Substituent Constant for Drug Design Studies Based on Properties of Organic Electron Donor–Acceptor Complexes

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Received April 11, 1977, from the **Chemistry Department, University of Dundee, Dundee, Scotland,* and the ‡*Wellcome Research Laboratories, Beckenham, Kent, England.* Accepted for publication January 17, 1978.

Abstract □ A new model chemical system based on organic electron donor–acceptor complexes is described. From values of equilibrium constants measured by an NMR technique, a predictable parameter for use in quantitative structure–activity relationship techniques is discussed.

Keyphrases □ Electron donor–acceptor complexes—as model system for structure–activity relationships □ Complexes, electron donor–acceptor—as model system for structure–activity relationships □ Structure–activity relationships—model system based on electron donor–acceptor complexes □ Models, chemical—for structure–activity relationships based on electron donor–acceptor complexes

In quantitative structure–activity relationships, a regression equation relating biological activity to chosen physicochemical properties is derived for a test set of compounds (1). If the properties are predictable, the regression equation may be used to predict the activities of other analogs prior to their synthesis. Physicochemical properties can best be predicted for substitution in the benzene ring, and the availability of accurately measurable substituent constants is crucial to the prediction of biological activity in these circumstances.

BACKGROUND

The approach can be described as semiempirical, and biological activity may be predicted when no mechanistic knowledge of the biosystem is available. Hence, a regression equation should never in itself be regarded as evidence for the involvement of a particular mechanistic step.

Nevertheless, it is desirable that the physicochemical model systems on which the substituent constants are based should be chosen for their relevance to biological systems. The most commonly used substituent constants (2, 3) are π (4), σ (5), F (6), R (6), and the bulk constant R_D (7). Although other model systems have often been used to provide information for predictive equations, they are rarely as useful as π and σ. In the present work, a model system based on the weak intermolecular interactions between organic electron donors and electron acceptors is proposed, and its potential uses are discussed.

Complexes of this type are often referred to as charge transfer complexes (8–10) because of a commonly observed property, *i.e.*, an electronic absorption attributable to an intermolecular charge transfer transition. However, charge transfer forces are only one component of the total molecular interaction in the ground state, and it is this total interaction that is measured and is of interest as a possible model system.

This type of interaction in biosystems (*vide infra*) has received considerable interest (11–13), and it is now proposed that a substituent constant should be defined as a measure of the capacity of a group to modify the stability of an electron donor–acceptor complex. To achieve this aim, it was necessary to choose a suitable model system, and the formation of complexes between a common acceptor and a series of monosubstituted benzenes was selected for this purpose.

The acceptor chosen was 1,3,5-trinitrobenzene (I), a sufficiently good electron acceptor to give measurable equilibrium constants with most monosubstituted benzenes. An aprotic solvent, carbon tetrachloride, was chosen to minimize undesirable solute–solvent hydrogen bonding effects. Such equilibrium constants can be determined by an NMR technique.

The model system should provide substituent constants applicable to a wide variety of environments. Some data demonstrating the effect of changing the common acceptor or the solvent are given under *Results*.

With this model system, a substituent constant, κ, is defined as:

$$\kappa = \log K_X - \log K_H \qquad \text{(Eq. 1)}$$

where K_X is the equilibrium constant for the formation of a complex between I and an x-substituted benzene and K_H is the equilibrium constant for the formation of a complex between I and benzene. The assumption has usually been made that equilibria of this type involve the formation of a 1:1 complex as shown in Scheme I:

$$A + D \rightleftharpoons AD$$

Scheme I

for which an equilibrium constant K_1 may be defined as:

$$K_1 = \frac{[AD]}{[A][D]} = \frac{[AD]}{([A]_0 - [AD])([D]_0 - [AD])} \qquad \text{(Eq. 2)}$$

where concentration terms with a subscript zero represent the total (free and complexed) concentration of that particular component. However, under the normal experimental conditions—*viz.*, $[D]_0 \gg [A]_0$, at least for π-donor–π^*-acceptor interactions the overall equilibrium may be better represented by assuming that not only a 1:1 complex but also a termolecular complex, AD_2, is formed. It may be written as shown in Scheme II:

$$AD + D \rightleftharpoons AD_2$$

Scheme II

and occur in addition to the first equilibrium (Scheme I). This second equilibrium constant, K_2, may be defined as:

