Table VII—Pharmacokinetic Model Parameters Derived from Fitting Multiple-Dose Phenytoin Data Assuming First-Order Absorption and Simultaneous Michaelis–Menten and First-Order Elimination

	K_m[a], mg/liter	V_m[b], mg/hr	V[c], liters	Cl[d], liters/hr	k_a[e], hr^{-1}
Computer estimate	20.7	0.605	72.7	7×10^{-4}	0.320
SD of estimate	5.6	0.119	2.5	4×10^{-4}	0.072

[a] Michaelis constant. [b] Maximum elimination rate. [c] Apparent volume of distribution. [d] First-order clearance. [e] First-order absorption rate constant.

tetracycline in 10 subjects increased from 6.3 hr (after the first dose) to 10 hr (after the eighth dose) during 4 days of repetitive oral dosing (every 12 hr). Although at least one blood sample was taken during each dosing interval, pharmacokinetic analysis was limited to the data obtained from the first and last doses.

The average serum concentration–time data from this study were fit using Program III, which accommodates dose-to-dose variation in the first-order elimination rate constant. The computer fit of the data is shown in Fig. 2. The correlation coefficient was 0.997. The parameter estimates are listed in Table VIII.

A substantial change in the elimination rate constant is evident when the first dose is compared with each subsequent dose. The elimination rate constants for Doses 2–8 tend to fluctuate about a mean value of 0.078 hr^{-1} ($t_{1/2} \simeq 9$ hr), which is much smaller than the value estimated after the first dose.

Table VIII—Pharmacokinetic Model Parameters Derived from Fitting Multiple-Dose Tetracycline Data Assuming Constant First-Order Absorption and Variable First-Order Elimination

Parameter	Computer Estimate	*SD* of Estimate
k_a[a]	0.563	0.046
V/F[b]	191.1	4.6
$K_{(1)}$[c]	0.164	0.010
$K_{(2)}$	0.083	0.007
$K_{(3)}$	0.086	0.014
$K_{(4)}$	0.066	0.007
$K_{(5)}$	0.061	0.010
$K_{(6)}$	0.089	0.006
$K_{(7)}$	0.099	0.005
$K_{(8)}$	0.061	0.003

[a] First-order absorption rate constant ($hours^{-1}$). [b] Ratio of the apparent volume of distribution, V, to the fraction, F, of the dose absorbed (liters). [c] The term $K_{(n)}$ denotes the first-order elimination rate constant after the nth dose ($hours^{-1}$).

The reason for this apparent change in half-life remains obscure. Whether it is due to a real change in the pharmacokinetics of the drug upon repetitive dosing or to a mathematical artifact that arises from selecting the wrong pharmacokinetic model (9) is not known. The present analysis suggests that if an inhibitory mechanism is operative, it is fully induced after the first dose.

The described analyses of hypothetical and experimental multiple-dose data further support the utility and flexibility of the recently reported digital computer-fitting method (1).

REFERENCES

(1) W. A. Colburn, D. Shen, and M. Gibaldi, *J. Pharmacokinet. Biopharm.*, **4**, 469 (1976).

(2) P. L. Morselli and A. Frigerio, *Drug Metab. Rev.*, **4**, 97 (1975).

(3) R. M. Welch, Y. E. Harrison, and J. J. Burns, *Toxicol. Appl. Pharmacol.*, **10**, 340 (1967).

(4) P. S. Misra, A. Lefevre, H. Ishii, E. Rubin, and C. S. Lieber, *Am. J. Med.*, **51**, 346 (1971).

(5) B. Solymoss, S. Toth, S. Varga, and M. Krajny, *Steroids*, **16**, 263 (1970).

(6) V. Nitti, F. Delli Veneri, A. Ninni, and G. Meola, *Chemotherapy*, **17**, 121 (1972).

(7) R. Sellman, J. Kanto, E. Raijola, and A. Pekkarinen, *Acta Pharmacol. Toxicol.*, **37**, 345 (1975).

(8) J. T. Doluisio and L. W. Dittert, *Clin. Pharmacol. Ther.*, **10**, 690 (1969).

(9) M. Gibaldi and H. Weintraub, *J. Pharm. Sci.*, **60**, 624 (1971).

(10) C. M. Metzler, Technical Report 7292/69/7292/005, The Upjohn Co., Kalamazoo, Mich.

