ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
25 (2001) 713–720

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Multi-task flow system for potentiometric analysis: its application to the determination of vitamin B_6 in pharmaceuticals

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Received 28 April 2000; received in revised form 23 November 2000; accepted 24 November 2000

Abstract

A flow set-up based on the sequential injection analysis concept was designed, aiming at increased automation and robustness of procedures related with potentiometric detection in pharmaceutical control. In this sense, programmable set-up calibration, ion-selective electrode characterization, standard addition techniques and titration procedures could be carried out without any stock solutions conventional handling or modification on the physical structure of the flow system. Evaluation of a flow-through vitamin B_6 selective electrode and its application to routine analysis of pharmaceuticals were selected as models to demonstrate the system potentialities. The system ability to generate in-line calibrating solutions was verified by comparing the results with those obtained with solutions prepared by the manual procedure. The vitamin B_6 determination in pharmaceutical products was carried out and in-line performed recoveries gave values within 97.4–103.5%. The system ability to perform titrations was ascertained using the precipitation reaction of vitamin B_6 with tetraphenylborate. Other profitable features such as lower reagent consumption with a low effluent generation volume were also achieved.

Keywords: Potentiometry; Vitamin B_6 selective electrode; Sequential injection analysis

1. Introduction

When analysis of any compound is intended, selection of the adequate technique, mainly considering its selectivity, price, analytical range and workload, is required. Potentiometry with ion-selective electrodes (ISEs) meets these requirements

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0731-7085/01/$ - see front matter
PII: S0731-7085(00)00581-1

becoming an advantageous analytical tool, especially when monitoring tasks and/or analyses of complex matrices such as pharmaceutical preparations are involved [1]. Therefore, the frequent appearance in the literature of new ISEs is well justified even knowing that every time a novel potentiometric sensor is prepared or is used for the first time, its evaluation involves different methodologies, the use of numerous solutions and laborious work.

Further improvement of analytical procedures was achieved by proposal of flow injection analysis based systems using ISEs as detection devices [2]. Enhancement of ISE behavior, such as increased sensitivity, decrease of chemical and mechanical interferences, shorter response time and higher reproducibility, was enabled. Flow-injection systems can also provide facilities in sample pretreatment like the addition of a reagent to the sample solution (for ionic strength and/or pH adjustment) rendering robust and less time-consuming procedures. Calibration using a single standard solution has also been exploited by several authors [3–6], although complex signal treatment is required. More commonly, different manifolds are usually referred for ISE study and sample analysis, and the number of solutions that should be prepared are most of times similar to the corresponding conventional procedures.

In this work, a simple flow set-up based on the sequential injection analysis principle [7] that can afford both ISE characterization or analytical determinations based on commonly used potentiometric methods is proposed. Sequential injection analysis is an expansion of the on-flow analysis concept based on the use of discrete solution volumes and variable flow conditions. In its basic configuration, a SI system requires a computer-controlled bi-directional flow-driven unit and a stream selecting multi-port valve. To minimize the workload related with solution preparation, a low-cost three-way solenoid actuated valve was coupled to a lateral port of the main stream selecting valve. The use of a solenoid valve under binary sampling conditions [8] provides reproducible mixing ratios of two solutions, being herein exploited in the preparation either of calibration solutions, and of reagent to sample additions directly from conventionally prepared stock solutions. The usefulness of the proposed system is evidenced in the determination of vitamin B_6 in multi-vitamin pharmaceutical preparations.

2. Experimental

2.1. Solutions

All solutions were prepared with distilled and de-ionized water (conductivity < 0.1 μS/cm). Analytical grade chemicals were used without further purification.

Pyridoxine hydrochloride (vitamin B_6; Roche Pharmaceutics, USA), lithium chloride (Merck), lithium hydroxide (Merck), formic acid (Riedel-deHaen) and sodium tetraphenylborate (Fluka) were used throughout.

For preparation of the selective membrane, 2-nitrophenyl octyl ether (Sigma), *bis*(triphenylphosphoranylidene) ammonium chloride (Sigma), high molecular weight poly(vinyl chloride) (PVC) (Fluka) and tetrahydrofuran (THF) (Riedel-deHaen) were used.

