ELSEVIER

International Journal of Pharmaceutics 168 (1998) 41–48

international journal of pharmaceutics

The solution stability of vancomycin in the presence and absence of sodium carboxymethyl starch

John S. Claudius [a,b], Steven H. Neau [a,*]

[a] *School of Pharmacy, University of Missouri-Kansas City, Kansas City, MO 64110, USA*
[b] *Hoechst Marion Roussel, Inc., Kansas City, MO 64137, USA*

Received 30 September 1997; received in revised form 5 January 1998; accepted 6 February 1998

Abstract

The purpose of this work was to study the potential for drug-excipient interactions to alter the stability of pharmaceutical drug products. Previous studies have demonstrated the interactions between glycopeptide antibiotics (e.g. vancomycin) and sodium carboxymethyl starch (NaCMS). A key objective of this work was to determine the effect of NaCMS binding on the solution stability of the glycopeptide antibiotic, vancomycin. The solution stability of vancomycin was studied under various pH and ionic strength conditions at physiological temperature (37°C). A stability-indicating HPLC method specific for vancomycin was developed and validated for use in this research. Vancomycin stability was evaluated alone and in the presence of NaCMS in various buffers from pH 2 to 9, and in simulated gastric and intestinal fluids to determine if NaCMS influences the degradation rate of vancomycin in solution. Results indicated that the pH stability profile of vancomycin at 37°C from pH 2 to 7 was slightly stabilized in the presence of NaCMS at the pH and ionic strength conditions where binding to NaCMS was shown to occur.

Keywords: Adsorption; Glycopeptide antibiotic; Vancomycin; Stability; Sodium carboxymethyl starch; Sodium starch glycolate

1. Introduction

Glycopeptide antibiotics are an important therapeutic class of compounds used for treating bacterial infections. Glycopeptides are potent antibiotics and have low minimum inhibitory concentrations for gram-positive strains (Biavasco et al., 1991). They are most commonly used in the treatment of virulent gastrointestinal or systemic infections (Gruneberg and Wilson, 1994; Daschner and Kropec, 1995), such as those elicited by staphylococcal and enterococcal organisms. Glycopeptide antibiotics such as vancomycin, ramoplanin, and teicoplanin are life-

* Corresponding author.

0378-5173/98/$19.00
PII S0378-5173(98)00080-5

saving drugs in certain clinical situations where first-line antibiotics (e.g. penicillins, cephalosporins) result in treatment failure (Sugarman and Pesanti, 1980; John, 1994).

The molecular structure of vancomycin hydrochloride is shown in Fig. 1. The stability of vancomycin in solution has been studied by various investigators at room temperature (Mathew and Das Gupta, 1995) and at 50°C (Antipas et al., 1994). The pH of maximum stability for vancomycin was found to be between pH 3.0 and 5.7 at room temperature. The primary route of vancomycin degradation occurs by the deamidation of the asparagine residue proceeding through two mechanisms: general acid- or base-catalyzed hydrolysis or by the formation of an imide intermediate to yield degradation products CDP-I or CDP-Im (Marshall, 1965). These two degradation products differ only in the orientation of the chlorine on the substituted tyrosine residue at the second amino acid position from the N-terminus. The glycosidic linkage between the heptapeptide backbone and attached sugars can also undergo hydrolysis to form aglycovancomycin.

A controlled study of the solution stability of vancomycin at physiological conditions (i.e. 37°C, pH 1–9) has not been reported in the literature. It would be worthwhile to determine the stability of vancomycin in solution at 37°C over a pH range of 2–9 and in simulated gastric (pH 1.2) and intestinal (pH 7.5) fluids. The study of vancomycin stability in these media could demonstrate the relevant importance of hydrolytic and enzymatic degradation mechanisms in the gastrointestinal tract.

