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Short Communication

Sensitive membrane electrodes for the determination of vitamin B_1 and vitamin B_6

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Introduction

The ion-selective membrane electrode technique has become a satisfactory tool for pharmaceutical analysis, although no pharmacopoeia has so far introduced their use for assays. Recent developments in pharmaceutical analysis with membrane electrodes [1–5] enable the activities of various drugs to be measured directly and selectively, and in most cases, without prior separation of the tested drug from the formulation matrix.

Ion-selective membrane electrodes sensitive to vitamin B_1 and vitamin B_6 based on ion-association extraction systems were first reported by Ishibashi *et al.* [6, 7]. Nitrobenzene and 1,2-dichloroethane were found to be adequate membrane solvents for the respective ion-association complexes with tetraphenylborate and dipicrylamine. Picrolonate [8] and tetra (*m*-methyl-phenyl) borate [9] were also investigated as site carriers for vitamin electrodes. Hassan *et al.* [10] applied the desulphurization procedures using solid potassium hydroxide and alkali plumbite solution to determine vitamin B_1, while Segopaul and Rechnitz [11] proposed for the same purpose a potentiometric method based on measuring the initial rate of carbon dioxide formation from a reaction sequence involving the recombination of thiamine pyrophosphate using pyruvate decarboxylase apoenzyme with the holoenzyme.

Many other recent analytical methods based on high-performance liquid chromatography (HPLC) [12–15], spectrometry [16–18], etc. have been developed for vitamin B_1 and B_6 assay in pharmaceuticals and clinical samples.

The new membrane electrodes, sensitive to vitamin B_1 and vitamin B_6 proposed in this paper, were successfully applied for assaying the respective vitamins in tablet and injectable solutions by standard addition method.

Experimental

Apparatus

The vitamin B_1 and B_6 membrane electrodes were used with a saturated calomel electrode (SCE) (Model 217, Dian Guang, Shanghai, China); pH measurements were performed with a combination glass electrode (Model 231, Dian Guang). E.m.f. values were measured with a pX-meter (Rex, pXSJ-216, Shanghai). All readings were recorded at room temperature under constant magnetic stirring.

Reagents and materials

Solutions of reagent-grade chemicals were prepared with distilled water. All reagents and materials used for membrane preparations were of analytical-reagent grade. The vitamin B_1 and B_6 were used as pure chemicals, such are currently available from China's pharmaceutical industry, and were used as hydro-

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chlorides. Pharmaceutical preparations were obtained from a local drugstore.

Stock solutions (0.1 mol l^{-1}) of vitamin B_1 hydrochloride (thiamine hydrochloride) and vitamin B_6 hydrochloride (pyridoxine hydrochloride) were prepared in distilled water and by keeping both the pH and ionic strength at constant values with acetate buffer solution (pH 3.5).

Electrode preparation

The basic principle of the PVC membrane electrode construction based on ion-association complexes embeded into plastic membrane has been described elsewhere [19–21] and the membrane compositions were: 6.7% electroactive site carrier (dinonylnaphthalenesulphonate and tetra (2-chlorophenyl) borate, respectively), 62.4% *o*-nitrophenyloctyl ether and 30.9% PVC (w/w). The internal filling solution was 10^{-2} mol l^{-1} in the respective vitamin hydrochloride of pH 3.5 (acetate buffer solution). The site carrier in the PVC membranes was converted to the ion-pair complex by soaking the electrode in the respective vitamin hydrochloride (10^{-2} mol l^{-1}) for 24 h. When not in use, the electrodes were stored in air.

Direct potentiometric assay of pharmaceutical preparations

(a) Vitamin B_1 and vitamin B_6 for injections. A 1.00-ml aliquot of the commercial product was diluted with distilled water to a final volume of 50 ml. 2.5 ml of this solution was diluted with distilled water and acetate buffer of pH 3.5 (10% buffer solution, v/v) to a 25-ml volumetric flask. This solution (V_x) was used for analysis. The appropriate vitamin electrode and SCE were immersed in this solution. After potential equilibration by stirring, the e.m.f. value was recorded. 2.5 ml of a 10^{-2} mol l^{-1} standard solution of the respective vitamin hydrochloride solution (pH 3.5) was added and the change in mV reading (accuracy ±0.1 mV) was recorded and used to calculate the vitamin concentration of the respective injectable solution.