Vitamin B_6 stock solutions of 1.0×10^{-1} and 1.0×10^{-3} mol/l were daily prepared by weighing the solid and dissolving it in a 0.1 mol/l LiCl solution used as ionic strength adjuster. For comparison purposes, less concentrated solutions were obtained after dilution of the previous ones.

Whenever simultaneous pH and ionic strength adjustments were required, the LiCl solution was replaced by a HCOOH/LiOH buffer solution (pH 4.5; ionic strength, 0.1 mol/l). These ionic strength or pH adjuster solutions were also used as carrier streams in the proposed flow set-up. A concentration of 1.0×10^{-6} mol/l vitamin B_6 was maintained in the carrier solutions in order to minimize the baseline drift and to preserve the lifetime of the selective membrane.

2.2. Electrode preparation

Tubular vitamin B_6 selective electrodes with a PVC membrane and without internal reference solution were constructed as previously described [9]. The sensor solution was prepared by mixing

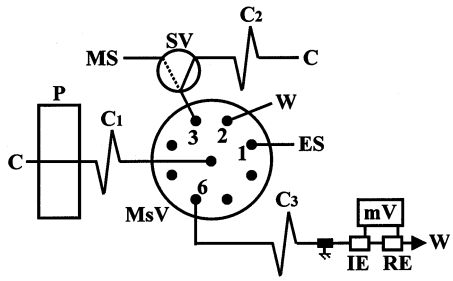

Fig. 1. System flow diagram. P, Peristaltic pump; C,carrier solution of ionic strength and/or pH adjuster; C_1, main holding coil; C_2, auxiliary holding coil; C_3, reaction coil; SV, solenoid valve; MsV, multi-port injection valve; MS, mother standard solution; ES, external standard solution; W, waste; IE, indicating electrode; RE, reference electrode; mV, decimilivoltammeter.

0.04 g of the *bis*(triphenylphosphoranylidene) ammonium pyridoxine in 2.0 g of 2-nitrophenyl octyl ether. Then, 0.18 g PVC amount was dissolved in 6 ml THF, and 0.4 ml sensor solution was added to it. This solution was dropwise applied over the inner wall of a central hole drilled in a graphite and epoxy resin conductive support. After complete drying, the membrane was maintained in contact with a 1.0×10^{-3} mol/l vitamin B_6 solution for about 12 h. The electrodes were kept in contact with the same solution when not in use.

2.3. Flow manifold

A schematic view of the proposed system is depicted in Fig. 1. It comprised a Minipuls 3 Gilson (Viliers-le-Bell, France) peristaltic pump with a PVC pumping tube (i.d., 1.65 mm) of the same brand, a VICI C25-3118.E, eight-port stream selecting valve (Valco Instruments, Houston, TX), a 161T031 NResearch three-way solenoid valve (Stow, MA), and a Crison MicropH-2002 potentiometer to which a 90-02-00 Orion AgCl/Ag reference electrode and the tubular detector were connected. Some homemade devices such as joint pieces, grounding electrode, supports for tubular and reference electrodes as described elsewhere [10] were also used. Coils C_1, C_2 (400 cm) and C_3 (100 cm), and flow lines were made of 0.8 mm i.d. PTFE tubing.

The rotation speed of the peristaltic pump (P), the rotor position of the eight-port valve (MsV) and the solenoid valve (SV) on/off switching were controlled through a PCL-711 Advantech interface card coupled to a microcomputer running a software written in QUICK BASIC 4.5.

2.4. Procedures

The manifold control parameters requested by the microcomputer before carrying out the proce-

Table 1
System control parameters used for direct potentiometric measurements of externally prepared solutions (steps 1 and 2), of in-line prepared solutions (steps 3, 4 and 5) and after in-line mixing of solutions at variable ratios (steps 6–9)

Step	Task	MsV		SV		Flow rate (ml/min)	Pumping direction[a]
		Port number	Time (s)	State	Time (s)		
1	Sampling	1	16	–	–	4	r
2	Detection	4	55	–	–	6	f
3	Filling C_2	3	28	–	–	4	f
4	Dilution	3	24	On Off	t_{on} $4-t_{on}$	4	r
5	Detection	4	55	–	–	6	f
6	Sampling	1	24	–	–	4	r
7	Filling C_2	3	24	–	–	4	f
8	Dilution	3	24	On Off	t_{on} $4-t_{on}$	4	r
9	Detection	4	55	–	–	6	f

[a] f, Forward; r, reversal.