The binding of vancomycin to sodium carboxymethyl starch (NaCMS) has been characterized and reported in the literature (Claudius and Neau, 1996). Vancomycin binds to NaCMS through an electrostatic attraction mechanism between the protonated amino groups of vancomycin and the carboxylate groups of NaCMS. Binding phenomena can, of course, affect physical and chemical characteristics of drugs as they relate to the stability, activity, and therapeutic properties of drugs (El-Shibini et al., 1971; Fung et al., 1974). Given this, the primary objective of this work was to determine whether the stability profile of vancomycin would be altered upon binding to NaCMS. In addition, since this binding process was shown to be electrostatic in nature, the effects of pH and ionic strength on the stability of vancomycin in the presence of NaCMS were also determined.

2. Materials and methods

2.1. Materials

Vancomycin was obtained as the hydrochloride salt form (MW 1485.67) from ICN Biochemicals (Los Angeles, CA) and was used without further purification. Sodium carboxymethyl starch was obtained from the Edward Mendell Co. (Patterson, NY) as Explotab®. Pepsin and pancreatin were of analytical purity and supplied by Mallinckrodt (St. Louis, MO). All other buffer reagents and solvents used were of analytical reagent grade quality.

2.2. Methods

An HPLC stability-indicating method was developed for the separation and detection of vancomycin in buffered aqueous media based on previous method development research (Inman, 1987). This previous work had demonstrated the need for gradient elution techniques to optimize the conditions for the isolation of vancomycin from its related substances.

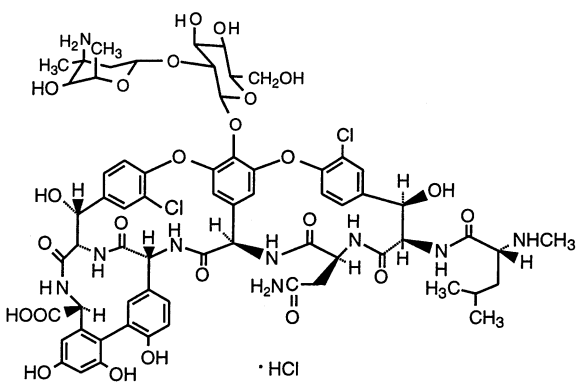


Fig. 1. The molecular structure of vancomycin hydrochloride.

Mobile phase consisted of 100 mM acetate buffer (pH 4.0 ± 0.1) and acetonitrile. Mobile phase was prepared by thoroughly mixing acetate buffer and acetonitrile in proportions of 5:95 for eluent A and 30:70 for eluent B. After mixing, each eluent was sonicated for 5 min and degassed with an equal volume of helium. The optimum gradient profile at a flowrate of 1.0 ml/min was determined to be the following: 0–15 min 100% A to 100% B; 15–20 min 100% B, and 20–25 min 100% B to 100% A. Fifteen minutes (or approximately five column volumes) was allowed for column equilibration before subsequent injections. The wavelength of detection was selected to be 280 nm (λ_{max}) to increase analyte specificity and minimize background interference while yet maintaining adequate detection sensitivity. Blank controls were run and showed minimal spectral interference during the gradient portion of the run.

2.3. Instrumentation

A Waters HPLC system (Milford, MA) was used for analytical method development and stability studies. The system consisted of a 600E system controller and quaternary pump, WISP 712 autosampler, and an Applied Biosystems Model 785A (Aston, PA) variable wavelength detector. A YMC Basic bonded-phase column (5 μm, 4.6×250 mm; Morris Plains, NJ) was used as the stationary phase for all analyses.

2.4. Method validation

Method linearity, accuracy, precision, ruggedness, and specificity were evaluated using a six-point standard curve performed in triplicate on three different days over the concentration range of 10–500 μg/ml.

2.5. Stability study design

The stability profile of vancomycin alone (control) and in the presence of NaCMS was determined at 37°C over a pH range of 2.0–9.0 and in simulated gastric (SGF) and intestinal (SIF) fluids. The following buffers were used: 0.01 N HCl (pH 2.0), citrate (pH 3.1), acetate (pH 4.7), carbonate (pH 6.9), Tris–HCl (pH 7.8), borate (pH 9.0). The initial and final pH of each stability sample was measured to ensure the adequacy of each buffer concentration. The effect of buffer concentration on vancomycin stability was studied using buffer concentrations from 10 to 20 mM. The ionic strength was held constant at each buffer concentration by adjusting with sodium chloride.