(b) Vitamin B_1 and vitamin B_6 tablets. At least 10 tablets were made into a powder. An appropriate amount of the powder, equivalent to *ca* 5 mg vitamin, was weighed and dissolved in a 50-ml volumetric flask; 5.0 ml of acetate buffer of pH 3.5 was added and the solution was made up to volume with distilled water. This solution was divided into 2 × 25 ml portions in which both the indicator and reference electrodes were immersed. After electrode equilibration by stirring and recording the e.m.f., 2.5 ml of 10^{-2} mol l^{-1} standard solution of the respective vitamin hydrochloride solution (pH 3.5) was added and the change in mV reading (accuracy ±0.1 mV) was recorded and used to calculate the vitamin content of the tablets.

Results and Discussion

Membrane materials

Vitamin B_1 and vitamin B_6, in protonated forms, as well as other amines or quaternary ammonium compounds, react with either DNNS or ClTPB to form more or less stable ion-pair complexes. The ion-pair complexes with both vitamins were obtained *in situ*, by soaking the site carrier-based membrane in appropriate 10^{-2} mol l^{-1} solution of vitamin hydrochloride. In all cases, 2-nitrophenyloctyl ether was chosen as plasticizer. The composition of the membranes are given in the Experimental section. When the concentration of the electroactive material in the membrane was varied from 2 to 8%, no significant changes or improvements in the electrode behaviour was noticed.

Electrode responses

The critical response characteristics for all four electrodes are shown in Table 1. The linear response ranges of vitamin B_6 electrodes, and consequently the detection limits are inferior than those of vitamin B_1 electrodes. This is because both ion-pair complexes with vitamin B_6 are more water-soluble due to more lipophilicity character of vitamin B_6. Among the two membrane electrodes sensitive to vitamin B_6, that based on ClTPB has better characteristics with respect to linear range, detection limit and selectivity. These characteristics agreed well with those reported previously [8, 9], when other site carriers were used for electrode construction. These confirm that the performance characteristics of an ISE are mainly related to the molecular structure (hydrophobicity character) of the ion of interest.

Effect of pH

To check the pH dependence of the e.m.f.

Table 1
Response characteristics for vitamin B_1 and B_6 membrane electrodes

Parameter	Vitamin B_1 electrode		Vitamin B_6 electrode	
	DNNS	CITPB	DNNS	CITPB
Slope (mV/log *a*)*	28.0 ± 0.4	27.1 ± 0.5	55.6 ± 0.7	55.3 ± 0.8
Linear range (mol l^{-1})	10^{-1}–10^{-5}	10^{-1}–10^{-5}	10^{-1}–7.1×10^{-4}	10^{-1}–1.2×10^{-4}
Detection limit (mol l^{-1})	5×10^{-6}	5.6×10^{-6}	2.5×10^{-4}	6.3×10^{-5}
(μg ml^{-1})	1.7	1.9	51.4	12.9
Potential drift† (mV h^{-1})	±0.4	±0.5	±0.6	±1.2
Reproducibility‡ (mV)	±0.6	±0.6	±1.2	±1.0
Life time	at least 2 months			
Response time	10–30 s in the concentrated solutions (10^{-1}–10^{-4}) and 3 min in more diluted solutions			

*Standard deviation of average slope value for multiple calibrations in 10^{-2}–10^{-4} mol l^{-1} (for DNNS-based vitamin B_6 electrode, the range was 10^{-2}–10^{-3} mol l^{-1}).
†In 10^{-3} mol l^{-1} solutions.
‡In 10^{-3}–10^{-4} mol l^{-1} solutions (n = 7–9).

readings of the vitamin B_1 and B_6 electrodes, potential–pH curves were constructed for 10^{-3} mol l^{-1} concentration. The plots showed that the potential is practically unaffected by changes in pH over the ranges 2–4.5 for vitamin B_1, and 2–4 for vitamin B_6. At higher pH values there is a gradual decrease in potential because of the gradual increase in the concentration of unprotonated vitamin. For vitamin B_1 in the pH range 2–4.5 the electrodes respond to the diprotonated cation of thiamine.

