Quantitative Analysis of Sodium Valproate in Pharmaceutical Preparations by a Valproate-Selective Electrode

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A simple and rapid method for the determination of sodium valproate in pharmaceutical preparations, without prior separation, has been developed using a valproate-selective electrode. A plastic liquid membrane electrode kit was used for the electrode based on the use of valproate: methyltris(tetradecyl)ammonium ion-pair complex in *n*-decanol and polyvinyl chloride matrix. The electrode showed a near-Nernstian response in the range of 10^{-2} to 2×10^{-4} M sodium valproate. Life span of the electrode was at least 1 month. The electrode was also used for the determination of the pK_a of valproic acid. Determination of 90 to 1500 $\mu\text{g/ml}$ of sodium valproate in aqueous solution showed an average recovery of 100.0% (mean standard deviation 0.4%) by direct potentiometry. Pharmaceutical excipients and diluents commonly used in drug formations did not interfere with the analysis. The selectivity of the electrode to some inorganic and organic anions was described. This assay was applied to determine sodium valproate in pharmaceutical preparations and the results compared favorably with those obtained by gas chromatography.

Keywords sodium valproate; valproic acid; valproate-selective electrode; ion-selective electrode; pharmaceutical analysis

Sodium valproate (VPA-Na) is an antiepileptic agent used alone or with other anticonvulsants in the prophylactic management of simple and complex absence seizures.¹⁾ Since VPA lacks ultraviolet (UV) absorbance at higher wavelengths ($>235\text{ nm}$), gas chromatography (GC) and titration in nonaqueous solutions has usually been used for the determination of VPA in pharmaceutical preparations. However, these methods have the disadvantages of requiring complicated and prior separations or non-specificity.

A potentiometric method using an ion-selective electrode has recently shown itself to be a viable means of analyzing pharmaceutical products and has been applied to the determination of active ingredients in pharmaceutical formulations.²⁾ These potentiometric methods can be easily used in the assay procedure without prior separations. The purpose of this study was to develop a simple and accurate assay system using a new VPA-selective electrode for the determination of VPA in pharmaceutical preparations. We used a plastic liquid membrane electrode kit³⁾ which has a polytetrafluoroethylene (PTFE) membrane as a carrier of a plastic matrix and VPA-methyltris(tetradecyl)ammonium as the electroactive material for the VPA-selective electrode.

Experimental

Reagents VPA-Na was purchased from Yodogawa Pharmaceutical Co., Ltd. Methyltris(tetradecyl)ammonium chloride (MTTDA-Cl), tri-*n*-octylmethylammonium chloride (Capriquat), *o*-nitrophenyl octyl ether (NPOE) and di-*n*-octylphenylphosphonate (DOPP) were obtained from Dojin Chemicals. Di-*n*-butyl phthalate (DBP), *n*-decanol and di-*n*-octyl phthalate (DOP) were from Wako Pure Chemical. Polyvinyl chloride (PVC; high relative molecular weight) was purchased from Aldrich. All other chemicals were of the best available commercial grade. Borax buffer (0.033 M, pH 9.3) was prepared from 12.6 g $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ made up to 1 l. Pharmaceutical preparations containing VPA-Na (Hyserenin fine granules®, Hyserenin fine granules 20®, Hyserenin tablets® and Hyserenin tablets 100®) were obtained from Kanebo, Ltd.

Apparatus Potentiometry: All potentiometric measurements were carried out using an Orion expandable ion analyzer (Model EA920). The VPA-selective electrode was used with an Orion Ag–AgCl double-junction reference electrode (Model 90-02) containing saturated $\text{Na}_2\text{B}_4\text{O}_7$ solution in the outer compartment as the reference. pH and temperature measurements were performed with a Horiba glass–calomel electrode (Model 6026) and an Orion automatic temperature compensator, respectively.

GC: A Hitachi gas chromatograph (Model 163) was equipped with a

flame-ionization detector and a Shimadzu integrator (Model C-R3A). The glass column, $2\text{ m} \times 3\text{ mm}$ i.d., was packed with 5% diethyleneglycol succinate (DEGS) + 1% H_3PO_4 phase on 60–80 mesh Chromosorb W (Shimadzu). The carrier gas (nitrogen) flow-rate was 35 ml/min. The system temperatures were: column, 150°C ; injector, 220°C ; detector 220°C .

