Thermodynamic Considerations in Co-ordination. Part XX.[1] A Computerised Approach as an Alternative to Graphical Normalised Curve Fitting as a means of detecting Oligonuclear Complexes in Metal Ion–Ligand Solutions and its Application to the Zinc(II)–, Lead (II)–, and Proton–Glycine Peptide Systems

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A computer program, PSEUDOPLOT, is reported as an alternative to the normalised-curves approach for selecting a set of formation constants necessary to define a system of competing equilibria. The selection of constants to describe the zinc(II) and lead(II) complexes of glycinate, glycylglycinate, and glycylglycylglycinate are reported as examples of the use of PSEUDOPLOT. Protonated and hydroxo-complexes have been identified.

COMPLEX formation between metal ions and ligands which are conjugate bases of weak acids is conveniently researched using glass-electrode pH potentiometry. The premier aim of such work is to completely define the system in terms of formation constants (β) for all the metal–ligand–proton complexes present. The visual representation of patterns amongst the experimental data (in terms of curves on graphs), and the degree of precision with which the formation constants produced can be used to calculate theoretical curves (which ought to superimpose exactly upon the experimental data), are critical parameters involved in the use of potentiometry.

The subject has evolved through various levels of sophistication. Initially it was common to plot titrant (cm^3) added against pH for ligand–proton or ligand–metal ion titrations. Next, formation curves were plotted: $\bar{Z}_h$ (the average number of protons per ligand) against ph, or $\bar{Z}$ (the average number of ligands per metal ion) against pa (−log [free ligand]).[2,3] If solely mononuclear metal–ligand species are present $\bar{Z}$ against pa curves obtained for different values of A and B (the total concentrations of ligand and metal respectively) are superimposable; if hydroxo-, protonated, or bi-, tri-, or oligo-nuclear species exist these formation curves form a complicated pattern (*i.e.* they are definitely not superimposable). Although various plots have been used to simplify these patterns, for example, Österberg plotted $Y\ [= (H - h)/A]$ (where H = total mineral acid present and h = free acid concentration) against ph,[4] the selection of species to describe these curves has been left to normalised curve analysis.[2]

Normalised, or standardised, curves are projection maps of model functions calculated on the assumption that certain species coexist (as defined by a set of β values †) and these are then compared with plots of the experimental data covering a wide range of concentrations. In this way it is possible to discover which species are probably present (and the position of best fit reveals the relevant values of the stability constants). Alternative sets of βs ought always to be tested since it is not always possible to find a unique set to interpret the data at the first normalisation. Unfortunately, each set of βs necessitates calculation of a unique pattern of normalised curves, each pattern being based on different mathematical relations.[5] For example, for the simple system in which just AH, A_2H, and A_2H_2 are postulated, the normalised curves are calculated from the relation (1) where $R = (2\bar{Z}_h - 1)/2\bar{Z}_h$ and $\boldsymbol{A}$ and $\boldsymbol{h}$ are normalised

$$\boldsymbol{A} = \frac{[\boldsymbol{h} - (1 + \boldsymbol{h})\bar{Z}_h][\boldsymbol{h}(1 - 2R) - 1]}{\boldsymbol{h}[2\bar{Z}_h(1 + R\boldsymbol{h}) - (1 + 2R\boldsymbol{h})]^2} \quad (1)$$

variables corresponding to A and h.[6,7] These curves apply to a set of just three constants (β_{101}, β_{201}, and β_{202}) that do not involve either metal ions or hydroxo-species. When more than three βs are examined the mathematics and algebra become more complex.[2,8]

The normalised-curves approach, as yet, has not been bettered, but now that several least-squares computer programs are available [9,10] there is a tendency to move directly from titration data to computations of ' best ' βs without reference to graphs exhibiting patterns in the data. Such a practice can cause some complex species to be overlooked. This paper reports a computerised approach which graphically is equivalent to the normalised-curves method and, by using a common series of relations (as distinct from a different series of equations for each set of βs), a computer program PSEUDOPLOT, and a graph plotter, is much faster than the normalised-curve approach.

