced phlebitis: a statistical validation of an in vitro model. *J Pharm Sci.* 2003;92:1574–1581.

13. Rowland M, Tozer TM. *Clinical Pharmacokinetics: Concepts and Applications*. Baltimore, MD: Lippincott Williams & Wilkins; 1995:138.

14. Abbott Laboratories NDA no. 13-025/S-035 submission. Available at: http://www.fda.gov/cder/ogd/rld/13025S35.pdf. Accessed: November 28, 2004.

15. Surakitbanharn Y, Simamora P, Ward GH, Yalkowsky SH. Precipitation of pH solubilized phenytoin. *Int J Pharm.* 1994;109:27–33.

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

17. Greenfield JC, Loux SJ, Sood VK, Jenkins KM, Davio SR. In vitro evaluation of the plasma and blood compatibility of a parenteral formulation for ditekiren, a novel renin inhibitor pseudopeptide. *Pharm Res.* 1991;8:475–479.

18. Faulding Pharmaceuticals. Editorial: vancomycin injection. *Pharm J.* 1999;263:880.

19. Kastango ES, Hadaway L. New perspectives on vancomycin use in home care, part 2: delivery systems. *Int J Pharm Comp.* 2002;6:55–57.