2.6. Sample preparation

Vancomycin samples, with and without NaCMS, were prepared in duplicate at a concentration of 0.5 mg/ml by dissolving it in the desired buffer in 25-ml type I clear glass vials (Wheaton Glass, Wheaton, IL). The concentration of NaCMS in each vial was 1 mg/ml. Vials had been rinsed with purified water, USP, and dried prior to use. Upon filling, all vials were sealed with Teflon-coated butadiene stoppers (The West Company, Millville, NJ) and covered with Parafilm® to minimize evaporation. Samples were stored in a static oven at 37°C and 1-ml aliquots were sampled, filtered and analyzed at 0, 2, 4, 6, 8, 10, 12, and 14 days. Samples containing NaCMS were diluted 1:1 (v/v) with 1 M NaCl prior to filtration for reversible desorption of vancomycin from NaCMS (Claudius and Neau, 1996). This procedure was validated to ensure total recovery of vancomycin from samples containing NaCMS. External standards were run in triplicate and used as a basis for calculating assay values of all stability samples.

2.7. Data analysis

Data from method validation runs were analyzed using Sigmaplot 3.0 (Jandel Scientific, San Rafael, CA). Data from stability studies were analyzed using Systat® statistical software (SYSTAT, Evanston, IL). Linear and log-linear regression models were used to estimate the values of method validation and stability parameters, respectively. One-way analysis of variance and analysis of covariance subroutines were used to determine the statistical significance of the esti-

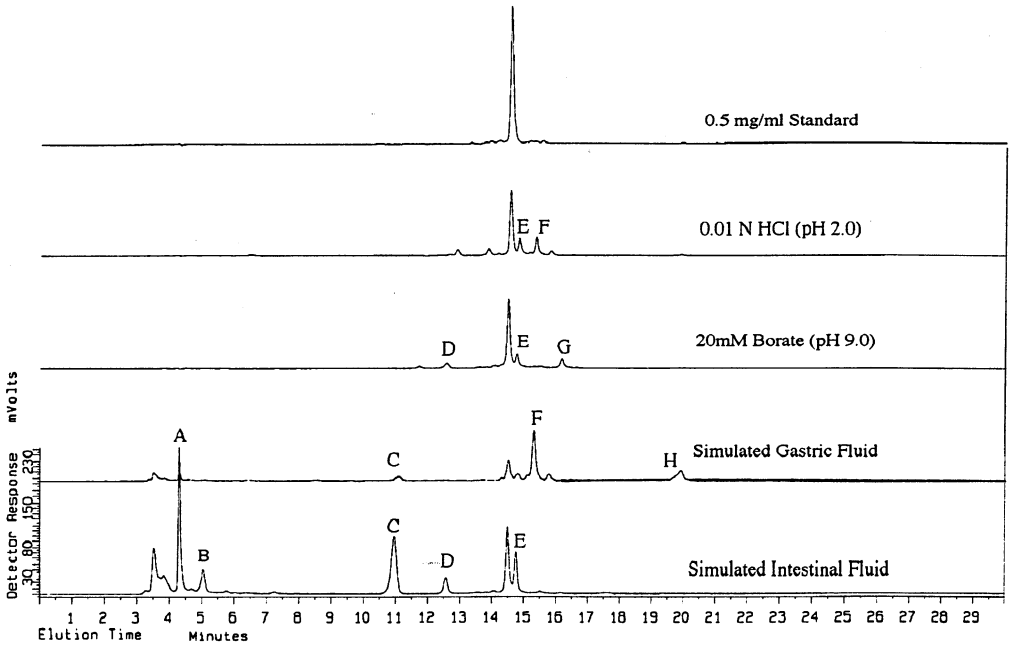


Fig. 2. Vancomycin chromatograms in various media after storage for 4 days at 37°C.

mated parameters. A critical probability level of $\alpha = 0.05$ was used to determine statistical significance.