† Throughout the paper, the term ' set of βs ' refers to the list of formation constants that represent the several complexes present in solution. The formation constant for the general complex $A_pB_qH_r$ is β_{pqr} where A = ligand, B = metal ion, and H = proton.

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RESULTS

*The PSEUDOPLOT Program.**—It is assumed that titrations have been carried out and the experimental data plotted as $\bar{Z}_h$ against p*h*, or $\bar{Z}$ against p*a* (for example by using the ZPLOT program [11,12]), the curves recorded for differing total metal or ligand concentrations not being superimposable but rather displaying a pattern. The PSEUDOPLOT program is a combination of Sillén and his co-workers' [13] HALTAFALL program and our ZPLOT program. The HALTAFALL portion uses, as input, the experimental conditions [*i.e.* concentrations in titrate, titrant, volumes (all *except* the p*h* readings)] and a selected set of β values. The output is simulated titrant (cm^3) added against p*h* data that could have been obtained had such a system been titrated experimentally. The program uses this data in the ZPLOT portion to produce $\bar{Z}_h$ against p*h* or $\bar{Z}$ against p*a* curves. These simulated titration curves are then compared with the experimental data and then additional sets of βs are tried until the ' best ' fit is obtained.

It is important to note that the mass-balance relations in ZPLOT, and in the ZPLOT part of PSEUDOPLOT, assume mononuclearity and absence of hydroxo- and protonated complexes. Thus, when these conditions are not valid, $\bar{Z}$ and p*a* (or p*h*) are really pseudo $\bar{Z}$ and pseudo p*a* (or p*h*), these functions being ideal for showing the degree of variation from mononuclearity, *etc.*, and for comparing experimental with simulated titrations. In general, the sharpest patterns and the most rapid comparison of experimental to simulated curves are obtained when as many parameters as possible are held constant throughout a titration. For example, rather than titrating a solution of A and B with just alkali, the A, and B, concentrations in the titrant ought to equal those in the titrate, the sole variant being the mineral acid content of these two solutions. This produces constant ligand and constant metal-ion concentration titrations.

Choice of Sets of β*s.*—In common with all mathematical treatments of non-simple mononuclear systems we are faced with the qualitative problem of finding the best set of βs and the quantitative task of assigning values to these βs. Qualitatively, the possible βs are limited by the co-ordination numbers of the metal ions and by the denticity of the ligands.[14] The concentration dependence of complexing is also a useful guide to approximate values of βs: $\bar{Z}$ against p*a* curves have characteristic mono, bis, and tris complex p*a* regions (*ca.* $\bar{Z} = \frac{1}{2}$, $1\frac{1}{2}$, and $2\frac{1}{2}$, respectively); low metal and ligand concentrations encourage mononuclearity, high concentrations encourage polynuclearity, titrations at acid pH values are used to study protonated-complex formation, and alkaline pH values encourage hydroxo-complexes. Thus, within a fairly large margin of error, lists of βs can be suggested and used to generate PSEUDOPLOT patterns of curves. The best set of βs, as judged from the best fit of PSEUDOPLOT curves to the experimental data, can then be carried forward to the more quantitative aspects in which the least-squares program SCOGS [15,16] or MINIQUAD [17] refines suggested values of βs to better values. These can be inserted into the PSEUDOPLOT program to reveal traces of any additional complexes present, all βs eventually being refined by SCOGS.

