A Potentiometric Study of the Zn(II)– and Pb(II)–Valproate Systems

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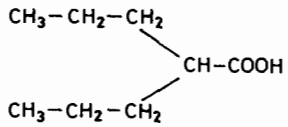
Valproic acid (2-propylpentanoic acid, **I**) was first synthesized by Burton in 1882 [1] although its therapeutic value as an anticonvulsant was not identified until 1962 [2]. Prior to this, the acid had been employed as a low toxicity organic solvent and it was in this rôle that the discovery was made [2]. Today, valproic acid and its sodium salt are effective agents in the management of epileptic seizures and, more recently, lead-induced convulsions in young infants [3].



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Unfortunately, as with other anticonvulsants, adverse reactions do occur with valproate; however, their incidence is considered to be low in comparison.

The most commonly reported problems are gastrointestinal disturbances which occur in 10–50% of patients [4]. In general, however, these effects tend to be transient. Other reported side effects include changes in appetite and metabolism, neutropenia, alopecia, birth defects, liver dysfunction and, more rarely, skin complaints, depression and leucopenia [5–19].

Many of these reactions could well be indicative of trace metal imbalances, in particular, zinc deficiency. It is noteworthy in this respect that valproate and many of its principal metabolites contain functional groups which may interact with trace metals *in vivo* and, thereby, cause their depletion.

The aims of this investigation were, therefore, to quantify the interactions of valproate and of its degradation products with zinc *in vitro* and, hence, to predict the ability of this drug and its metabolites to compete for this metal ion with the naturally occurring ligands in plasma. The study also serves to examine the rôle of valproate in controlling the lead-induced seizures in neonates.

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This note accordingly reports formation constant values for the proton–, Zn(II)–, and Pb(II)–valproate systems and examines the effect of this new data on the distribution of zinc and lead in blood plasma using the ECCLES (Evaluation of Constituent Concentrations in Large Equilibrium Systems) computer model [20].

Experimental

Sodium valproate was provided by Labaz, Sanofi U.K. Ltd., and used without purification (C, H analysis: Found: C, 58.2; H, 9.2. Calc. for $C_8H_{18}O_2Na$: C, 57.5; H, 9.6%). Due to the hygroscopic nature of valproate, solutions were prepared by direct weighing in a dry nitrogen atmosphere.

Acidified stock solutions of zinc chloride and lead chloride were prepared using AnalaR grade reagents (BDH Chemicals Ltd) and subsequently analysed by EDTA complexometric titration for metal ion content [21] and by strong acid *versus* strong base titration for hydrogen ion concentration.

All solutions were prepared using distilled, degassed, doubly deionised water (resistivity >2 MΩ). Sodium chloride, AnalaR grade (BDH Chemicals Ltd) was employed to maintain a background electrolyte concentration of 150 mmol dm^{-3} chloride in all solutions.

Measurement of Formation Constants

Potentiometric titrations were performed as outlined by Filella *et al.* [22]. To avoid solubility problems, ligand protonation studies were carried out with an alkaline solution of ligand as the titrand and hydrochloric acid as the titrant. The metal ligand titrations were performed in an analogous manner; although in this case the acidified metal solutions were used as the titrant. Various total ligand, metal and proton concentrations were employed throughout, a summary of these being given in Table I.

Computing

The protonation and formation constants were evaluated from the potentiometric titration data using the OBJE task of the ESTA (Equilibrium Simulation for Titration Analysis) library of computer programs [23]. The ZBAR and QBAR tasks were employed to graphically check the chosen formation constants. Species distribution profiles were generated using the SPEC task. The ECCLES program was used to compute the distributions of zinc and lead in the presence of valproate in plasma.

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TABLE I. Summary of Titration Data Used in Formation Constant Calculations. Initial Total Concentrations of Vessel Valproate (C_L) and Mineral Acid (C_H^V) and Burette Metal (C_M) and Mineral Acid (C_H^B), also pH Range Investigated. All Concentrations are expressed in mmol dm^{-3}

System	C_L	C_H^V	C_M	C_H^B	pH Range
Proton–valproate	16.88	−13.04		100.0	4.4–11.44
	16.88	−13.04		100.0	4.47–11.50
	8.44	−13.04		100.0	2.27–11.43
	8.82	−9.09		100.0	2.35–11.37
	4.41	−9.09		100.0	2.26–11.28
Zn(II)–valproate	14.75		20.23	40.11	2.91–6.26
	9.83		20.23	40.11	2.74–6.14
	14.75		20.23	40.11	2.90–6.26
	6.55		20.23	40.11	2.73–6.16
	9.83		20.23	40.11	2.74–6.15
Pb(II)–valproate	10.10		5.00	40.02	2.53–6.15
	10.10		5.00	40.02	2.53–6.13
	7.57		5.00	40.02	2.40–6.09
	7.57		5.00	40.02	2.49–6.08
	5.05		4.94	19.61	2.69–6.10
	5.05		4.94	19.61	2.69–6.10