Table 2
Steps sequence according to the selected procedure

Selected procedure	Steps performed								
	1	2	3	4	5	6	7	8	9
Direct measurements of samples	x	x							
In-line calibration			x	x	x				
Separated solutions method			x	x	x				
Fixed interference method						x	x	x	x
Standard additions method						x	x	x	x
Titration						x	x	x	x

dures are defined in the columns of Table 1. Table 2 depicts the step sequence according to the selected procedure.

Initially, the holding coil (C_1) and transmission line from the eight-port MsV up to the detection system (C_3) were filled with the carrier solution (C). This was achieved by selecting port 6 of the MsV and positioning the peristaltic pump in the forward pumping mode until the achievement of a detector stable signal. Afterwards, the pump movement was reversed, and ports 1 and 3 of the MsV were sequentially selected in order to fill the respective access channels with the external prepared solution and stock solution. When port 3 was selected, the SV was switched on in order to fill the conduit connected to its normally closed entry with the stock solution. In these two steps, after solutions reached the holding coil C_1, they were discharged towards waste (W) by selecting MsV port 2 and driving the pump in the forward mode.

The effect of the inserted sample volume was investigated by loading an aliquot of an externally prepared vitamin standard solution (ES) into C_1 (Tables 1 and 2, step 1) and directing it towards the detector (Tables 1 and 2, step 2). These two steps were repeated but with increase of sampling time from 4 to 28 s with increments of 4 s. Experiments to check the overall electrode behavior were performed by running a set of ES vitamin B_6 calibration solutions and by settling the sampling time interval at 16 s.

By means of software selection of an in-line calibration sub-routine, the inner calibration solutions (IS) were on-line prepared by dilution of the stock solutions (MS), performed with the sequence assigned in Table 2. The auxiliary holding coil (C_2) was loaded with carrier solution (step 3), afterwards step 4 was carried out and, at the same time, a set of electric pulses were sent to the SV valve. Under this condition, slugs of the MS solution (1.0×10^{-1} or 1.0×10^{-3} mol/l) were inserted into the coil C_1 in tandem with slugs of the carrier solution aspirated from C_2 while SV was off. To generate three different dilution degrees, the time interval to switch the SV on was settled to 1.0, 5.0 and 10.0% of each SV on/off cycle period (t_1). These experiments were performed with flow rates of 1, 2, 4 and 6 ml/min and SV cycle periods (t_1) of 1.0, 2.0, 4.0, 6.0 and 8.0 s.

The study of the potential interfering ions on the vitamin B_6 determination was performed by employing the separated solutions and the fixed interference methods [11]. After selecting the sub-routine corresponding to the separated solutions method, two sets of IS solutions were on-line prepared following steps 3, 4 and 5 (Table 2) and using as MS solutions either a 0.1 mol/l vitamin B_6 solution or the interfering ion solution with the same concentration. The t_1 period was fixed at 4.0 s and the time intervals to switch SV on (t_{on}) were 1.0, 5.0 and 10.0% of the t_1 period, maintaining the flow rate at 4.0 ml/min. In order to implement the fixed interference method, the auxiliary coil (C_2) was filled with the interfering ion solution (ca. 1.7 ml) presented to port 1 of the MsV valve (Tables 1 and 2, steps 6 and 7). To this slug was then added vitamin B_6 solution by performing steps 8 and 9.

The system operation cycle comprising steps 6–9 were also carried out by selecting the standard additions or titration analytical applications. The sampling period (t_1) was fixed at 4 s and the time interval to switch SV on (t_{on}) was settled in order to achieve either the appropriated standard addition to the sample contained in C_2 or the addition of a MS titrant solution.

