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Vitamins B_1 and B_6 tubular electrodes as FIA detectors; their use in the analysis of pharmaceutical products*

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Abstract: Ion-selective electrodes without an inner reference solution and tubular potentiometric detectors for the determination of vitamins B_1 and B_6 in pharmaceutical preparations by flow injection analysis (FIA) are reported. The membranes were prepared with the vitamin tetra(2-chlorophenyl)borate (TCPB) dissolved in *o*-nitrophenyloctyl ether (*o*-NPOE) and immobilized on PVC.

Intrinsic behaviour of the tubular detectors was assessed using a low-dispersion single-channel FIA manifold and was compared with conventionally-shaped electrodes using the same membrane.

Data obtained in the determination of vitamins B_1 and B_6 in pharmaceutical preparations with a double channel flow injection manifold incorporating the tubular detectors are presented and compared with those obtained by the U.S. Pharmacopeia method and by direct potentiometry with conventionally-shaped electrodes.

Keywords: *Vitamins B_1 and B_6; tubular electrodes; flow injection analysis; pharmaceutical preparations.*

Introduction

The advantages of using ion-selective electrodes for determining organic species in pharmaceutical preparations are well-known as they may be an expedient alternative to the time-consuming and tedious procedures suggested in the pharmacopeias [1–4].

The advantages of the potentiometric measurements can be increased if we associate these to those of flow injection analysis (FIA). In effect, the fact that potentiometry may perform determinations over a large concentration range without any change in sensitivity does not imply the use of complex flow injection analysis manifolds as would be the case with other detection processes [5, 6].

The use of an FIA manifold with potentiometric detection may present some difficulties, generally of a mechanical nature, when conventionally-shaped electrodes with a cascade arrangement [7] are used. These can be overcome by using tubular detectors which are fixed to the manifold, as we have shown previously, thus building units which are sensitive to anionic organic compounds of pharmaceutical interest [8–10].

Experimental

Reagents and solutions

All solutions were prepared with deionized water (specific conductance less than 0.1 μS cm^{-1}). All chemicals were of analytical reagent grade without further purification.

Vitamin B_1 and B_6 were used as pure chemicals; these were obtained from Roche Pharmaceutics (Nutley, NJ, USA).

Standard vitamin B_1 and B_6 solutions were prepared daily by carefully weighing the respective hydrochlorides; dilutions were made whenever necessary. When not in use, the vitamin solutions were protected from light.

Apparatus

Evaluation of the conventionally-shaped ion-selective electrodes was done with standard equipment and techniques.

The FIA manifold (Fig. 1) included a Gilson Miniplus peristaltic pump (Middleton, WI, USA), Teflon tubing (0.8 mm i.d.) for connections and some auxiliary home-made devices, namely joints, grounding electrode and reference electrode supports, constructed as reported previously [11].

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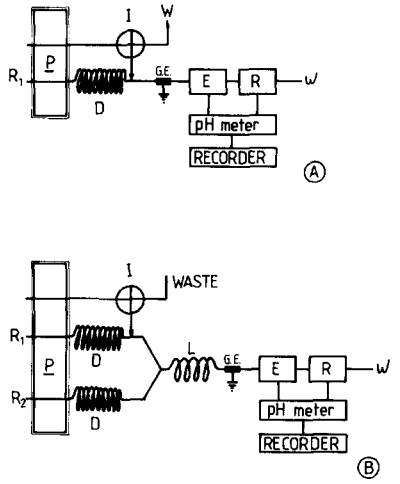

Figure 1
Flow injection low dispersion manifold used in the tubular electrode evaluation (A) and in the vitamin B_1 and B_6 determination in pharmaceutical preparations (B). P, Peristaltic pump; I, injection valve (loop 200 μl); G.E., grounding electrode; L, dilution coil (50 cm length); D, pulse damper coil (2 m); E, tubular electrode; R, reference electrode; W, waste. The total flow rate was 6.3 ml min^{-1}.

Samples were inserted in the manifold using a Rheodyne 5020 valve (six-port three-way Rheodyne, Cotatis, CA, USA) or a Hamilton HVLX 8/7 (eight-port and four-way, Hamilton, Reno, NV, USA).

For the determinations of vitamin B_1 and vitamin B_6 by the US Pharmacopeia method, a Shimadzu, model RF-540 spectroflurimeter (Shimadzu, Columbia, MD, USA) and Philips model PU 8625 spectrophotometer (Philips, Eindhoven, The Netherlands) were used.