$$K_2 = \frac{[AD_2]}{[AD][D]} \qquad \text{(Eq. 3)}$$

If this second equilibrium is ignored and the whole system is treated as if only the first equilibrium occurs, then experimental data yield a value for the association constant that is not equal to K_1. This value is termed the apparent association constant, K_{app}. There is little doubt that many literature values claimed to be K_1 values are really K_{app} values. Thus, Eq. 1 should be more correctly written as:

$$\kappa = \log_{10} K_{app,x} - \log_{10} K_{app,H} \qquad \text{(Eq. 4)}$$

If the difference between the chemical shift of the proton singlet absorption of I in a solution where the donor concentration is $[D]_0$ and the chemical shift of I in a solution containing no donor is Δ, it may be shown that (14):

$$\Delta/[D]_0 = -K_{app}\Delta + K_{app}\Delta_0 \qquad \text{(Eq. 5)}$$

where Δ_0 is the chemical shift of the measured nucleus that the complex would have in solution, if there were no dissociation, relative to the chemical shift of the same nucleus in completely uncomplexed I in solution.

A plot of $\Delta/[D]_0$ *versus* Δ gives a straight line of gradient $-K_{app}$ and an intercept with the ordinate of $(K_{app}\Delta_0)$, from which K_{app} and Δ_0 may be evaluated. In practice, a least-squares computer program is used to obtain these two parameters.

The determination of K_{app} for complexes formed between electron donors and the acceptor 2,3,5,6-tetrafluoro-*p*-benzoquinone (II) may be similarly determined from observations of the chemical shift of the ^{19}F-singlet absorption of II.

EXPERIMENTAL

The values of K_{app} were obtained from measurements of the variation in the line positions of a nuclear magnetic resonance in the acceptor as

0022-3549/78/0900-1310$01.00/0

Table I—Benzene Concentrations ($[D]_0$) and Chemical Shift Differences (Δ) of I

$[D]_0 \times 10^2$, moles/kg	Δ, Hz	$\Delta/[D]_0$, Hz kg/mole
4.00	2.3	57.5
11.76	6.5	55.27
24.98	13.1	52.24
49.18	23.0	46.77
57.47	25.9	45.07
33.57	17.0	50.64
18.75	10.0	53.33
40.60	19.6	48.28
	$K_{app} = 0.53 \pm 0.06$	
	$\Delta_0 = 112 \pm 12$	

Table II—Benzene Concentrations ($[D]_0$) and Chemical Shift Differences (Δ) of I

$[D]_0 \times 10^2$, moles/kg	Δ, Hz	$\Delta/[D]_0$, Hz kg/mole
8.11	4.6	56.72
21.78	11.5	52.80
43.19	20.7	47.93
30.22	15.2	50.30
38.78	18.8	48.48
17.59	9.5	54.01
56.80	25.7	45.25
	$K_{app} = 0.54 \pm 0.06$	
	$\Delta_0 = 109 \pm 11$	

the concentration of the electron donor was varied under the conditions $[D]_0 \gg [A]_0$.

Solutions of different donor concentrations (~0.05–0.8 *M*), but of approximately constant acceptor concentration (~10^{-3} *M*), were prepared gravimetrically using a stock solution of acceptor, with all concentrations of donor being expressed in moles per kilogram. The carbon tetrachloride solutions also contained 2% (v/v) tetramethylsilane. Measurements were made at 90.0000 MHz for the ^{1}H-resonances and at 63.5305 MHz for the ^{19}F-resonances.

The line positions of the ^{1}H-resonance of I and the ^{19}F-resonance of II were measured four times with respect to the internal reference for each solution. Where the solvent was carbon tetrachloride, tetramethylsilane was used as the internal reference; for cyclohexane solutions, the ^{1}H-resonance of the solvent itself was used. The arithmetic mean of the measurements of the line position for a given solution was taken; in all cases, the individual line positions were within ±0.1 Hz of this mean.

Under the conditions of these experiments, the values of K_{app} were independent of the particular internal reference used (15).

All measurements were made at 33.4 ± 0.1°. This temperature was maintained by a built-in temperature control and checked before and after each set of measurements with an external copper–constantan thermocouple in a dummy tube. At least two separate determinations of K_{app} were made for each system. Tables I and II contain typical data for two such determinations for the benzene–I system.

RESULTS

Tables I and II show data and the computed parameters for two separate determinations of K_{app} for the benzene–I system in carbon tetrachloride at 33.4°. The combined data gave the following values of K_{app} and Δ_0 by least-squares treatment: $K_{app} = 0.53 \pm 0.03$ kg/mole and $\Delta_0 = 111 \pm 7$ Hz.