TABLE II. Formation Constants for Proton–, Zn(II)– and Pb(II)–Valproate Interaction at 37 °C[a]

Interaction	Species			lg β_{pqr}	Standard deviation	Objective function (U)	R_{factor}	Number of points	Number of titrations
	p	q	r						
Valproate protonation	0	1	1	4.645	0.004	279	0.005	196	5
Zn(II)–valproate	1	1	0	1.70	0.01	116	0.005	218	5
	1	1	−1	−3.65	0.01				
Pb(II)–valproate	1	1	0	2.34	0.01	116	0.004	330	6
	1	2	−1	−0.06	0.01				

[a] I = 150 mmol dm^{-3} chloride; $\beta_{pqr} = [M_pL_qH_r]/[M]^p[L]^q[H]^r$.

Results and Discussion

The valproate protonation constant is shown in Table II, along with associated statistical output and the formation constants for its interactions with zinc and lead. The species distribution profiles of both the zinc and lead systems are given in Figs. 1 and 2.

The ability of valproate to mobilize Zn(II) and Pb(II) *in vivo* was evaluated over a range of drug concentrations using the ECCLES Plasma Mobilizing Index [24]. The findings of the study indicate zero mobilization of both these ions from the labile protein complexes in plasma.

The most important conclusion of this *in vitro* study is that metal mobilization by valproate does not contribute to the side effects observed in some patients, even those with an inherently low trace metal status. The inability of valproate to mobilize Pb(II) implies that the seizure control achieved in neonates is probably through the action of valproate on aberrant metabolic processes in the brain rather than the removal of, or reduction in, the level of Pb(II) ions.

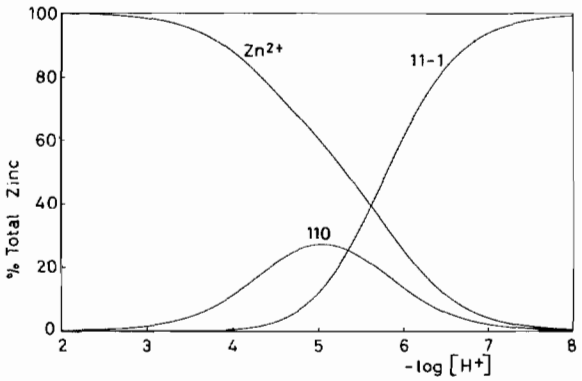

Fig. 1. Zinc(II)–valproate species distribution ([L]:[M] = 15:5 mmol dm^{-3}).

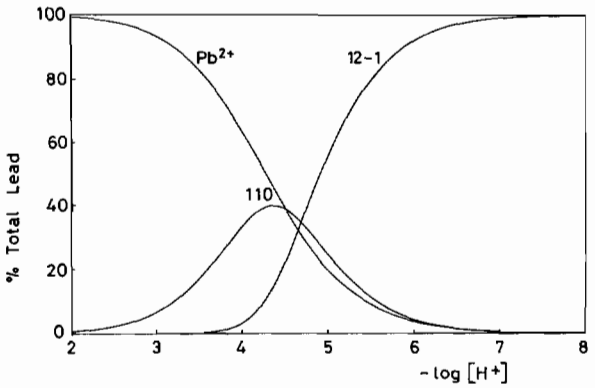

Fig. 2. Lead(II)–valproate species distribution ([L]:[M] = 15:5 mmol dm^{-3}).

The study was also extended to incorporate the major metabolite of valproate, 2-propyl pentenoic acid. The formation constants for the interaction of this ligand with Zn(II) and Pb(II) ions were considered to be equivalent to those obtained for valproate, due to the similarity of their chemical structures. The results of this investigation show the metabolite is unlikely to mobilize these metal ions. In view of this, the presence of this degradation product, and others of a similar structure, are considered not to enhance the excretion of Zn(II) and Pb(II) from plasma.

Ideally, the findings of the computer simulation studies ought to be validated clinically by, for example, urine analyses. However, possible pre-existing variations in trace metal status due to disease or other drugs should be taken into account.

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