(11) J. Christiansen, *Numer. Math.*, **14**, 317 (1970).

(12) A. J. Glazko, in "Bioavailability of Drugs," B. B. Brodie and W. M. Heller, Eds., S. Karger, New York, N.Y., 1972, p. 170.

(13) N. Gerber and J. G. Wagner, *Res. Commun. Chem. Pathol. Pharmacol.*, **3**, 455 (1972).

(14) G. E. Mawer, P. W. Mullen, M. Rodgers, A. J. Robins, and S. B. Lucas, *Br. J. Clin. Pharmacol.*, **1**, 163 (1974).

(15) L. K. Garrettson and W. J. Jusko, *Clin. Pharmacol. Ther.*, **17**, 481 (1975).

ACKNOWLEDGMENTS AND ADDRESSES

Received March 25, 1976, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214.*

Accepted for publication May 27, 1976.

Supported in part by Grant GM-20852 from the National Institute of General Medical Sciences, National Institutes of Health.

[x] To whom inquiries should be directed.

Metal-Ion Interaction with Penicillins: Kinetics of Complexation of Nickel(II)

G. V. FAZAKERLEY [x] and G. E. JACKSON

Abstract □ The kinetics of complexation of nickel(II) with some penicillins and related compounds show that the zwitterionic form of the ligand has very low reactivity compared to the anionic form. The resolved rate constants are interpreted in terms of binding to the ring nitrogen and carboxyl group and not to the side chain.

Keyphrases □ Penicillin G—and related compounds, complexation with nickel(II) ions, reaction kinetics □ Nickel(II)—complexation with penicillin G and related compounds, reaction kinetics □ Complex formation—penicillin G and related compounds with nickel(II) ions, reaction kinetics □ Kinetics, reaction—complexation of nickel(II) with penicillin G and related compounds □ Metal ions—nickel(II), complexation with penicillin G and related compounds, reaction kinetics □ Antibacterials—penicillin G and related compounds, complexation with nickel(II) ions, reaction kinetics

Penicillin antibiotics interact with metal ions in a complex manner. Copper(II) has a very pronounced effect on the hydrolysis rate of the β-lactam ring (1, 2), which is not shown by other first-row transition metal ions (3, 4).

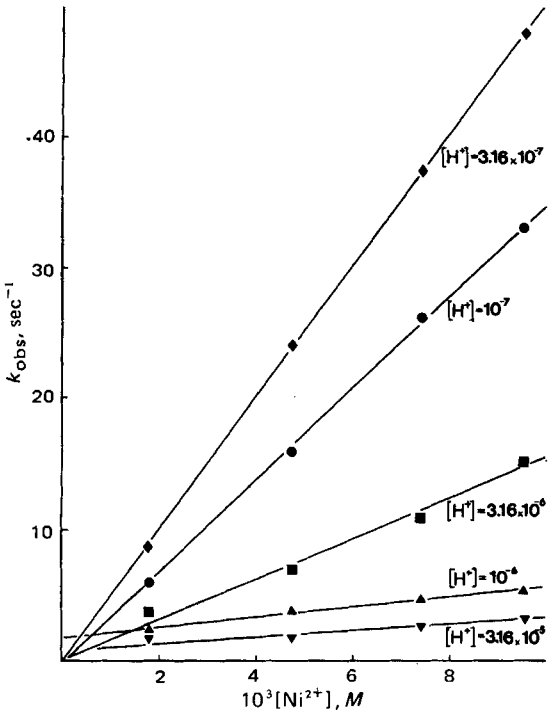

Figure 1—*Plot of* k_{obs} versus $[Ni^{2+}]$ *at constant* $[H^+]$ *for penicilloic acid.*

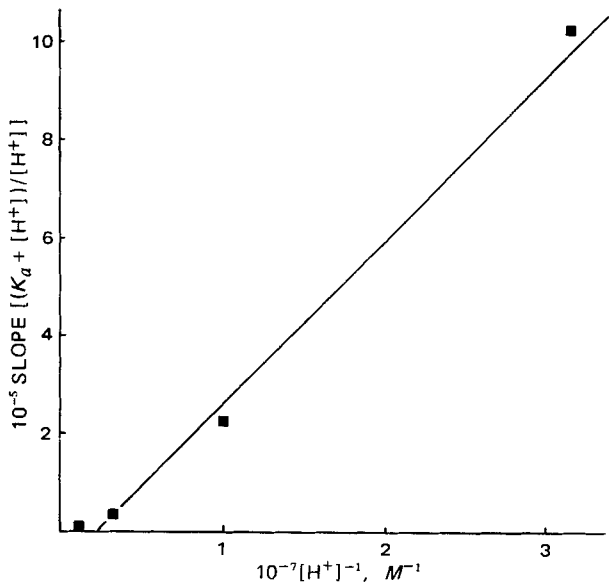

Figure 2—*Plot of slope* $(K_a + [H^+])/[H^+]$ versus $1/[H^+]$ *for penicilloic acid.*