Preparation of conventionally-shaped and tubular electrodes

Ion-association complexes were prepared by dissolving potassium tetra(2-chlorophenyl) borate (350 mg) in the minimum amount of acetone to give a clear solution. An aqueous solution of vitamin B_1 or B_6 (0.1 M, 25 ml) was added to form a white precipitate. The acetone was removed by evaporation at 40°C. The solid precipitate remaining in the aqueous phase was recovered by centrifugation, redissolved in minimum acetone, and washed again with the 0.1 M vitamin solution. The process of acetone evaporation, centrifugation, and redissolving in acetone was repeated four times to remove any excess tetra(2-chlorophenyl)borate.

In order to prepare the membranes, sensors whose preparation has been mentioned above, were dissolved in *o*-nitrophenyloctyl ether (*o*-NPOE) and PVC to create membranes with the following percentages: vitamin B_1 sensor–*o*-NPOE–PVC (6.9:62.1:31.0, %w/w) and vitamin B_6 sensor–*o*-NPOE–PVC (2.3:66.7:31.0, %w/w).

Using membranes with the sensor solution immobilized in PVC, previously-described procedures were used in the construction of the conventionally-shaped electrodes [12] and tubular detectors [13].

Procedures for the direct potentiometric and FIA assay of pharmaceutical preparations

In order to perform direct potentiometric or FIA assay, significant amounts (10 ampoules, one bottle of syrup, or at least 20 tablets) of pharmaceutical preparations were used, from which an amount corresponding to approximately 10 mg of the vitamin to be determined was removed and dissolved in deionized water in a 50.0 ml volumetric flask.

Direct potentiometry determinations were done on samples of the aforementioned solution to which an equal volume of a 0.2 M formic acid solution (pH = 4 adjusted with LiOH) was added as a pH and ionic strength adjuster.

Regarding the FIA determinations, the aqueous solution resulting from the dissolution of the pharmaceutical product was introduced without any prior treatment into the manifold. In the case of tablets, the passage of undissolved solids was avoided by previously filtering the sample or by placing a filter at the manifold point of entry.

Results and Discussion

Behaviour of conventionally-shaped electrodes

The overall operating characteristics of conventionally-shaped electrodes without an internal reference solution were assessed twice a day on the basis of repeated tracing of the calibration curves during the lifetime of the electrodes. Regarding the vitamin B_1- and B_6-sensitive electrodes, we determined the lower limit of linear response (LLLR), the practical limit of detection (PLD), the slope (S), the response time and potential stability (Table 1).

The tracing of the variation curves of the potential with respect to the pH of the solutions with a fixed concentration of the principal ion (10^{-2} M) enabled us to identify an interval of 2–4 pH units for the vitamin B_1 and

Table 1
Response characteristics for vitamin B_1 and B_6 conventionally-shaped electrodes without an inner reference solution*

Parameter	Vitamin B_1 electrode	Vitamin B_6 electrode
LLLR† (M)	8.0×10^{-6}	2.0×10^{-5}
PDL‡ (M)	1.0×10^{-6}	3.9×10^{-6}
Slope (mV dec^{-1})	32.6 ± 0.9	62.6 ± 0.3
Response time (s)	~15	~15
Potential stability (mV day^{-1})	±0.7	±1.2
Life-time (months)	>10	>8

*Obtained in solutions with the ionic strength adjusted to 0.1 M with LiCl.
†Lower limit of linear response.
‡Practical limit of detection.

Table 2
Potentiometric selectivity coefficients (log K^{pot}) for vitamin B_1 and B_6 conventional and tubular electrodes*

	Vitamin B_1		Vitamin B_6	
Interferent	Conventional	Tubular	Conventional	Tubular
Sodium	+0.68 ± 0.09†	−2.91 ± 0.09‡	−2.38 ± 0.05‡	−2.13 ± 0.06‡
Potassium	−0.36 ± 0.03†	−2.64 ± 0.06‡	−1.34 ± 0.02‡	−1.04 ± 0.04‡
Ammonium	−0.43 ± 0.02†	−2.78 ± 0.05‡	−1.62 ± 0.01‡	−1.17 ± 0.02‡
Proton	−0.21 ± 0.03†	−0.58 ± 0.03‡	−2.08 ± 0.06‡	−1.07 ± 0.07‡
Lithium	−0.54 ± 0.04†	—	−2.38 ± 0.06†	—
Magnesium	−3.91 ± 0.06‡	−2.57 ± 0.07‡	−3.66 ± 0.03†	−3.19 ± 0.05†
Calcium	−3.11 ± 0.01‡	−2.39 ± 0.05‡	−3.2 ± 0.07†	−2.81 ± 0.07†
Pyridoxine (B_6)	−0.41 ± 0.08†	−1.02 ± 0.08‡	—	—
Nicotinamide	−0.47 ± 0.05†	−3.74 ± 0.04‡	−2.23 ± 0.04‡	−1.83 ± 0.08‡
Cyanocobalamine	−1.32 ± 0.02†	−2.89 ± 0.07†	−2.24 ± 0.05†	−2.18 ± 0.03†

*Average of two determinations with three electrodes.
†Mixed solution method with interferent ion concentration fixed at 5×10^{-3} M.
‡Separated solution method with the primary ion and interferent concentration fixed at 5×10^{-3} M.