In Fig. 1, the equilibrium constants, K_{app}, for complex formation between I and a set of monosubstituted benzenes are compared with constants for complex formation between II and the same monosubstituted benzenes in the same solvent. The line shown is the least-squares fit straight line ($r = 0.97$). A comparison of equilibrium constants for interactions between I and a set of monosubstituted benzenes in carbon tetrachloride with the corresponding constants for the interactions in cyclohexane is shown in Fig. 2. The least-squares fit straight line has a correlation coefficient of 0.99.

Table III shows the values of K_{app}, $\log_{10}K_{app}$, and κ obtained for 26 monosubstituted benzenes in the chosen model system. Table IV shows the results of a comparison of κ with four commonly used substituent constants.

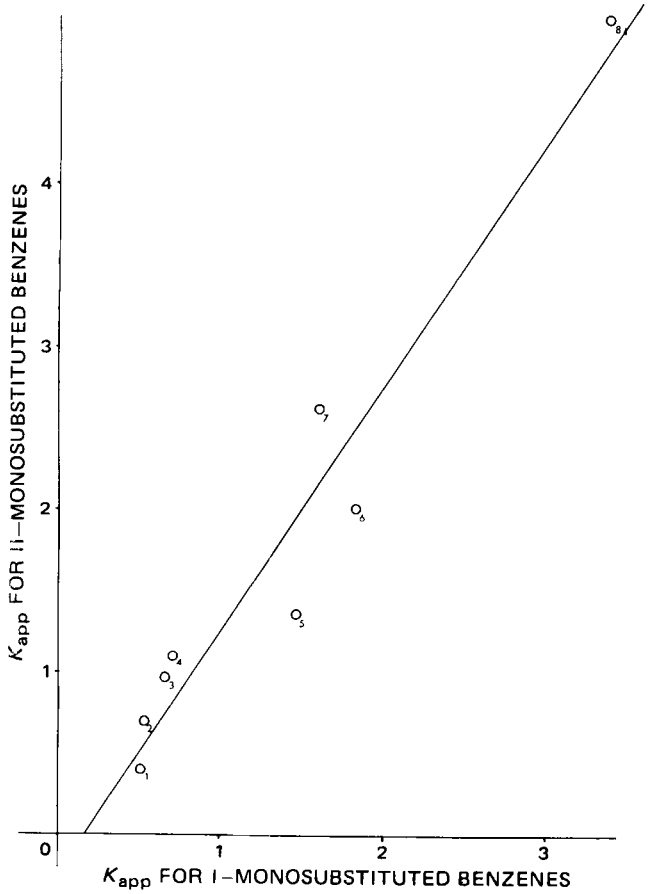

Figure 1—*Correlation between II complexes and the corresponding I complexes with the following monosubstituted benzenes: 1, Cl; 2, H; 3, CH_3; 4, CH_2CH_3; 5, OCH_3; 6, $COOCH_2CH_3$; 7, $COCH_3$; and 8, $N(CH_2CH_3)_2$.*

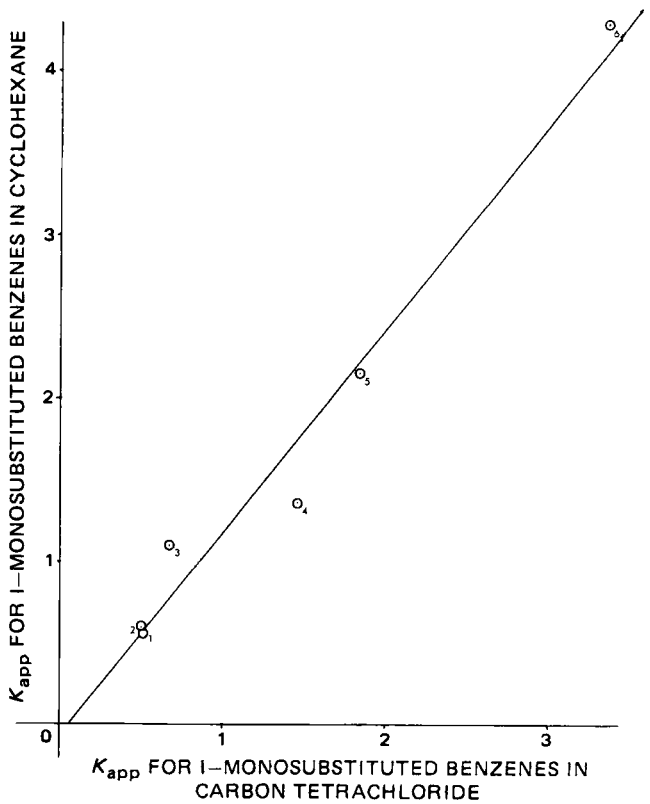

Figure 2—*Correlation between I complexes in cyclohexane and the corresponding complexes in carbon tetrachloride with the following monosubstituted benzenes: 1, Cl; 2, H; 3, CH_3; 4, OCH_3; 5, $COOCH_2CH_3$; and 6, $N(CH_2CH_3)_2$.*