Examples of PSEUDOPLOT Fitting: Zn^{II} *and* Pb^{II} *Glycine Peptide Systems.*—As part of our programme to design improved lead-chelating drugs,[18] we required formation constants for *all* complexes present in the zinc(II)–, and also the lead(II)–, glycinate, glycylglycinate, and glycylglycylglycinate systems. PSEUDOPLOT was a convenient means of establishing these sets of β_{pqr} and their numerical values. Formation curves were established for protonating the ligands. For each ligand, curves for different values of *A* were superimposable and the ' best ' SCOGS constants are listed in Table 1; β_{101} refers to protonating the primary amine group whereas K_2 for the second step (β_{102}/β_{101}) refers to protonating the carboxylate group. Neither the zinc nor the lead complex-formation curves were superimposable because of the presence of metal hydroxo-complexes and protonated metal–ligand species. The following log βs for hydroxo-complexes were assumed: $Zn(OH)_2$ −20·10; [19] $[Pb(OH)]^+$ −7·9; $[Pb_4(OH)_4]^{4+}$ −19·25; $[Pb_3(OH)_4]^{2+}$ −22·87; $[Pb_6(OH)_8]^{4+}$ −42·14.[20] Then the PSEUDOPLOT approach was applied.

TABLE 1

Log formation constants (β_{pqr}) * for ligand protonation at 37 °C and I = 0·15M-$NaClO_4$ and at 25 °C and I = 3M-$NaClO_4$; n = number of experimental observations and s denotes the standard deviation

Ligand	Conditions	p	q	r	log β	s	n
Glycinate	37 °C, 0·15M	1	0	1	9·173	0·003	179
		1	0	2	11·511	0·003	
	25 °C, 3M	1	0	1	10·070	0·007	117
		1	0	2	12·752	0·012	
Glycyl-glycinate	37 °C, 0·15M	1	0	1	7·739	0·001	230
		1	0	2	10·843	0·002	
	25 °C, 3M	1	0	1	8·562	0·007	115
		1	0	2	12·072	0·009	
Glycylglycyl-glycinate	37 °C, 0·15M	1	0	1	7·589	0·006	173
		1	0	2	10·695	0·013	
	25 °C, 3M	1	0	1	8·601	0·008	101
		1	0	2	12·234	0·012	

* β_{pqr} Refers to the general complex $A_pB_qH_r$ where A = ligand, B = metal ion, and H = proton.

Taking the lead(II)–glycylglycinate system (25 °C, I = 3·00M $NaClO_4$) † as an example, the ZPLOT points are shown in Figure 1. Step by step, PSEUDOPLOT was applied to the data using all possible combinations of β_{pqr} (for $p = 0 \rightarrow 3$, $q = 0 \rightarrow 2$, and $r = -2 \rightarrow 3$). The best fit is shown as full lines in Figure 1, the SCOGS log β_{pqr} values used being log $\beta_{00-1} = -14{\cdot}22$, log $\beta_{101} = 8{\cdot}562$, log $\beta_{102} = 12{\cdot}071$, log $\beta_{110} = 3{\cdot}375$, log $\beta_{111} = 9{\cdot}907$, log $\beta_{01-1} = -7{\cdot}900$, log $\beta_{03-4} = -22{\cdot}87$, log $\beta_{04-4} = -19{\cdot}25$, and log $\beta_{06-8} = -42{\cdot}14$. Figure 2 is a far poorer fit because β_{111} was omitted from the above set of βs and log β_{110} then converged in SCOGS to a value of 2·821. Simple amino-

* The program and input details are to be found in Supplementary Publication No. SUP 21191 (75 pp.). For details see Notice to Authors No. 7, *J.C.S. Dalton*, 1973, Index issue.

† 1M = 1 mol dm^{-3}.

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acids and tripeptides could be PSEUDOPLOT fitted with a similar degree of precision (*e.g.* see Figure 3).