Preparation of Electroactive Material One milliliter aliquot of *n*-decanol was mixed with 130 mg of MTTDA-Cl and shaken with an 8 ml aliquot of a 0.1 M VPA-Na aqueous solution. The organic phase was washed twice with distilled water and used for preparation of the electrode.

Electrode Preparation A liquid membrane electrode kit (Denki Kagaku Keiki Co.) was used as described previously.³⁾ The electroactive material (100 mg) and PVC (50 mg) were dissolved in 1.5 ml of tetrahydrofuran. A PTFE membrane filter was dipped in the mixture and placed on top of the chip of the electrode kit. Several drops of the mixture were applied to the PTFE membrane and the membrane was air dried at room temperature. The internal reference solution of the electrode was a 10^{-2} M VPA-Na solution of 0.033 M borax buffer (pH 9.3). The chip was conditioned by soaking in 10^{-2} M VPA-Na solution (pH 9.3).

Potential Measurements Cell assemblies of the following type were used: Ag–AgCl | 10^{-2} M VPA-Na solution (borax buffer, pH 9.3) || PVC membrane || test solution (borax buffer, pH 9.3) | Ag–AgCl double-junction reference electrode. The potential values were measured for the stirred solution at $25 \pm 1^\circ\text{C}$.

Selectivity Coefficients The selectivity coefficients were evaluated by the fixed interference method according to IUPAC recommendations. The potential was measured with solutions of a constant level of interference (10^{-2} M) and varying concentrations of VPA-Na from 10^{-2} to 2×10^{-4} M.

Assay by Potentiometry Standard Preparation: Two standard preparations, I and II, in 0.033 M borax buffer (pH 9.3) having known concentrations of VPA-Na 160 and 200 $\mu\text{g/ml}$, respectively, were prepared.

Assay Preparation: Pharmaceutical preparations of VPA-Na were finely powdered. An accurately weighed portion of the powder, equivalent to about 0.1 g of VPA-Na, was added to about 40 ml of borax buffer (pH 9.3) and sonicated, and borax buffer was added to make exactly 50 ml.

Procedure: The VPA-selective electrode and the Orion double-junction reference electrode were immersed successively in standard preparation I, the assay preparation and standard preparation II. The respective electron motive force (emf) values of these solutions were determined. The quantity was calculated by comparing the measured emf values of the assay preparation with the calibration line calculated from the standard preparations.

Assay by GC Pharmaceutical preparations of VPA-Na were finely powdered. An accurately weighed portion of powder, equivalent to about 0.1 g of VPA-Na, and 5 ml of standard solution (VPA-Na 10 mg/ml) were transferred to two different separators and treated identically as follows: Five milliliters of internal standard solution (diphenyl 10 mg/ml in chloroform) and 15 ml of 1 N hydrochloric acid were added and the solution was extracted with 20 ml of chloroform, centrifuged, and dehydrated by anhydrous sodium sulfate; the organic layer was then injected.

Results and Discussion

Selection of Ion-Pairing Agent Quaternary ammonium species,⁴⁾ cationic dyes and ferroin-compound cations⁵⁾ and metalloporphyrines⁶⁾ have been used as ion-pairing agents for acidic drugs. In the present study, we tested quaternary ammonium species (MTTDA, Capriquat) and ethyl violet because these compounds were commercially available and ethyl violet has large extractability of an ion-pair into nonpolar solvents.⁷⁾ The membranes of the electrode contained PVC as a plastic matrix and *n*-decanol as a solvent. The response characteristics for the VPA-selective electrodes are summarized in Table I, and the effect of concentration of ion-pair in the solvent is also shown. The MTTDA-based electrode showed the lowest limit of linear range. This is probably because MTTDA forms the most hydrophobic ion-pair with VPA and extractability of the ion-pair is the best. The concentration of ion-pair in the membrane was increased, resulting in excellent performance. Based on these results, we selected the 0.2M MTTDA-VPA ion-pair in *n*-decanol as the membrane material.

Selection of Solvent As the solvent in the membrane material, we tested *n*-decanol, DOPP, NPOE, DBP and DOP using 0.2M MTTDA-VPA ion-pair in these solvents. The response characteristics for these electrodes revealed the one containing *n*-decanol to be excellent (Table II). The composition of around 70 : 30 percent solvent (plus sensor): polymer was shown to be most appropriate for the PVC electrodes.⁸⁾ Therefore, we selected the following membrane compositions for the VPA-selective electrode: 67% (w/w) 0.2M VPA-MTTDA in *n*-decanol, and 33% (w/w) PVC.