Table III—Measured Association Constants (Moles per Kilogram) for I Complexes of Monosubstituted Benzene Donors in Carbon Tetrachloride at 33.5°

Substituent	K_{app}	$Log_{10}K_{app}$	κ
H[a]	0.53	−0.28	0.00
CH_3[a]	0.67	−0.17	0.11
CH_2CH_3[b]	0.71	−0.15	0.13
$CH_2CH_2CH_3$[b]	0.58	−0.24	0.04
$CH(CH_3)_2$[b]	0.61	−0.22	0.06
CF_3[c]	0.43	−0.37	−0.09
OCH_3[e]	1.46	0.16	0.44
NH_2[d]	2.38	0.38	0.66
NO_2[c]	0.96	−0.02	0.26
Br[e]	0.53	−0.28	0.00
CN[c]	0.90	−0.05	0.23
$CH_2CH_2CH_2CH_3$[b]	0.62	−0.21	0.07
$C(CH_3)_3$[b]	0.45	−0.35	−0.07
C_6H_5[e]	1.48	0.17	0.45
OH[e]	1.31	0.12	0.40
OCH_2CH_3[e]	1.25	0.10	0.38
CHO[e]	1.14	0.06	0.34
$COCH_3$[e]	1.60	0.20	0.48
$COOCH_3$[e]	1.65	0.22	0.50
$COOCH_2CH_3$[e]	1.83	0.26	0.54
$NHCH_3$[d]	2.80	0.45	0.73
$NHCH_2CH_3$[d]	3.20	0.51	0.79
SCH_3[e]	1.31	0.12	0.40
F[e]	0.36	−0.44	−0.16
Cl[e]	0.51	−0.29	−0.01
I[e]	0.54	−0.27	−0.01

[a] From Ref. 14. [b] From Ref. 16. [c] From Ref. 17. [d] From Ref. 18. [e] Present work.

DISCUSSION

It is apparent from the high linear correlation shown in Fig. 1 that the relative values of equilibrium constants of donors are independent of the choice between acceptor I and acceptor II. Likewise, Fig. 2 indicates that the relative values of the equilibrium constants are independent of the particular "inert" solvent used. Thus, it is possible to change both solvent and common acceptor while still retaining a substituent constant well correlated with the original. This finding suggests that the values of κ do not reflect undesirable specific solvent–solute or donor–acceptor interactions to an unacceptable extent.

In addition to the validity of the model system, a substituent constant intended for use in quantitative structure–activity relationships should be judged by four criteria prior to its adoption. These criteria are: plausibility, predictability, availability, and orthogonality. The substituent constant κ is now considered in the light of these criteria.

Plausibility—The involvement of electron donor–acceptor interactions in molecular processes of relevance to the biophase was suggested previously (11, 12, 19). In the work of Cilento and Tedeschi (20), for example, on the system glyceraldehyde 3-phosphate dehydrogenase–NAD, absorptions assignable to charge transfer transitions were observed in the UV spectrum. There is also evidence of the relevance of electron donor–acceptor interactions to some processes involving biological macromolecules and exogenous species (*i.e.*, drug molecules). Hetnarski and O'Brien (21) postulated the involvement of electron donor–acceptor interactions in the action of some arylmethyl carbamates as inhibitors of cholinesterase. Measurements of the UV spectra of the compounds themselves provided results consistent with this theory.

Large numbers of naturally occurring moieties exist whose properties suggest their suitability for participation in electron donor–acceptor complexes. Of the protein residues alone, potential electron donors are aspartate, glutamate, cystine, methionine, and tyrosine; potential electron acceptors are cysteine, arginine, and lysine. Other groups such as histidine, asparaginine, and tryptophan may act as both electron donors and acceptors.