Previous kinetic studies dealt exclusively with the hydrolysis rate in the presence of metal ions rather than with the complexation reaction itself. The differing effects of metal ions have been explained in terms of two possible binding sites (1). This paper describes the complexation reaction kinetics of nickel(II) with penicillin G and some related compounds.

EXPERIMENTAL

Penicillin G sodium, ampicillin, and thiaproline were commercial products used without further purification. Penicilloic acid was prepared by the method of Rapson and Bird (5). Nickel(II) perchlorate was prepared from nickel(II) sulfate and sodium bicarbonate by treating the nickel bicarbonate produced with perchloric acid. Excess nickel bicarbonate was filtered off, and nickel perchlorate was crystallized. Solutions of nickel perchlorate were standardized by adding a known concentration of edetate and back titrating[1] with standard magnesium sulfate.

Reactions were followed at 220 nm for thiaproline and penicillin G, 225 nm for ampicillin, and 250 nm for penicilloic acid on a stopped-flow spectrophotometer at 298 ± 0.5° K. The ionic strength was adjusted to 0.15 *M* with sodium perchlorate, and a 0.02 *M* sodium borate–mannitol buffer was used to maintain the pH.

The pH measurements were made using a glass–calomel electrode in which the potassium chloride had been replaced by sodium chloride. The meter was calibrated at pH 4 and 7. Reactions were carried out in the following pH regions: penicillin G, pH 6.1–7.8; thiaproline, pH 4.5–6.0; ampicillin, pH 6.7–7.4; and penicilloic acid, pH 4.5–6.5.

Ligand concentrations were 10^{-4} *M*. Reactions were run under pseudo-first-order conditions with nickel(II) concentrations in 10–100-fold excess to ensure that only 1:1 species were formed. Oscilloscope traces yielded excellent first-order rate constants, linear for at least 90% completion of the reaction. Value of k_{obs} used in evaluating further parameters were an average of three to five individual determinations.

RESULTS AND DISCUSSION

Penicilloic Acid, Ampicillin, and Thiaproline—All three ligands showed the same behavior in that the observed rate of reaction, k_{obs}, increased markedly with increasing pH at constant nickel(II) concentration. Figure 1 shows the effect of pH upon the reaction rate with penicilloic acid. The reactions showed the same characteristics as those of nickel(II) with amino acids (6) and were accounted for by the increasing concentration of the deprotonated zwitterion at high pH.

The reactions are shown in Scheme I, where L is the singly charged anion for thiaproline and ampicillin and the doubly charged anion for penicilloic acid. The pKa's for thiaproline, penicilloic acid, and ampicillin are 6.1 (7), 5.19 (7), and 7.25 (5), respectively.

$$Ni^{2+} + L \xrightarrow{k_1} [NiL]^{2+}$$

$$Ni^{2+} + LH^+ \xrightarrow{k_2} [NiL]^{2+} + H^+$$

$$LH^+ \overset{K_a}{\rightleftharpoons} L + H^+$$

Scheme I

The usual rate equation (6) is:

$$k_{obs} = \frac{k_1K_a + k_2[H^+]}{K_a + [H^+]}[Ni^{2+}] \qquad \text{(Eq. 1)}$$

Thus, the slopes in Fig. 1 are equal to $(k_2[H^+] + k_1K_a)/(K_a + [H^+])$. Since the intercepts are not experimentally different from zero, the dissociation reactions can be ignored.

Rearranging Eq. 1 gives:

$$\text{slope}\frac{(K_a + [H^+])}{[H^+]} = \frac{k_2 + k_1K_a}{[H^+]} \qquad \text{(Eq. 2)}$$

Figure 2 shows a graph of the left-hand side of Eq. 2 against $1/[H^+]$ for penicilloic acid. The slope is k_1K_a, and the intercept is k_2. However, the intercepts are not experimentally different from zero, so only the overall forward rate constant, k_f, is determined (Table I).