3. Results and discussion

3.1. Method validation

Validation data revealed that the vancomycin HPLC method demonstrates excellent linearity ($r^2 > 0.99999$) over the concentration range of 10–500 μg/ml. Method precision was deemed adequate as measured by inter-day (0.9% CV) and intra-day (0.7% CV) variability.

Typical chromatograms of a freshly prepared vancomycin standard and samples degraded under various conditions are shown in Fig. 2. There were several low level peaks, assumed to be process impurities, present in the original material. Additional peaks observed in the simulated gastric and intestinal fluid samples were not detected in the buffered solutions at similar pH and, thus, are an indication of enzymatic degradation. This is a clear indication of the different degradation mechanisms occurring across the range of pHs studied and in simulated physiological fluids. The relative retention times and amounts of the main degradation products of vancomycin present in these samples after 4 days storage at 37°C are listed in Table 1.

3.2. Stability studies

The initial and final pH of each stability sample was measured and found to vary by only ± 0.2 pH units (except for the carbonate buffer samples which varied by ± 0.5 pH units). The stability profiles of samples degraded under the extremes of the pH range studied and in simulated fluids are shown as log-linear plots in Fig. 3. These stability profiles demonstrate the magnitude of degradation at extreme conditions of the study over a 14-day period. The degradation of vancomycin is enhanced due to the presence of the enzymes pepsin (in SGF) and pancreatin (in SIF).

The degradation rate of vancomycin is adequately described by a pseudo first-order kinetic model for all stability samples ($r \geq 0.95$). First order degradation rate constants for the control

Table 1
Relative retention times (RRT) and amounts of vancomycin main degradation products after 14-day storage at 37°C

Peak (RRT)	Vancomycin main degradation products (peak area %)							
	A (0.30)	B (0.34)	C (0.76)	D (0.87)	E (1.02)	F (1.06)	G (1.12)	H (1.37)
pH 2.0	n.d.	n.d.	n.d.	4.6	12.3	12.7	n.d.	n.d.
pH 9.0	n.d.	n.d.	n.d.	6.2	11.5	n.d.	9.4	n.d.
SGF	n.d.	n.d.	3.8	n.d.	n.d.	30.6	n.d.	10.5
SIF	20.0	6.6	17.0	3.7	7.6	n.d.	n.d.	n.d.

n.d., none detected.

samples (no NaCMS added) are included in Table 2 and have been estimated from the slopes of their corresponding log-linear plots. Half-lives (t_{50}) have also been calculated and are presented. The probabilities of catalysis occurring from 10 to 20-mM buffer concentrations at each pH are included.

All rate constants in Table 2 are within one order of magnitude of each other. The standard error for all rate constants was on the order of 10^{-5}, indicating minimal variability in the estimation of these parameters. From this data, the pH of maximum stability appears to be from pH 4.7 to pH 7.8. There was no buffer catalysis observed in the vancomycin control samples from buffer concentrations of 10–20 mM based on analysis of covariance (ANCOVA). A probability level of $p = 0.05$ or greater indicates that no significant difference exists between the degradation rate

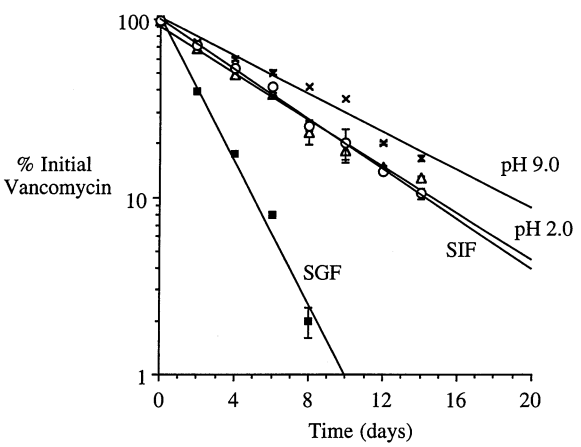


Fig. 3. Degradation profile of vancomycin samples stored for 14 days at 37°C.