B_6 electrodes, within which variations in the acidity of solutions did not alter the electrode potential. Using the separate solution method [14] for those cases where the interfering and principal ions had the same charge, and the mixed solution method [14] for those in which the respective charges were different, we also determined the corresponding values for the potentiometric selectivity coefficients for both types of electrodes (Table 2).

The operating characteristics of those electrodes whose construction and assessment are referred to herein, compare favourably to those of other electrodes reported in the literature and sensitive either to vitamin B_1 [15–23] or vitamin B_6 [17–19]. We make particular note of the improvements regarding selectivity, speed of response, potential reproducibility, and the lifetime of the units. These results are a consequence of the choice of the sensor system and the type of construction used.

Behaviour of tubular electrodes

Intrinsic operating characteristics of the tubular detectors were assessed with a low-dispersion single channel FIA manifold (Fig. 1A) which was set up in such a manner to allow the sample plug to undergo practically no dilution between the injection point and the detector with practically no dispersion in the system. The carrier (6.3 ml min^{-1}) was a (0.1 M) LiCl solution with a fixed vitamin concentration (5×10^{-5} M) in which the vitamin standards (200 µl) were injected having a 0.1 M ionic strength adjusted with LiCl. Tubular detectors sensitive to vitamins B_1 and B_6 assessed under these experimental conditions presented operating characteristics very similar to those of conventionally-shaped electrodes when evaluated by the batch method.

The extremely high speed of response of the FIA manifold permitted sampling rates of approximately 180 samples per hour for both type of detectors. This characteristic is a consequence of the rapid response of the detection system upon the arrival of the sample plug and the equally rapid return to the baseline due to the fact that the internal

diameter of the tubular detectors create practically no distortion of the hydrodynamic characteristics of the sample.

In spite of the vitamin background concentration in the carrier, the tubular electrodes, particularly those sensitive to vitamin B_6, presented a baseline drift attributed to a certain degree to the ion-association complex solubility which is felt more strongly with continuous flow systems than when used in batch procedures.

Determination of vitamins B_1 and B_6 in pharmaceutical products

With a view to the automatic determination of vitamins B_1 and B_6 in pharmaceutical products, a double-channel FIA manifold (Fig. 1B) was set up to obtain, without any prior preparation, the pH adjustment and ionic strength necessary within the system to adjust the composition of the samples to the requirements of the measurements in the potentiometric detectors. We assessed the influence of the several flow system parameters, namely the injection volume, the length of the reaction coil and the flow rate, on the response of the tubular detectors. The results for the vitamin B_1- and B_6-sensitive detectors were similar to those obtained with the same tubular electrodes when these were incorporated in a low dispersion manifold. The only difference being that which resulted from the dilution of the sample plug in the double-channel system, namely the lower limit of linear response. Thus, for the double-channel system, we created experimental conditions which would mimic those used for a single channel manifold. An injection volume of 200 μl and an overall flow of 6.3 ml min^{-1} were used whereby both channels contributed equally to the total flow. A solution with an ion concentration double to that used in the single channel system was introduced through the auxiliary channel, as this would be diluted by half at the confluence point (Fig. 1B).

With the double channel manifold we noted a baseline drift which was more significant with the vitamin B_6 tubular detector. This would suggest that systematic calibrations of the system ought to be done. This difficulty which would affect the rate and, eventually, the quality of the determinations was overcome by using an eight-port and four-way injection valve which, as demonstrated in a previous paper with a spectrophotometric detector [24],

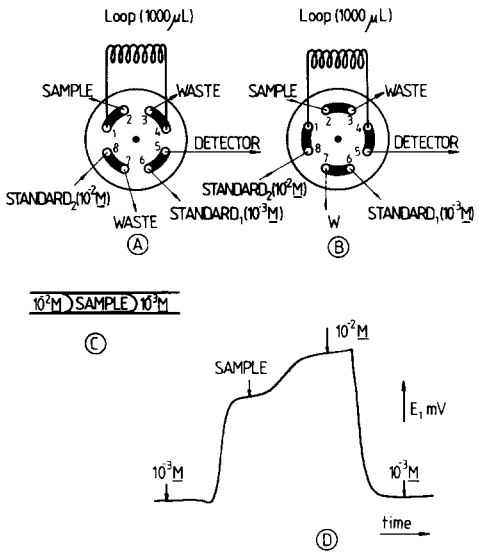

Figure 2
The eight-port injection valve behaviour: (A) load position; (B) injection position; (C) relative position of samples and standards in the manifold after injection; (D) typical recorder output.