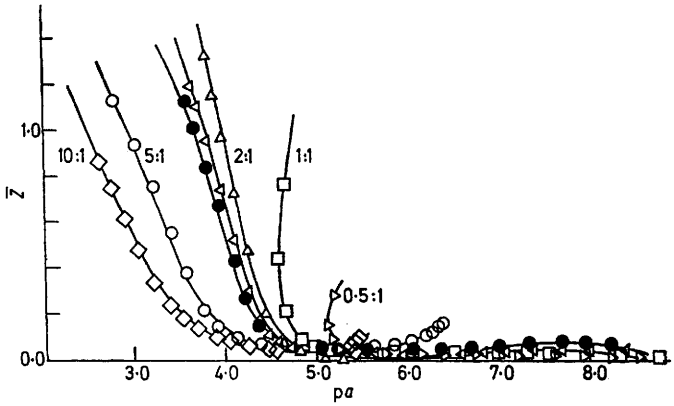

FIGURE 1 PSEUDOPLOT curves for the best set of βs plotted on the experimental ZPLOT points. $A : B$ Ratios are noted on the curves

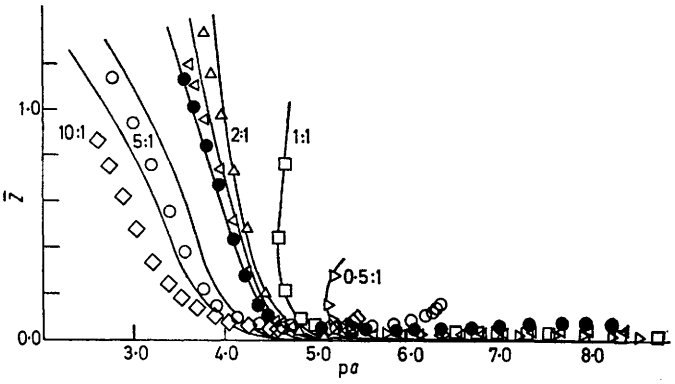

FIGURE 2 PSEUDOPLOT curves for the best set of βs plotted on the experimental ZPLOT points, β_{111} being omitted from the calculations. $A : B$ Ratios are noted on the curves

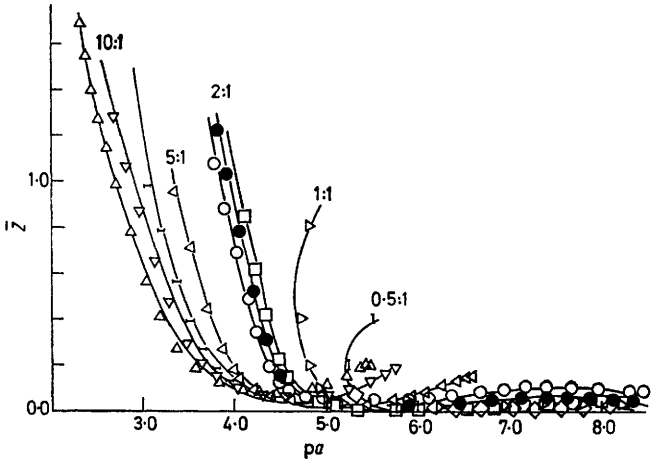

FIGURE 3 PSEUDOPLOT curves for the best set of lead(II)–glycylglycylglycinate βs plotted on the experimental ZPLOT points. $A : B$ Ratios are noted on the curves

Figures 1 and 3 have some interesting features. (*i*) The $\bar{Z}$ hump for $pa \geqslant 6$ and when $A : B \geqslant 2 : 1$ arises from the presence of the protonated complex ABH (it is not present in the PSEUDOPLOT curves of Figure 2). For $A : B = 1 : 1$ and $\frac{1}{2} : 1$ the trait is for the formation curve to lean over backwards. (*ii*) The overall spread in the curves, which is $A : B$ ratio dependent, is caused by metal hydroxide complex formation; however if the major complexes present are protonated, then the high to low $A : B$ ratio spread is the converse of Figure 1 (*i.e.* from right to left). (*iii*) Each $A : B$ ratio can have its own particular spread, for example see $A : B = 2 : 1$, depending on the value of B (for $A : B = 2 : 1$, from left to right, $B = 12$, 6, and 3mM).