constants estimated at 10, 15, and 20-mM buffer concentrations in control samples. As is shown in Table 2, the p value for all control samples is greater than 0.05 (except for the Tris–HCl buffer) indicating that the rate constants estimated at different buffer concentrations are not significantly different. The probability value for the Tris–HCl buffer system is 0.019. This probability value indicates that the rate constants estimated for the three buffer concentrations are statistically different from one another. However, in practical terms, the rate constants from all three Tris–HCl buffer concentrations are within a range of 11%.

The first order degradation rate constants for vancomycin samples containing NaCMS are shown in Table 3. The rate constants for each buffer concentration were significantly different using ANCOVA and as a result, all probabilities for buffer concentrations as a covariate term were equal to zero. As such, the rate constant estimated for each buffer concentration and pH is listed. In general, the rate of degradation is slightly less in the samples containing NaCMS as compared to control samples. The rate constants tend to increase slightly with increasing buffer concentration. A similar level of sample variability was observed in those samples containing NaCMS, as compared to control samples.

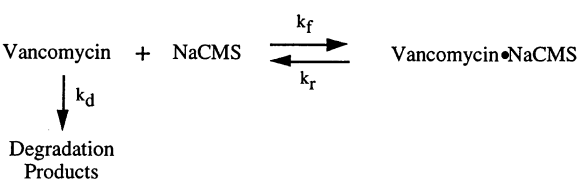
The pH stability profiles of vancomycin alone (control) and in the presence of NaCMS at different buffer concentrations are shown in Fig. 4. As previously mentioned, control samples demonstrated no buffer catalysis whereas the effect of buffer concentration was significant in samples containing NaCMS.

Table 2
First order degradation rate constants of vancomycin control samples

pH	Buffer (M)	Probability (p)	Observed rate constant (h^{-1})	t_{50} (days)	Model fit (Pearson r)
2.0	0.010–0.020	0.251	2.67×10^{-3}	10.8	0.982
3.1	0.010–0.020	0.153	2.04×10^{-3}	14.2	0.975
4.7	0.010–0.020	0.369	1.29×10^{-3}	22.4	0.976
6.9	0.010–0.020	0.082	1.33×10^{-3}	21.7	0.946
7.8	0.010–0.020	0.019	1.00×10^{-3}	28.9	0.994
9.0	0.010–0.020	0.375	2.08×10^{-3}	13.9	0.967
SGF	—	—	7.54×10^{-3}	3.8	0.999
SIF	—	—	2.96×10^{-3}	9.8	0.995

The stability profile of the control samples exhibits a complex relationship in which vancomycin stability is highly dependent upon its ionization state in solution. From the pH stability profiles in Fig. 4, it is apparent that the addition of NaCMS alters the stability of vancomycin in solution to a slight degree. This could be due to the competing dynamic process of adsorption onto NaCMS. The pH stability profiles for 10, 15, and 20-mM buffer concentrations in the presence of NaCMS are directly correlated with the stabilizing of vancomycin in solution at the pH range where binding has been shown to occur (pH ~ 2–7). In other words, vancomycin is stabilized and protected from degradation reactions by adsorption onto NaCMS at these pH and ionic strength conditions. This is indicated by the reduced degradation rate constants at lower buffer concentrations in the presence of NaCMS.