TABLE I. Response Characteristics for Various PVC: *n*-Decanol Based VPA-Selective Electrodes (Borax Buffer, pH=9.3)

Ion-pairing agent (M)	Slope ^{a)} (mV/log C)	Correlation coefficient	Intercept (mV)	Lower limit of linear range (M)
MTTDA				
0.05 ^{b)}	-47.03 ± 1.21 ^{c)}	0.9998	144 ± 3.1	2 × 10 ⁻⁴
0.1	-57.29 ± 0.33	0.9998	128 ± 1.8	2 × 10 ⁻⁴
0.2	-58.26 ± 0.02	0.9999	115 ± 0.9	2 × 10 ⁻⁴
Capriquat				
0.2	-56.79 ± 0.81	0.9999	119 ± 1.0	5 × 10 ⁻⁴
Ethyl violet				
0.2	-54.97 ± 0.64	0.9998	128 ± 3.4	5 × 10 ⁻⁴

a) Average value calculated for the range of 10⁻²M—lower limit of linear range. b) Concentration of ion-pairing agent in *n*-decanol. c) Average value and standard deviation (S.D.) of values (*n*=3) obtained from the same electrode.

TABLE II. Response Characteristics for Various PVC: MTTDA Based VPA-Selective Electrodes (Borax Buffer, pH=9.3)

Solvent	Slope ^{a)} (mV/log C)	Correlation coefficient	Intercept (mV)	Lower limit of linear range (M)
<i>n</i> -Decanol	-58.26 ± 0.02 ^{b)}	0.9999	115 ± 0.9	2 × 10 ⁻⁴
DOPP	-55.86 ± 0.84	0.9997	122 ± 3.7	2 × 10 ⁻⁴
DBP	-54.7	0.998	122	5 × 10 ⁻⁴
NPOE	-53.9	0.998	124	5 × 10 ⁻⁴
DOP	-52.9	0.998	126	5 × 10 ⁻⁴

a) Average value calculated for the range of 10⁻²M—lower limit of linear range. b) Average value and S.D. of values (*n*=3) obtained from the same electrode.

The effect of quantity of membrane material mounted on the electrode to the unit's performance is shown in Table III. In this range the slope changed little but the response time of the electrode increased with increasing quantity. Therefore, we mounted 12 mg of membrane material on the electrode. Data obtained from five electrodes (Table III) indicates the high reproducibility of performance among them.

Influence of pH The influence of pH on the potential of the VPA-selective electrode was investigated. The pH was varied by adding sulfuric acid or sodium hydroxide solution dropwise to VPA-Na aqueous solution. The results are shown in Fig. 1. At lower pH (pH < 6.5), the potential increases with the decrease in the pH because the protonated VPA species gradually decrease. From these potential-pH curves *pK_a* of VPA was evaluated. The *pK_a* is equal to the pH value when the electrode potential increases by $-S \times \log 2$ mV (*S*=electrode slope).⁹⁾ The *pK_a* values obtained from the curves of 10⁻³ and 5 × 10⁻³ M VPA-Na in the figure were 4.9 and 4.7, respectively, which were in agreement with the literature (a value of 4.8).¹⁾ At higher pH values, the potential increases with the increase in the pH. This might be due to the increasing of ion strength with the addition of sodium hydroxide, because in the case of high ion strength like 10⁻³ M VPA-Na in 0.033M borax solution, the potential was not changed up to pH 11.5.

Influence of Interfering Compounds The selectivity coef-

TABLE III. Response Characteristics for Various Quantities of Membrane Construction (Borax Buffer, pH=9.3)

Quantity (mg)	Slope ^{a)} (mV/log C)	Correlation coefficient	Intercept (mV)	Lower limit of linear range (M)
7	-54.86 ± 0.24 ^{b)}	0.9992	141 ± 0.9	2 × 10 ⁻⁴
12	-58.26 ± 0.02 (-57.90 ± 1.07) ^{c)}	0.9999 (0.9996)	115 ± 0.9 (117 ± 10.6)	2 × 10 ⁻⁴
24	-57.00 ± 0.20	0.9996	120 ± 0.6	2 × 10 ⁻⁴
36	-58.17 ± 1.05	0.9996	127 ± 4.5	2 × 10 ⁻⁴

a) Average value calculated for the range 10⁻²M—lower limit of linear range. b) Average value and S.D. of values (*n*=3) obtained from the same electrode. c) Average value and standard deviation of values obtained from five different electrodes.