DISCUSSION

PSEUDOPLOT Program.—The two main advantages of this program are its speed of use and the visual representation of errors. The speed arises because it is unnecessary to derive different normalised functions for different sets of βs, and also the plotting is done mechanically. Although least-squares programs such as SCOGS produce numerical values of 'standard deviations in titres' and 'residuals in titres,' (*a*) the human mind prefers to see such errors in *diagrammatic* form and, once having seen these discrepancies in the PSEUDOPLOT fits, it is then in a position to suggest a β to correct them (from observing the area of the plots where the data and calculated curves are most mismatched), and (*b*) all calculated 'residuals in titres,' *etc.* are based on the set of βs being offered to the computer program. Until least-squares programs have automatic species selectors included in their functioning, PSEUDOPLOT could be widely used to advantage.

Ligand Protonations.—The proton-attracting power of ligand primary-amine groups decreases with molecular weight (see β_{101} in Table 1). This trend supports a previous observation for substituted ligands.[21] However, the pK values of protonating free-ligand carboxylate groups and of protonating 1 : 1 metal complexes increase with peptide size (Zn: 4·39, 5·58, 5·71; Pb: 5·80, 6·53, 6·64 for glycinate, glycylglycinate, and glycylglycylglycinate), the latter suggesting carboxylate sites as the ones protonated. Clearly, calorimetric investigations could clarify this site identification. In a similar vein, the stepwise constants for adding a hydroxide group to AB are of the same order as for forming BH_{-1} (*e.g.* $[Pb(OH)]^+$ —7·9, and pK^{OH} —7·7 and —7·5 for lead(II)–glycinate and –glycylglycylglycinate).

Metal Complexing.—Complexes formed by zinc(II) and glycinate are more stable than zinc peptide complexes (see Table 2). Bidentate glycinate clearly bonds through the amine and carboxylate groups, but when the anion is unidentate (for example, when the ABH complex is present) carboxylate–proton and amine–zinc bonds are possibly present. Glycylglycinate and glycylglycylglycinate zinc complexes involve the primary amine and either the oxygen or possibly the nitrogen atom of the nearest peptide linkage; *i.e.* five-membered chelate rings are formed.

The literature contains a paucity of data on lead(II)–glycinate and peptide investigations (see Table 2).

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One cannot unambiguously assign peptide–lead binding sites without more evidence, but from other bivalent metal ions the general consensus is in favour of the primary amino-group in conjunction with *either* the peptide oxygen or, according to Kim and Martell,[22] both the terminal carboxylate group and the amide nitrogen (*i.e.* tridentate). Lead(II) is a HSAB softer acceptor than the cations studied and so ought to incline towards nitrogen rather than the harder oxygen donors.[23] Whichever the mode of bonding, the similarity between the formation constants for the glycylglycinate and glycylglycylglycinate complexes suggests that the atoms

TABLE 2

Log formation constants (β_{pqr}) for the metal complexes at 37 °C and I = 0·15M-$NaClO_4$ and at 25 °C and I = 3M-$NaClO_4$; n = number of experimental observations and s denotes the standard deviation