As the buffer concentration increases, the rate of vancomycin degradation increases until the rate is essentially the same as the control sample. The exception for this rank order is the Tris–HCl buffer system where the rate constant associated with the control sample is intermediate to the rate constants associated with the samples containing NaCMS. In the neutral region of the pH stability profile, a pronounced flattening of the middle portion of the curve has occurred with lower buffer concentrations in samples containing NaCMS. This indicates that the reactivity of the different ionic forms of vancomycin present (i.e. monocation, zwitterion, monoanion) is relatively the same. As shown with the control profile, the inherent relative reactivity of these different ionic species is not the same without NaCMS present. This may suggest that the ionization of some functional groups (i.e. amines) may be inhibited by the presence of NaCMS in a way, such as binding, that may reduce the ionization of these forms of vancomycin. This is substantiated by the mechanism that has been previously established, i.e. that amine groups are involved in the binding process with the carboxylate groups on NaCMS. The schematic below describes the proposed mechanism for the stability of vancomycin in solution in the presence of NaCMS:



Of course, this mechanism assumes that vancomycin does not degrade in the adsorbed state. The degradation of vancomycin in the adsorbed state could occur depending on, for example, the accessibility of water to the reaction site in the case of hydrolysis reactions.

In practical terms, it should be noted that all rate constants lie within a range of less than one order of magnitude. This indicates that any stabilizing effects contributed by adsorption to NaCMS are minimal and should not have any direct therapeutic implications in terms of solution stability.

Table 3
First order degradation rate constants of vancomycin samples containing sodium carboxymethyl starch (NaCMS)

pH	Buffer (M)	Observed rate constant (h^{-1})	t_{50} (days)	Model fit (Pearson r)
2.0	0.010	2.42×10^{-3}	11.9	0.999
	0.015	2.42×10^{-3}	11.9	0.999
	0.020	2.67×10^{-3}	10.8	0.998
3.1	0.010	1.67×10^{-3}	17.3	1.000
	0.015	1.71×10^{-3}	16.9	0.999
	0.020	1.83×10^{-3}	15.8	0.999
4.7	0.010	1.00×10^{-3}	28.9	0.996
	0.015	1.08×10^{-3}	26.7	0.997
	0.020	1.21×10^{-3}	23.9	0.998
6.9	0.010	1.04×10^{-3}	27.8	0.992
	0.015	1.21×10^{-3}	23.9	0.992
	0.020	1.42×10^{-3}	20.3	0.990
7.8	0.010	1.04×10^{-3}	27.8	0.991
	0.015	1.21×10^{-3}	23.8	0.996
	0.020	1.29×10^{-3}	22.4	0.998
9.0	0.010	2.08×10^{-3}	13.9	0.999
	0.015	2.38×10^{-3}	12.1	0.998
	0.020	2.46×10^{-3}	11.7	0.991
SGF	—	8.33×10^{-3}	3.5	0.994
SIF	—	3.32×10^{-3}	8.7	0.966

In summary, there are competing processes that occur when vancomycin is in the presence of NaCMS. Unbound vancomycin degrades in solution as evidenced by the control samples with no NaCMS present. In the presence of NaCMS, there is a dynamic equilibrium established between unbound and bound vancomycin based on the conditions of pH and ionic strength at physiological temperature.

4. Conclusions

The stability of vancomycin in solution alone and in the presence of NaCMS has been determined at 37°C over the pH range of 2.0–9.0. In addition, vancomycin stability was evaluated in simulated gastric fluid and simulated intestinal fluid to determine the relative magnitude of enzymatic degradation as it relates to vancomycin stability. Buffer catalysis was shown not to occur in control samples (no NaCMS) over the buffer concentration range from 10 to 20 mM. However, there was a slight stabilizing of vancomycin when NaCMS was present at the pH (approximately 2–7) and ionic strength (less than or equal to 20 mM) conditions where binding to NaCMS was shown to occur. This effect decreased with increasing buffer concentration.

In practical terms, the stabilizing effects contributed by adsorption to NaCMS are minimal and should not have any direct therapeutic implications in terms of solution stability. However, possible analytical and formulation complications such as low assay and dissolution release could result from these interactions. Therefore, given the choice of other pharmaceutical disintegrants (e.g. sodium croscarmellose, sodium crospovidone), caution is warranted on the use of sodium carboxymethyl starch as a disintegrant in solid dosage forms containing glycopeptide antibiotics.