Interactions between a drug molecule and any of these species could, in principle, be involved in either the formation of a drug–receptor complex or drug transport to the site of action. Although the possibility of such a direct relationship between molecular processes in the biosystem and the physicochemical model is desirable, it should not be a *sine qua non* in assessing plausibility. It would have been sufficient to justify an electron donor–acceptor parameter if it could merely be regarded as a "probe" for more fundamental properties, *i.e.*, if it could be postulated that fundamental properties contributing to log $K_{app}(\kappa)$ might also be of relevance to biological molecular interactions.

In both respects, there are grounds to regard κ as a highly plausible substituent constant for quantitative structure–activity relationship studies.

Table IV—Results of Regression Analysis: Correlation Matrix for the Parameter Set for the 26 Substituents in Table III[a]

	F	R_D	R
R_D	−0.37		
R	0.34	0.05	
π	−0.22	0.58	0.39

[a] The best equations of the analysis are:

$\kappa = -0.186\ (0.05)\pi + 0.34$
$r = 0.634 \quad F = 16.09$

$\kappa = -0.330\ (0.03)\pi + 0.037\ (0.004)R_D + 0.059$
$r = 0.935 \quad F = 79.68$

$\kappa = -0.323\ (0.03)\pi - 0.036\ (0.07)R$
$0.037\ (0.004)R_D + 0.054$
$r = 0.936 \quad F = 51.42$

Predictability—The κ values described are derived from monosubstituted benzenes. Their applicability to polysubstituted ring systems has not yet been assessed. Early results indicate that relative log K_{app} values for members of some congeneric series may be derivable from the original data base using simple additivity rules. This may be partly attributable to the fact that the π-electron system as a whole contributes to binding, whereas the effect of the substituent relative to a particular position in the ring is important for a quantity such as σ. The treatment of some diverse sets of electron donor–acceptor complex data by Charton (22–24) using linear free energy relationships is encouraging in this context.

Availability—Values of $\log_{10}K_{app}$ (hence κ) can be easily and accurately measured using an NMR spectrometer. Substituent constants based on 26 monosubstituted benzenes are quoted here, and work is being performed on polysubstituted benzenes.

Orthogonality—To assess whether a new parameter contributes anything extra to existing parameter sets, regression analyses must be performed. If the new parameter correlates with a single well-used parameter, then it clearly has little to offer. If it is completely orthogonal even to a linear combination of all of the parameters in a working set (3), then it can be used as an addition to the set. If, however, it is uncorrelated with any one parameter but correlated with a linear combination of two or more, then it might be considered as a replacement for the "two or more" in situations where a paucity of compounds in the test set limits the number of parameters.

A commonly used set of parameters is: π, derived from octanol–water partition coefficients (4); F and R, field and resonance components, respectively, of Hammett's σ constants (6); and R_D, the molar refraction (7), which is currently used as a bulk parameter.

Regression analysis was performed on the data for the substituent set in Table III to assess the orthogonality of κ with respect to π, F, R, and R_D (Table IV). For the set of 26 substituted benzenes studied, κ is well correlated with a linear combination of π and R_D.

The significance of this equation is such that κ may be said to contribute little extra information to the linear combination of π and R_D for this data set. Thus, in general, κ would not be used as an addition to the above-mentioned four-parameter set unless a correlation matrix for a real subset of compounds indicated the contrary. However, for situations where the number of parameters that may be used is restricted by statistical considerations, κ might be considered as a replacement for π and R_D. Thus, κ–σ regressions may be of value in these circumstances.

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ACKNOWLEDGMENTS

The authors thank Dr. P. J. Goodford for advice and criticism and Mr. J. A. Chudek for assistance.

Interactions between Polymyxin B and Divalent Nickel in Near-Neutral Aqueous Media