Metal ion	Conditions	Ligand	p	q	r	log β	s	n	Literature data (θ_c/°C, I/M, log β)	Ref.
Zn^{2+}	37 °C, 0·15M	Glycinate	1	1	0	4·909	0·021	123	37, 0·15 (KNO_3), β_1 4·90, β_2 9·01, β_3 11·31, β_{11-1} −8·89	*a*
			2	1	0	8·997	0·043			
			3	1	0	11·306	0·035			
			1	1	1	9·297	0·239		25, 0·1 (KNO_3), β_1 5·03, β_2 9·30	*b*
			1	1	−1	−2·706	0·384			
									25, ⟶ 0, β_1, 5·52, β_2 9·96	*c*
									20, 0·5 (KNO_3), β_1 4·80, β_2 8·94, β_3 11·50	*d*
									25, *ca.* 0·01, β_1 5·33, β_2 9·72	*e*, *f*
									20, *ca.* 0·01, β_1 5·2, β_2 9·3	*g*, *h*
									25, 0·01, β_1 5·0	*i*
									20, 0·1 (KCl), β_1 5·16, β_2 9·50	*j*
									25, 0·15 (KNO_3), β_1 5·42, β_2 9·94	*k*
									30, 75% dioxan, β_1 8·3, β_2 15·1, β_3 18·9	*l*
									22, 0·01 ($ZnSO_4$), β_2 9·2	*m*
									20, 0·1 (KNO_3), β_1 5·9, β_2 10·1, β_3 13·2	*n*
									10, 25, 0·65 (KCl), β_1 (10 °C) 4·96, β_2 9·24, β_3 11·9; β_1 (25 °C) 4·88, β_2 9·01, β_3 11·0	*o*
									15–40, 0·2 (KCl), β_1 (15 °C) 5·27, β_2 9·58; β_1 (25 °C) 5·19, β_2 9·4; β_1 (40 °C) 5·07, β_2 9·14	*p*
									25, 0·5 (KCl), β_1 4·88, β_2 9·01, β_3 11·02	*q*
									25, 0·5 (KCl), β_1 4·88, β_2 9·11, β_3 11·56	*r*
		Glycylglycinate	1	1	0	3·574	0·037	129	37, 0·15 (KNO_3), β_1 3·24, β_2 5·88	*s*
			2	1	0	5·880	0·056			
			3	1	0	8·015	0·137		0–25, 0·058 (KCl), β_1 (0 °C) 4·06, β_2 7·56	*t*
			1	1	1	9·150	0·098		β_1 (25 °C) 3·91, β_2 7·22	
									25, 0·01, β_1 3·6	*i*
									25, ⟶ 0, β_1 3·80, β_2 6·57	*e*
									21, 0·01 ($ZnSO_4$), β_2 6·4	*m*
		Glycylglycylglycinate	1	1	0	3·378	0·033	166	37, 0·15 (KNO_3), β_1 3·00, β_2 5·34	*s*
			2	1	0	5·395	0·147			
			1	1	1	9·087	0·083		25, 0·15 (KNO_3), β_1 3·18	*u*
			1	1	−1	−4·677	0·483			
									25, ⟶ 0, β_1 3·33, β_2 6·32	*v*
									25, 0·01 ($ZnSO_4$), β_1 2·6	*i*
Pb^{2+}	25 °C, 3M	Glycinate	1	1	0	5·600	0·034	289	25, 0·1 (KNO_3), β_2 7·7	*b*
			1	1	1	11·396	0·127			
			1	1	−1	−2·142	0·035		25, 0·1 (KNO_3), β_2 7·4	*b*
									25, ⟶ 0, β_1 5·47, β_2 8·86	*c*
									25, ⟶ 0, β_1 5·17	*w*
									25, *ca.* 0·01, β_1 5·53, β_2 9·98	*e*
									22, 0·01 [$Pb(NO_3)_2$], β_2 9·3	*m*
									30, 1·0 (KNO_3), β_1 5·11, β_2 7·08	*x*
		Glycylglycinate	1	1	0	3·375	0·039	176	25, ⟶ 0, β_1 3·23, β_2 5·93	*c*
			1	1	1	9·907	0·032			
									21, 0·01 [$Pb(NO_3)_2$], β_2 5·8	*m*
		Glycylglycylglycinate	1	1	0	3·767	0·039	192	25, ⟶ 0, β_1 3·02, β_2 5·75	*v*
			1	1	1	10·403	0·037			
			1	1	−1	−3·761	0·040			

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involved in their chelate rings are the same in both instances.