Acknowledgements

The authors are grateful to Hoechst Marion Roussel, Inc., for funding this research. The authors also wish to thank Dr Gregory Beck for his helpful suggestions during the development and validation of the HPLC method.

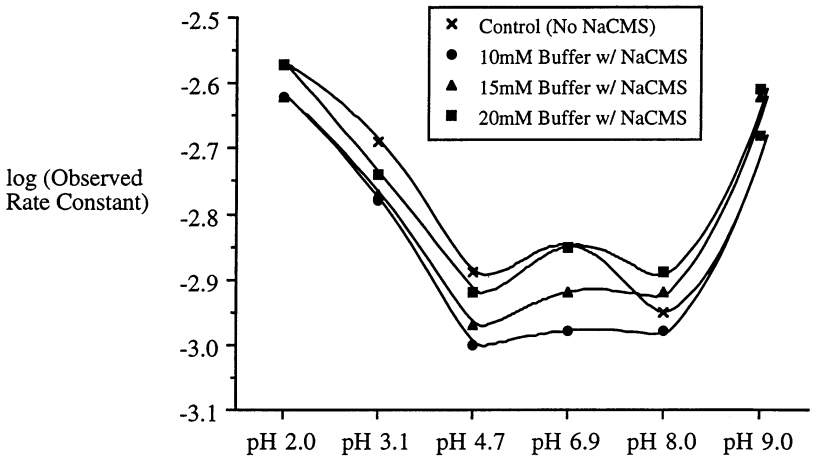


Fig. 4. Vancomycin pH stability profile alone (control) and in the presence of NaCMS in different buffer concentrations at 37°C.

References

Antipas, A.S., Vander Velde, D., Stella, V.J., 1994. Factors affecting the deamidation of vancomycin in aqueous solutions. Int. J. Pharm. 109, 261–269.

Biavasco, F., Manso, E., Varaldo, P.E., 1991. In vitro activities of ramoplanin and four glycopeptide antibiotics against clinical isolates of *clostridium difficile*. Antimicrob. Agents Chemother. 35, 195–197.

Claudius, J.S., Neau, S.H., 1996. Kinetic and equilibrium characterization of interactions between glycopeptide antibiotics and sodium carboxymethyl starch. Int. J. Pharm. 144, 71–79.

Daschner, F.D., Kropec, A., 1995. Glycopeptides in the treatment of staphylococcal infections (review). Eur. J. Clin. Microbiol. Infect. Dis. 14 (Suppl. 1), S12–17.

El-Shibini, H.A.M., Abdel-Nasser, M., Motawi, M.M., 1971. Streptomycin alginate as potential long-acting preparation. Pharmazie 26, 630–632.

Fung, H.-L., Yap, S.K., Rhodes, C.T., 1974. Development of a stable sublingual nitroglycerin tablet I. Interaction of nitroglycerin with selected macromolecules. J. Pharm. Sci. 63, 1810–1811.

Gruneberg, R.N., Wilson, A.P., 1994. Anti-infective treatment in intensive care: the role of glycopeptides (review). Intensive Care Med. 20 (Suppl. 4), S17–22.

Inman, E.L., 1987. Determination of vancomycin related substances by gradient high-performance liquid chromatography. J. Chromatogr. 410, 363–372.

John, C.C., 1994. Treatment failure with use of a third-generation cephalosporin for penicillin-resistant pneumococcal meningitis: case report and review. Clin. Infect. Dis. 18, 188–193.

Marshall, F.J., 1965. Structure studies on vancomycin. J. Med. Chem. 8, 18–22.

Mathew, M., Das Gupta, V., 1995. Stability of vancomycin hydrochloride solutions at various pH values as determined by high-performance liquid chromatography. Drug Dev. Ind. Pharm. 21, 257–264.

Sugarman, B., Pesanti, E., 1980. Treatment failures secondary to in vivo development of drug resistance by microorganisms. Rev. Infect. Dis. 2, 153–168.