would permit the placing of the sample plug between two different solutions. When this valve is placed in the load position (Fig. 2A), the flow consisted of a fixed concentration vitamin solution (10^{-3} M standard) produced a signal which formed the baseline (Fig. 2D). In the injection position (Fig. 2B), the sample (1000 μl) was introduced into the system together with another standard solution (10^{-2} M), thereby reaching the detector between the two standards (Fig. 2C). A recorder output on which the potential values corresponding to those portions of the solutions which sequentially come into contact with the detector was obtained (Fig. 2D).

In order to obtain a well-defined signal corresponding to the vitamin concentration levels which come into contact with the detector the use of a relatively large (1000 μl) injection volume was required. This value was determined to be the minimum necessary volume for a complete separation of the respective analytical signals (Fig. 2D), thereby reducing the sampling rate.

Double-channel FIA manifolds with potentiometric detection were used for the determination of vitamin B_1 in pharmaceutical preparations, parallel to direct potentiometric determinations with conventionally-shaped electrodes and by the method suggested by the US Pharmacopeia [25] (Table 3). Regarding

Table 3
Vitamin B_1 determination in pharmaceuticals by direct potentiometry, FIA with tubular potentiometric detectors* and U.S. Pharmacopeia method†

Commercial product	Vitamin B_1 content (% w/w)		
	Potentiometry	FIA*	USP*
Totaforte (2.6%)	2.6 ± 0.2	2.5 ± 0.1	2.5 ± 0.2
Dragavit B_1 retard (53.9%)	52.9 ± 0.6	54.9 ± 1.1	53.8 ± 2.2
Becozyme xarope (0.10%)	0.11 ± 0.02	—	0.11 ± 0.03
Vigorvil xarope (0.68%)	0.92 ± 0.01	—	0.93 ± 0.08
Vitamin B_1 fortíssima (5%)	5.2 ± 0.5	5.7 ± 0.5	4.6 ± 1.3
Aboplex (1.5%)	1.61 ± 0.09	—	1.56 ± 0.18
Bê–Cê oral (0.20%)	0.26 ± 0.02	—	0.29 ± 0.04

*Average of six determinations.
†Average of four determinations.

the potentiometric determinations, the values presented are the result of six determinations with two electrodes or tubular detectors, whereas the values presented for the reference method correspond to an average of four determinations.

Few determinations of vitamin B_6 were done as both the electrodes and the tubular detectors could not be used in polyvitaminic preparations given their low selectivity for other vitamins, namely vitamin B_1. Nevertheless, in the case of capsules containing vitamin B_6 only as the principle active vitamin (Bénedon), our results were 38.8 ± 0.6 mg for direct potentiometry, 39.1 ± 0.2 mg for FIA and 39.1 ± 0.9 mg for the US Pharmacopeia method. In all cases in which vitamins B_1 and B_6 were determined in pharmaceutical preparations, the results obtained by all three methods were in good agreement with each other. Additionally, we stress the greater precision of the results obtained with the two potentiometric procedures.

Conclusions

The conventionally-shaped electrodes without an inner reference solution described herein present good operating characteristics which are at least, as good as those of the best available electrodes sensitive to this species [15–23]. We particularly note the long lifetimes of the electrodes, especially as these incorporate mobile carrier membranes. The construction procedure used makes it possible, without resorting to sophisticated technical means, to prepare tubular detectors which can be easily fixed to the FIA manifold, thereby creating a stable set-up which is easily handled and free of mechanical problems, thus making potentiometric detection useful for routine determinations.

Operating characteristics of the tubular detectors are very similar to those of conventionally-shaped electrodes when the former are used in a low dispersion FIA manifold which mimics the batch conditions of conventionally-shaped electrodes. Results of the vitamin B_1 and B_6 determinations in pharmaceutical preparations obtained with a FIA manifold with tubular potentiometric detectors demonstrates the feasibility of substituting normally slow and costly reference methods with a rapid and easily automated procedure.

Finally, we stress that the eight-port and four-way injection valve used for this study is a good solution for those difficulties resulting from the baseline drift in certain potentiometric detectors, making it possible for a simultaneous calibration of the system with each sample injection.

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