One of the reasons for studying zinc and lead complexes stems from their pharmacological relevance. Both *in vivo* zinc administration and lead removal require the presence of neutral complexes for effective

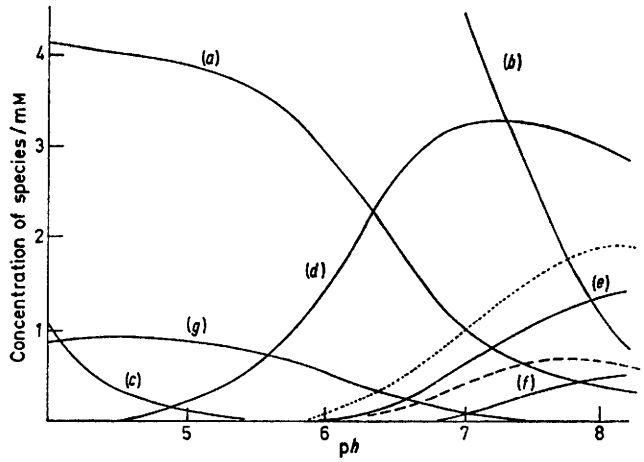

FIGURE 4 COMPLOT calculation of pH dependence for complexes present in the zinc–glycylglycinate(A)–proton system when $A = 10$ and $B = 5$mM: (*a*), Zn^{2+}; (*b*), HA; (*c*), H_2A^+; (*d*), $[ZnA]^+$; (*e*), $[ZnA_2]$; (*f*), $[ZnA_3]^-$; (*g*), $[Zn(HA)]^{2+}$. For comparison purposes the concentrations of bis(glycinato)zinc(II) (· · · ·) and bis(glycylglycylglycinato)zinc(II) (— — —) are also plotted

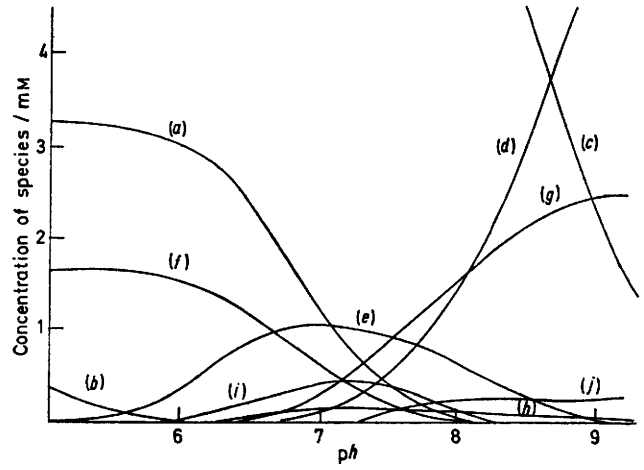

FIGURE 5 COMPLOT calculation of pH dependence for complexes present in the lead–glycylglycylglycinate(A)–proton system when $A = 10$ and $B = 5$mM: (*a*), Pb^{2+}; (*b*), H_2A^+; (*c*), HA; (*d*), A; (*e*), $[PbA]^+$; (*f*), $[Pb(HA)]^{2+}$; (*g*), [PbA(OH)]; (*h*), $[Pb(OH)]^+$; (*i*), $[Pb_4(OH)_4]^{4+}$; (*j*), $[Pb_3(OH)_4]^{2+}$ and $[Pb_6(OH)_8]^{4+}$ superimposed

lipid–protein membrane solubility and permeability. COMPLOT [24] computations established the distribution of the metals between various complexes (Figures 4 and 5). The amount of uncharged A_2B zinc complex present parallels the size of the peptide. Lead does not form such a neutral A_2B complex, but it is important to record that the peptides complex less of the total lead than does their parent simple amino-acid anion.