The unreactivity of the protonated species has been explained (6) in

Table I—Rate Constants for the Formation (k_f) and Dissociation (k_d) of Nickel(II) Complexes at 25° and Ionic Strength 0.15 *M*

Ligand	Reacting Form of Ligand	k_f, M^{-1} sec^{-1}	k_d, sec^{-1}
Thiaproline	L^-	7.5×10^2	0.15[a]
Penicilloic acid	L^{2-}	5.2×10^3	4.3[a]
Ampicillin	L^-	6.9×10^2	—
Penicillin G	L^-	2.3×10^3	19.3

[a] Calculated using $k_d = k_f/K$ (9).

[1] Using Erichrome Black T as indicator.

terms of an extreme sterically controlled substitution (SCS) mechanism, since, upon protonation, first-bond formation must switch from the amino group to the carboxylic group. The rate of ring closure is comparable with, or smaller than, the rate at which the first bond is broken.

Penicillin G—The reaction was again found to be first order with respect to ligand concentration, the metal being in at least 10-fold excess. It was also first order with respect to the metal-ion concentration observed in the linearity of the k_{obs} *versus* $[Ni^{2+}]$ graph. In the region of study, pH 6.1–7.8, k_{obs} was independent of $[H^+]$. This finding was expected because the reactions were carried out well above the pKa (2.73) and, thus, in the absence of the protonated species.

The reaction shown in Scheme II:

$$[Ni^{2+}] + L^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [NiL]^+$$

Scheme II

gives the rate equation (8):

$$k_{obs} = k_1[Ni^{2+}] + k_{-1} \qquad \text{(Eq. 3)}$$

The forward and dissociation rate constants are shown in Table I.

At lower pH values, there was no observable reaction.

The rate constant for thiaproline was much lower than that expected for "normal" substitution (9). There are two possible explanations for this finding: a shift to another rate-determining step (an SCS mechanism) occurs or hydrogen bonding between a water molecule in the metal-ion coordination sphere and the sulfur atom disorients the ligand for attack on the metal. The value of k_f was an order of magnitude less than for proline and hydroxyproline (9). Some reduction in k_f was observed with hydroxyproline relative to proline and was attributed to a hydrogen bonding mechanism. However, the large drop with thiaproline cannot be explained in this way because hydrogen bonding through sulfur is weaker than that through oxygen. Thus, a shift to another rate-determining step is postulated.

The inductive effect of sulfur is known (10) to reduce the basicity of nitrogen. This effect increases the rate at which the first bond formed is broken. This result is shown by the dissociative rate constant (0.15), which was higher than that for proline (0.024) and hydroxyproline (0.014).

The forward rate constant for penicillin G was also lower than that for normal substitution mechanisms but higher than that for thiaproline. If reaction occurs at the ring nitrogen and carboxyl group, then, by analogy with thiaproline, an SCS mechanism will lower the rate. The increase in rate over thiaproline can be explained by hydrogen bonding of the β-lactam carbonyl to water around the metal ion, which promotes the correct orientation of the ligand for attack in an internal conjugate base type of mechanism (11).

The alternative site, β-lactam carbonyl and side-chain amide nitrogen, would react as a neutral species for which the normal substitution rate is about 3×10^3, in good agreement with the observed rate. However, this normal value was obtained for strong nitrogen chelates. If comparison is made with acetylacetonate in the enol form, a value of 5.0 is found (12) because an SCS mechanism is operative. From this discussion, a similar mechanism seems to be operative, suggesting a forward rate constant much lower than 10^3 for coordination at the side chain. For this reason, and by comparison with penicilloic acid, the former postulate is favored.

The reaction rate with penicilloic acid was 5.2×10^3. A potentiometric study (7) indicated that penicilloic acid acts as a bidentate ligand, with one carboxyl group not being involved in coordination. The lower than normal rate is attributed to the nitrogen basicity.

The reactions with ampicillin are known (13) to involve coordination only at the side chain through the amino and carbonyl groups. As an effectively neutral donor involving no ring strain, a forward rate constant of $3–4 \times 10^3$ is expected. Since the pKa of the amino group is 7.25, an SCS mechanism is not likely. The decreased reactivity must be due to hydrogen bonding.