Selectivity

The interference of various cations was studied by the mixed solution method and calculated as previously described [22]. While vitamin B_1 and B_6 membrane electrodes are reasonably selective over many organic compounds such as amino acids, nicotinamide, caffeine, etc., they are affected in their response by various beta-blocker-drugs (see Table 2). Vitamin B_1 is also an interferent for both vitamin B_6 membrane electrodes. Since the selectivity of these membrane electrodes is related to the free energy of transfer of thiaminate and pyridoxinate anions, respectively, between aqueous and organic phases, the poor selectivity of vitamin B_6 membrane electrodes confirms that the ion-pair com-

Table 2
Selectivity coefficients for vitamin B_1 and vitamin B_6 membrane electrodes*

Interfering species, J	Selectivity coefficient			
	Vitamin B_1 electrode		Vitamin B_6 electrode	
	DNNS	CITPB	DNNS	CITPB
Alanine	$<10^{-4}$	$<10^{-4}$	1.8×10^{-2}	1.3×10^{-3}
Histidine	$<10^{-4}$	$<10^{-4}$	9.6×10^{-2}	9.7×10^{-4}
Lysine	$<10^{-4}$	$<10^{-4}$	6.1×10^{-2}	1.6×10^{-3}
Nicotinamide	$<10^{-4}$	$<10^{-4}$	1.3×10^{-2}	1.5×10^{-2}
Caffeine	$<10^{-4}$	$<10^{-4}$	2.2×10^{-2}	9.2×10^{-2}
Vitamin B_1	—	—	0.35	0.43
Vitamin B_6	1.3×10^{-4}	1.4×10^{-4}	—	—
Atropine	5.6×10^{-2}	5.0×10^{-2}	6.8	12.0
Metoprolol	0.12	0.1	19.4	31.7
Propranolol	42.3	41.7	443	607

*In all cases pH 3.5 (acetate buffer).

Table 3
Determination of vitamin B_1 and vitamin B_6 in pharmaceuticals with vitamin membrane electrodes by standard addition method

Product		Recovery (% of nominal)*	Standard deviation (%)
Vitamin B_1	Tablets (10 mg/tablet)	102.4	2.9
	Injectable solutions (100 mg/2 ml)	102.2	2.5
Vitamin B_6	Tablets (10 mg/tablet)	100.2	1.6
	Injectable solutions (50 mg/2 ml)	101.2	1.5

*All values are average of 6–7 determinations.

plexes of this anion with both DNNS and CITPB site carriers are less oil-soluble than those formed by vitamin B_1.

Analytical applications

All membrane electrodes proved useful in the potentiometric determination of the respective vitamins in the drug substances as well as pharmaceuticals (tablets and injections). Results for measurements of the pure vitamin solutions at $\mu g\ ml^{-1}$ range were performed with good recovery and precision (recovery, 100.5 and 99.5%; standard deviation, 2.2 and 2.1% for vitamin B_1 and vitamin B_6, respectively).

Table 3 shows the analysis results of vitamin determinations by the direct potentiometric method (standard addition) with membrane electrodes. As can be seen in the table, vitamin B_1 was determined with a lower precision than vitamin B_6. This is because the larger error is encountered with a divalent selective electrode (e.g. vitamin B_1 electrodes).

In contrast to the most common methods used for the determination of these vitamins in pharmaceuticals, which are time consuming and require sample pretreatment, the electrode method is simple, fast and selective.

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