3. Results and discussion

3.1. Electrode evaluation

When an electrode is employed as the detector in a flow system, the transient signal can be affected by some factors comprising the kind of detector used and its response time, the volume of the sample solution injected and also the manifold design. When considering a single line flow configuration, by increasing the inserted sample aliquots, the signals provided by the detector became similar to those obtained when the electrodes are operated under stationary conditions. In this sense, the analytical signal depended only on the analyte concentration and on the detector features. In the proposed system, this condition was attained by inserting vitamin B_6 solution volumes greater than 800 μl and directing the solution towards the flow-through electrode at 6 ml/min flow rate (steps 2, 5, 9; Tables 1 and 2). Aiming at the developed electrode evaluation, the system was operated in the aforementioned conditions by implementing an analytical cycle comprising steps 1 and 2 referred to in Tables 1 and 2. Thus, 1067 μl aliquots of ES vitamin B_6 calibrating solutions were inserted into the holding coil C_1 and afterwards directed towards the detector, yielding part of the results shown in Table 3. The repeatability of the potential readings was also assessed by calculation of the standard deviation of the signals produced by performing 15 analytical cycles, at three concentration levels (Table 3). Considering the slopes of the analytical curves and repeatability assays, no significant differences were observed between results obtained with and without pH adjustment and those previously reported for the same ISE unit [12].

The potentialities of the proposed system were then exploited to accomplish in-line preparation of calibrating solutions and to reduce reagents consumption. To evaluate the system ability to perform this task, a control cycle sub-routine was developed following steps 3, 4 and 5 of Tables 1 and 2. During aspiration of the MS solution into the coil C_1 (step 4), the SV was switched on/off several times, according to dilution required by the operator, in order to enable a string comprising slugs of the MS solution in tandem with slugs of the carrier solution. The flow reverse taking place between the solution aspiration and carry forward towards the detector (step 5) promoted the dispersion of the Ms slugs in the carrier aspirated through C_2 during the off periods of the SV activation cycles. To find the experimental conditions that would produce an IS solution with the intended concentration, the t_1 period and aspiration flow rate were both varied from 1 to 8 s

Table 3
Characteristics of the vitamin B_6 selective tubular electrode working under adjusted ionic strength (I) or simultaneous pH and ionic strength (II) conditions, and for on-line generated (IS) or externally prepared (ES) solutions

	I		II	
	IS	ES	IS	ES
Linearity range (mol/l)	5×10^{-5}–1×10^{-2}	5×10^{-5}–1×10^{-2}	1×10^{-4}–1×10^{-2}	1×10^{-4}–1×10^{-2}
Slope (mV/decade)	57.0 ± 0.2	57.1 ± 0.6	55.5 ± 0.6	56.5 ± 0.6
Repeatability ($\pm mV$)				
5.0×10^{-3} mol/l	±0.58	±0.50	±0.36	±0.52
1.0×10^{-2} mol/l	±0.60	±0.66	±0.47	±0.56
5.0×10^{-2} mol/l	±0.32	±0.53	±0.43	±0.42

and from 1 to 6 ml/min, respectively, while the aspirated sample volume (step 4 delay) was selected in order to ensure the steady-state signals. It was found that longer periods corresponded to an oscillating analytical signal that resulted either from the lack of a homogeneous dispersion over the IS solution or from the short detector response time. The effect was corroborated by the behavior observed when higher flow rates were adopted. For shorter SV on/off periods, the IS solutions generated for ratios of t_1/t_{on} between 10 and 100 promoted analytical signals with a negative bias tendency when compared with those obtained for ES solutions, regarding the inertia of liquids during the selected t_{on} pulses. One could also observe this effect minimized for aspiration flow rates higher than 4 ml/min.