Choice of Experimental Conditions.—As a subsidiary part of this work we attempted to resolve some of the dichotomies involved in the choice of an ideal background salt and temperature for potentiometric studies, in particular 3M-$NaClO_4$ at 25 °C against 150mM-$NaClO_4$ at 37 °C. Thus, the protonation βs in Table 1 are reported for both these conditions and then the particular problems are discussed. The disadvantages of using $I = 3{\cdot}00$M-$NaClO_4$ and 25 °C are: (*i*) these are far removed from biological blood-plasma conditions (which approximate to 37 °C and $I = 150$mM-Cl^-); (*ii*) the final traces of impurity remaining in the sodium perchlorate are emphasised when the background salt is 3M; (*iii*) even though the perchlorate ion has little tendency to ion pairing, at 3M concentration some may still occur; [25] and (*iv*) the lower the temperature the higher the amine pK values (see Table 1). Sometimes this can take them outside the working range of glass electrodes. The disadvantages of using $I = 150$mM and 37 °C are: (*i*) a considerable quantity of volumetric glassware needs recalibrating; (*ii*) unless the complete system of vessel and electrodes are thermostatted at 37 °C condensation occurs in the cooler parts of the system and the electrothermal effect in the electrodes can cause an error of up to 3 mV; [26] (*iii*) the tubing linking the burette to the titration vessel also needs to be maintained at 37 °C to minimise temperature fluctuations in the vessel; (*iv*) $I = 150$mM permits only a 8mM change in ion concentration without significantly changing the activity coefficients; [27,28] (*v*) ion-responsive electrodes, such as amalgams, are less stable at higher temperatures; (*vi*) the Sillén school have reported many metal-ion hydrolysis constants for 3M-ClO_4^- and 25 °C but relatively few ions have been studied at 150mM-ClO_4^- and 37 °C (this means that ion-hydrolysis studies may be necessary before further complexing studies are undertaken); and (*vii*) the higher temperature accelerates the rate of peptide hydrolysis.

Clearly, there is no ideal medium, each set of conditions having certain merits and problems. This listing has two uses: (*i*) conditions ought to be specifically selected for each investigation to minimise as many of these disadvantages as possible, and (*ii*) being aware of these difficulties, a more realistic consideration of experimental errors is possible.

EXPERIMENTAL

The following ligands were used: glycine (Fisons A.R.) (Found: C, 32·1; H, 6·50; N, 18·5. Calc. for $C_2H_5NO_2$: C, 32·0; H, 6·70; N, 18·6%); glycylglycine (Koch–Light) (Found: C, 36·3; H, 6·40; N, 21·1. Calc. for $C_4H_8N_2O_3$: C, 36·4; H, 6·10; N, 21·2%); and glycylglycylglycine (Koch–Light) (Found: C, 38·0; H, 6·10; N, 22·1. Calc. for $C_6H_{11}N_3O_4$: C, 38·1; H, 5·85; N, 22·3%). Zinc perchlorate (G. F. Smith Chemical Co.) solution was

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prepared and analysed as in ref. 29 and lead perchlorate as in ref. 18.

Methods.—The potentiometric approach was as described in ref. 12, the protonation and lead studies being carried out at 25 °C and I = 3·00M-$NaClO_4$, and the protonation being repeated with the zinc studies at 37 °C and I = 150mM-$NaClO_4$. To within the limits dictated by hydrolysis, each system was studied using a pattern of titrations whereby both A and B were held constant and equal in the titrant and titrate, these two solutions differing solely in their total acid contents, and for each B value of 1, 10, 20mM, *etc.* titrations were executed for $A : B$ = 10 : 1, 5 : 1, 2 : 1, 1 : 1, and $\frac{1}{2}$: 1. In this manner it was possible to detect all complexes formed, hydroxo-, polynuclear, and protonated. (For the protonation work, B was set equal to 0.) Concentration formation constants were refined from this titration data using the SCOGS computer program [15] and the resultant 'best' constants are shown in Tables 1 and 2.

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