Fig. 1. Effect of pH on the Potential of the VPA-Selective Electrode for Different VPA-Na Concentrations

TABLE IV. Selectivity Coefficients for VPA-Selective Electrode

Interferent	$K_{i,j}$
NO_3^-	4.9×10^{-1}
SO_4^{2-}	6.6×10^{-2}
Cl^-	6.0×10^{-2}
CO_3^{2-}	5.4×10^{-2}
HPO_4^{2-}	3.8×10^{-2}

Fig. 2. Plot of $\log K_{i,j}$ against the Number of Carbon Atoms of Unbranched Alkanoic Acid

TABLE V. The Allowable Limit of Excipients

Excipient	Excipient weight ^{a)} / VPA-Na weight ^{b)}
Corn starch, JP	>20
Potato starch, JP	>20
Microcrystalline cellulose, JP	>20
Synthetic aluminium silicate, JP	>20
Sucrose, JP	>20
Lactose, JP	10
D-Mannitol, JP	5
Magnesium aluminometasilicate	1
Magnesium stearate, JP	>2
Hydroxypropylcellulose, JP	>2
Hydroxypropylmethylcellulose 2910, JP	>2

a) Excipient weight which gives an error of 1%. b) Concentration of VPA-Na is 200 $\mu\text{g/ml}$.

ficients, $K_{i,j}$ of the VPA-selective electrode determined by the fixed interference method according to IUPAC recommendations are listed in Table IV. A plot of $\log K_{i,j}$ versus the number of carbon atoms in unbranched alkanic acids gave a straight line as shown in Fig. 2. This indicates that selectivity of the VPA-selective electrode is governed by hydrophobicity of ions, that is, extractability of ions into the membrane in a manner similar to other electrodes based on quaternary ammoniums.¹⁰⁾ The influence of some excipients normally used in pharmaceutical preparations was also examined. Table V shows the quantity of excipients against VPA-Na which gives an error of 1% to the assay of VPA-Na by potentiometry. These excipients demonstrated no interference in the quantity usually used.

Assay of VPA-Na In Table VI, reproducibility and accuracy determined by the direct potential method for VPA-Na solution in the range of 90–1500 $\mu\text{g/ml}$ are shown. The results show an average recovery of 100.0% and a mean standard deviation of 0.4%. The response time

TABLE VI. Determination of VPA-Na in Solution by VPA-Selective Electrode

VPA-Na ($\mu\text{g/ml}$)		Recovery (% ^{a)})	Standard deviation
Added	Found		
89.9	89.7	99.7	0.51
149.8	150.2	100.3	0.72
299.6	199.5	100.0	0.15
599.2	599.2	100.0	0.26
898.9	895.6	99.6	0.76
1198.5	1202.2	100.3	0.36
1498.1	1498.3	100.3	0.36

a) Average of three measurements; average recovery $\pm$ mean S.D. = $100.0 \pm 0.4\%$.

TABLE VII. Determination of VPA-Na in Pharmaceutical Preparations by VPA-Selective Electrode and GC

Sample	Electrode method		GC method	
	Recovery (%)	S.D. (n=6)	Recovery (%)	S.D. (n=6)
Hyserenin fine granules [®]	99.2	1.2	97.5	2.0
Hyserenin fine granules 20 [®]	104.6	1.3	103.0	1.5
Hyserenin fine tablets [®]	97.7	0.5	97.5	1.4
Hyserenin fine tablets 100 [®]	100.8	0.7	101.3	1.0

of the VPA-selective electrode in this range was short, viz., within 60 s.

To check the ISE (ion-selective electrode) method recovery efficiency, we spiked VPA-Na with placebo excipients used in Hyserenin fine granules[®], Hyserenin fine granules 20[®], Hyserenin tablets[®] and Hyserenin tablets 100[®]. The average recoveries and mean standard deviation of VPA-Na from these mixed samples were $99.8 \pm 0.2\%$, $100.0 \pm 0.7\%$, $100.4 \pm 0.4\%$ and $100.3 \pm 0.5\%$, respectively.

Commercially available preparations containing VPA-Na were determined by the ISE method and compared with GC. The results obtained by both methods (Table VII) show good agreement and high precision. The present procedure is sufficiently simple, rapid, quantitative, and reproducible for the assay of VPA-Na in pharmaceutical preparations. The ISE described is easily constructed and a potentiometric apparatus is not too costly. Furthermore, since the ISE method does not require complicated prior separation, it can be used for routine quality control.

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