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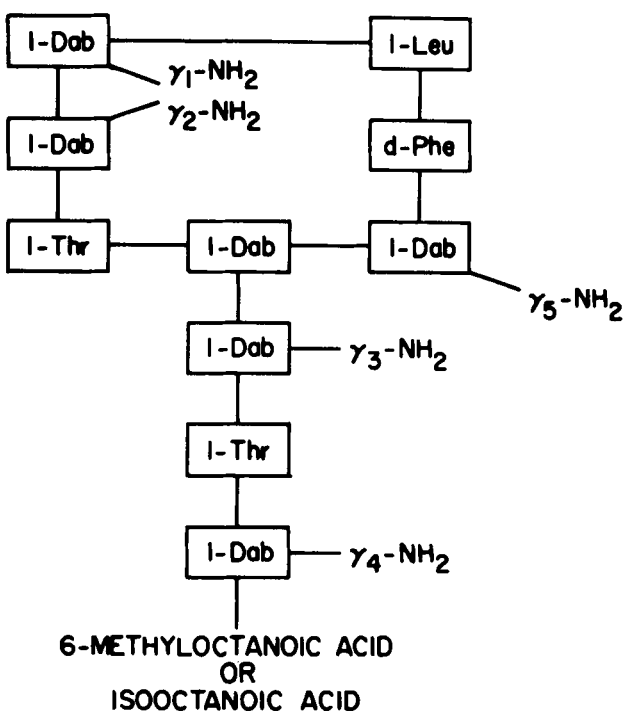
Received September 12, 1977, from the *Department of Chemistry, Brandeis University, Waltham, MA 02154.* Accepted for publication January 24, 1978.

Abstract □ Stopped-flow and temperature-jump methods were used to determine the complexation constants for the reactions of polymyxin B with Ni^{2+} at 25° and 0.1 *M* ionic strength [$N(CH_3)_4NO_3$]. At pH 6.4–7.1 and with the ligand in excess, the formation of a 1:1 metal–ligand complex according to the reaction scheme $Ni^{2+} + H_3L^{3+} \rightleftarrows NiH_3L^{5+}$ and $Ni^{2+} + H_4L^{4+} \rightleftarrows NiH_3L^{5+} + H^+$ (k_1 and k_1' are respective forward rate constants; k_{-1} and k_{-1}' are respective reverse rate constants) is consistent with the data. The determined rate constants are $k_1 = (3.8 \pm 0.2) \times 10^3$ M^{-1} sec^{-1} and $k_{-1} = (1.2 \pm 0.1) \times 10^{-1}$ sec^{-1}. Upper and lower limits for k_1' of 2.4 and 0.6 M^{-1} sec^{-1}, respectively, were set. The stability constant ($K_1 = k_1/k_{-1}$) of the 1:1 complex determined by potentiometric titration is $(3.3 \pm 0.1) \times 10^4$ *M*; the four pKa values of polymyxin B agree with the literature values. At pH 7.1–7.9 and with the metal ion in excess, the described scheme and a binuclear complex reaction step, $Ni^{2+} + NiHL^{3+} \rightleftarrows Ni_2HL^{5+}$ (k_2 is the forward rate constant; k_{-2} is the reverse rate constant), fit the data. Upper and lower limits for k_2 of 1×10^4 and 3×10^3 M^{-1} sec^{-1}, respectively, were set for the formation of the binuclear complex. Comparison with model system complex formation rate constants indicates that the forward rate constants evaluated in this study are enhanced. This effect may be interpreted in terms of an internal conjugate base mechanism.

Keyphrases □ Polymyxin B—complexation with nickel(II) in aqueous media, effect of pH and ligand concentration □ Nickel(II)—complexation with polymyxin B in aqueous media, effect of pH and ligand concentration □ Complexation—polymyxin B and nickel(II) in aqueous media, effect of pH and ligand concentration □ Antibacterials—polymyxin B, complexation with nickel(II) in aqueous media, effect of pH and ligand concentration □ Metals—nickel(II), complexation with polymyxin B in aqueous media, effect of pH and ligand concentration

Polymyxin B is a surface-active polypeptide antibiotic exhibiting potent bactericidal activity against most Gram-negative bacilli (1). The addition of polymyxin B to cell suspensions of these bacilli results in a leakage from the cells of pentose, phosphate, and substances with absorption maxima at 260 nm (1, 2). The mechanism of action is thought to involve combination of the antibiotic with charged groups on the cell membrane surface, resulting in disorganization of the membrane structure (1–3). The bactericidal activity of polymyxin B is influenced strongly by various metal ions in several complicated modes of interaction (1, 2).

The proposed structure of polymyxin B is shown in Structure I and Fig. 1 (4, 5). Polymyxin B is a cyclic hexapeptide with a tripeptide side chain *N*-acylated by 6-methyloctanoic acid (polymyxin B_1) or isooctanoic acid (polymyxin B_2); it is a mixture of B_1 and B_2 in the ratio 65:35, respectively (6). Each component has one D-amino



I: Amino acid sequence of polymyxin B. Key: l-Dab, *l*-α,γ-diaminobutyric acid; l-Thr, *l*-threonine; d-Phe, *d*-phenylalanine; and l-Leu, *l*-leucine.

0022-3549/78/0900-1313$01.00/0