As a compromise between the opposite described effects, the SV on/off period (t_1) was maintained at 4 s and, to generate four different IS solutions, the time interval to switch on SV (t_{on}) was respectively settled at 1.0, 5.0, 10.0 and 50% of t_1. For the time delay of 24 s of step 4, it was possible to repeat sequentially the SV on/off cycle six times. By adopting a constant flow rate of 4 ml/min (66.7 μl/s), the overall solution volume loaded into the holding coil C_1 was 1600 μl, which determines analytical signals similar to those obtained by injection of 1067 μl ES solutions. Two sets of IS solutions with concentrations 1.0×10^{-5}, 5.0×10^{-5}, 1.0×10^{-4}, 5.0×10^{-4} mol/l and 1.0×10^{-3}, 5.0×10^{-3}, 1.0×10^{-2}, 5.0×10^{-2} mol/l vitamin B_6 were obtained using the 1.0×10^{-3} mol/l MS solutions or 1.0×10^{-1} mol/l vitamin B_6, respectively. The results obtained with these IS solutions are shown in Table 3. By comparison of the results obtained with IS and ES solutions, the linear equations E_{IS} (mV) = 1.02 ($\pm$0.07) E_{ES} (mV) = −2 ($\pm$8); ($R = 0.9997$) and E_{IS} (mV) = 0.98 ($\pm$0.02) E_{ES} (mV) + 0 ($\pm$3); ($R = 0.9992$) were obtained under adjusted ionic strength and simultaneous pH and ionic strength, respectively. Both equations allow one to conclude of the statistical similarity between the two procedures. Moreover, the volumes of each MS solution inserted to generate in-line IS solutions were 16.0, 80.0, 160 and 800 μl. The total volume of the MS solution used to prepare four IS solutions was 1056 μl, which almost matches each aliquot of ES necessary to provide a steady-state signal in the detection device.

3.2. Selectivity coefficient evaluation

Selectivity is one important feature of ISE electrodes that is expressed by the potentiometric selectivity coefficients with regard to the ions usually present in real samples. For this evaluation, the separated solutions or the fixed interference methods are usually employed.

The first approach was performed with the proposed system by simply replacing the vitamin B_6 solution by another MS solution of the interfering ion under study. Changing the t_{on} of the SV valve enabled the quick evaluation of the interfering ion at several main and interfering ion-matched concentration values. As in the previous section, for a t_1 period of 4 s, the t_{on} of the SV was varied in order to produce solutions with different concentrations of the interfering species. For inorganic interfering ions, MS solutions at two concentration levels were selected, involving five different MS solutions. The results were compared with those obtained with the system operating in the conventional way using ten ES solutions (Table 4). If a higher number of concentration levels were selected, the difference in the number of solutions that must be prepared in the batch mode would increase as the number of MS solutions required would probably still be the same.

For the fixed interference method, six different concentrations of vitamin B_6 solutions were in-line prepared, maintaining a background concentration of the interfering ion at 5.0×10^{-3} mol/l. For this, coil C_2 was previously loaded with solution of interfering ion by carrying out steps 6 and 7 described in Tables 1 and 2. The results are presented in Table 4 and, as can be seen, they are in a good agreement with those obtained with solutions prepared by the conventional way, thus proving the effectiveness of the proposed system to prepare in-line mixed solutions, also useful when sample matrix simulation is required.

Table 4
Potentiometric selectivity coefficients attained for the vitamin B_6 selective electrodes using ES or IS solutions and the separated solutions (*) or fixed interference methods

Interferents	1×10^{-3} mol/l concentration			5×10^{-3} mol/l concentration		
	IS	ES	RD (%)	IS	ES	RD (%)
H^{+}*	$+0.777 \pm 0.004$	$+0.775 \pm 0.034$	−0.26	$+0.579 \pm 0.018$	$+0.573 \pm 0.030$	−0.97
Na^{+}*	-1.751 ± 0.044	-1.829 ± 0.005	+4.45	-2.380 ± 0.034	-2.346 ± 0.023	−1.76
K^{+}*	-1.487 ± 0.031	-1.491 ± 0.032	+0.23	-2.007 ± 0.034	-2.038 ± 0.068	+1.52
NH_4^{+}*	-1.466 ± 0.105	-1.508 ± 0.041	+3.11	-2.043 ± 0.089	-2.043 ± 0.081	−0.01
Ca^{2+}[a]	–	–	–	-2.584 ± 0.021	-2.549 ± 0.141	−1.37
Mg^{2+}[a]	–	–	–	-2.369 ± 0.028	-2.374 ± 0.035	0.21

[a] Interfering ion concentration, 5×10^{-3} mol/l.