The two most likely coordination metal sites on penicillin are: A, the β-lactam carbonyl and the side-chain amide nitrogen; and B, the carboxyl group and the ring nitrogen. Structure A has been used to explain the rapid hydrolysis rate in the presence of certain metal ions of penicillin G compared to penicillanic acid (2). This interpretation is suspect because the hydrolysis rate of acylamino-substituted β-lactams is known to be several orders of magnitude faster than that of unsubstituted β-lactams in the absence of metal ions (14). Structure B is supported by potentiometric studies of nickel(II) with *N*-benzylpipecolic acid and *N*-hippurylpipecolic acid (15). The two complexes have similar stability constants, although one has the penicillin G side chain and the other does not.

Amide nitrogens are poor donors because binding results in a loss of the amide resonance stabilization energy through change in hybridization from sp^2 to sp^3. The ring nitrogen of penicillin G is already much distorted from an sp^2 planar structure. The nitrogen lies 0.4 Å out of the plane and close to the position (0.56 Å) expected for sp^3 (16). Consequently, the amide resonance is already largely lost even before coordination, making this nitrogen a better donor.

Furthermore, molecular models show that whereas it is possible to accommodate a metal ion in Structure B with normal metal ion–ligand bond lengths, a very long (~3.4 Å) and consequently weak metal nitrogen bond would result from Structure A.

From these results and those from potentiometry (7) and NMR (13) studies, it is clear that the dominant binding site for penicillin G is at the ring nitrogen and carboxyl group. In itself, this finding does not explain the different hydrolysis rates in the presence of different metal ions. This result must arise from the nature of the binding rather than a shift in the site of binding. Copper(II) will form a stronger bond to the ring nitrogen than nickel(II). The stronger tendency of copper(II) to remove electrons from the nitrogen will weaken the β-lactam ring and promote hydrolysis.

REFERENCES

(1) W. A. Cressman, E. T. Sugita, J. T. Doluisio, and P. J. Niebergall, *J. Pharm. Pharmacol.*, **18**, 801 (1966).

(2) W. A. Cressman, E. T. Sugita, J. T. Doluisio, and P. J. Niebergall, *J. Pharm. Sci.*, **58**, 1471 (1969).

(3) G. Gunther, *Pharmazie*, **5**, 577 (1950).

(4) J. Johnson, H. Clarke, and R. Robinson, "The Chemistry of Penicillin," Princeton University Press, Princeton, N.J., p. 14.

(5) H. Rapson and A. Bird, *J. Pharm. Pharmacol., Suppl.*, **15**, 222T (1963).

(6) J. C. Cassatt and R. G. Wilkens, *J. Am. Chem. Soc.*, **90**, 6045 (1968).

(7) G. V. Fazakerley, G. E. Jackson, and P. W. Linder, *J. Inorg. Nucl. Chem.*, **38**, 1397 (1976).

(8) F. Basolo and R. G. Pearson, "Mechanics of Inorganic Reactions," 2nd ed., Wiley, New York, N.Y., 1967.

(9) K. Kustin and S. Liu, *J. Chem. Soc. Dalton*, **1973**, 278.

(10) J. Johnson, H. Clarke, and R. Robinson, "The Chemistry of Penicillin," Princeton University Press, Princeton, N.J., p. 417.

(11) D. B. Rarabacher, *Inorg. Chem.*, **5**, 1891 (1966).

(12) R. Pearson and D. Anderson, *ibid.*, **9**, 39 (1970).

(13) G. V. Fazakerley and G. E. Jackson, *J. Inorg. Nucl. Chem.*, **37**, 2371 (1975).

(14) J. Johnson, H. Clarke, and R. Robinson, "The Chemistry of Penicillin," Princeton University Press, Princeton, N.J., p. 978.

(15) A. Weiss and S. Fallab, *Helv. Chim. Acta*, **40**, 611 (1962).

(16) R. Sweet and L. Dahl, *J. Am. Chem. Soc.*, **92**, 5489 (1970).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 24, 1975, from the *Department of Inorganic Chemistry, University of Cape Town, Rondebosch, 7700, South Africa.*

Accepted for publication May 26, 1976.

The authors thank the University of Cape Town and the Council for Scientific and Industrial Research for financial support and for an AE&CI fellowship (to G. E. Jackson).

[x] To whom inquiries should be directed.