3.3. Analytical applications

The analytical feasibility of the system was ascertained by applying it to vitamin B_6 determination in a set of multi-vitamin formulations. Direct potentiometric measurements were performed by following in-line system calibration and afterwards following steps 1 and 2 for sample analysis (direct measurements of samples; Table 2). The accuracy of results was assessed employing the standard addition technique. Samples plus standard IS solutions were in-line prepared by following the same strategy described for ISE selectivity evaluation using the fixed interference method (Tables 1 and 2, steps 6–9). In the present situation, steps 6 and 7 enabled one to fill the C_2 coil with each tested sample. The results obtained are presented in Table 5. Some organic compounds present in the samples could exert strong interference on the selected ESI detector [12]; nevertheless, this effect was not observed considering that results present a recovery ranging from 97 to 103%.

Taking into account the fact that vitamin B_6 presents acidic behavior and can be precipitated by tetraphenylborate, a titration procedure was implemented in order to demonstrate the multitask ability of the system. Experiments were performed using a 5.0×10^{-3} mol/l tetraphenylborate solution as titrant (MS) prepared in 0.1 mol/l LiCl solution. The titration procedure was implemented following the subroutine including steps 6–9 (Tables 1 and 2) and resorting to a 1×10^{-4} mol/l vitamin B_6 solution as sample. Aliquots of the titrant solution were added to the sample zone while step 8 was performed. To change its concentration into the sample bulk, the time interval (t_{on}) to switch on SV was varied from 4 to 40% of the sampling period (t_1) that was maintained at 4 s. Six sampling periods were carried out to insert a solution volume (sample plus titrant) of 1600 μl into the holding coil (C_1). Under this condition, measurements were carried out at steady-state condition, yielding results shown in the Fig. 2.

Table 5
Vitamin B_6 determinations in several commercial pharmaceutical preparations and the corresponding recoveries using the proposed procedure

Pharmaceutical	Found	Recovery (%)
Benadon, tablets (300 mg/tablet)	302.12 ± 13.15	103.5 ± 2.5
Ergitone, injections (50 mg/injection)	49.81 ± 3.98	103.3 ± 3.6
Dragavite, tablets (5 mg/tablet)	4.98 ± 0.35	103.4 ± 1.4
Complexo B, tablets (2 mg/tablet)	1.92 ± 0.21	97.4 ± 2.3
Becozyme, syrup (40 mg/100 ml)	49.79 ± 6.49	102.1 ± 1.9
Complexan, syrup (40 mg/100 g)	41.89 ± 3.45	98.1 ± 0.8

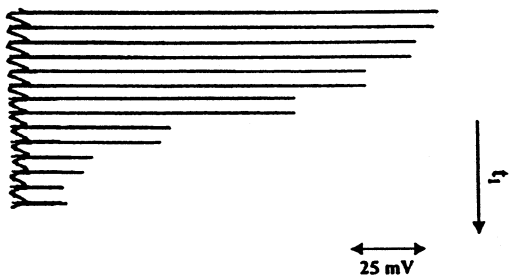

Fig. 2. Recorder tracing of signals obtained in a titration procedure. A 5×10^{-3} mol/l tetraphenylborate solution and a 1×10^{-4} mol/l vitamin B_1 solution were used as titrant and titrand, respectively. Each volume ratio titrant/titrand was processed twice.

4. Conclusions

By implementing a sequential injection set-up with an additional binary sampling approach, the system versatility was widened both by accomplishment of diverse analytical procedures and by handling of stock solutions, usually performed in a batch mode. In this way, in-line solution preparation, ISE electrode characterization procedures, direct potentiometric measurements, standard addition techniques, and potentiometric titrations can be afforded in the same system, using simple steps sequences and saving analytical costs. The ability to generate in-line solutions for set-up calibration, for standard addition techniques, and for ISE electrode characterization using only one or two MS solutions resulted in a remarkable decrease on the reagent consumption and bench-top work.

Furthermore, the obtained results stresses the system robustness as all the measurements could be taken under minimum dispersion conditions that are more easy to attain.

Acknowledgements

Praxis/P/QUI/10109/1998 (Portugal), CAPES and FINEP/PRONEX (Brazil) research projects supported this work.

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