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Determination of potassium ions in pharmaceutical samples by FIA using a potentiometric electrode based on ionophore nonactin occluded in EVA membrane

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Abstract

A simple and rapid method was developed for the K^+ ions determination employing a flow injection system using a flow-through electrode based on the naturally-occurring antibiotic ionophore nonactin occluded in a polymeric membrane. The nonactin ionophore was trapped in poly(ethylene-*co*-vinyl acetate) (EVA) matrix (40% w/w in vinyl acetate) and dispersed on the surface of a graphite-epoxy tubular electrode. The plasticizer-free all-solid-state potassium-selective electrode showed a linear response for K^+ concentrations between 5.0×10^{-5} and 5.0×10^{-2} M ($r = 0.9995$) with a near-Nernstian slope of 51.5 mV per decade, when Tris–HCl buffer (pH 7.0;0,1 M) was employed as a carrier. The potentiometric-FIA system allows an analytical frequency of 120 samples per hour with a precision of 3.6%. The relative standard deviations (R.S.D.) for K^+ determination in pharmaceuticals samples, without any previous treatment, were lower than 4.0%, comparable to those obtained by flame photometry. Ammonium is the main analytical interference and the electrode response time was 5 s at 25 °C. The useful lifetime of the tubular sensor is longer than 3 months in continuous use.

Keywords: Potassium determination; Potentiometric electrode; Pharmaceutical samples

1. Introduction

Biological active-transport systems involving ions, in particular K^+, have important functions in the organism, which are essential for regulation of many intracellular activities. These systems are related in the transmission of information by the nervous system and in the excitation and relaxation cycle of muscle tissue [1,2]. Besides, some enzymes require K^+ as a cofator for the maximum catalytic activity, while metabolically supported gradients of K^+ and Na^+ across the cell mem-

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brane are involved in the maintenance of the membrane potential caused by an abrupt increase in the permeability of the membrane when it is stimulated [2,3].

In despite of these facts, alterations in skeletal muscle function during chronic K^+ deficiency have been described [4]. The changes may be associated with abnormalities of muscle membrane permeability and cellular function. Thus, several procedures are extensively employed for the K^+ determination, including spectrochemical methods [5–8], liquid chromatography [9,10] and potentiometry [11–14], considering the important role of this alkali metal cation in the organism.

Particularly, the ion-selective electrodes (ISEs), which are able to monitor ions of pharmacological interest, have been very employed mainly coupled with flow injection analysis (FIA) due to the versatility obtained in some analytical steps [15,16]. Several all-solid-state electrodes based on polymeric membranes incorporating ionophores for K^+, especially valinomycin [17,18] and rifamycin [19], were utilized in potentiometric determinations regarding the construction simplicity, robustness and lifetime of these sensors.

Ionophores, ligants that selectively associate themselves with ions, were very studied in potentiometric analysis, based on the potential difference associated with the interaction [20]. Typically, they are macrocyclic molecules with an ion linked in a cavity well defined. The selectivity is based on the relation between the dimensions of the ion and the ligant site. Crown ethers constitute the best-known class, although other types of molecules, of synthetic or natural occurrence, act as ionophores. In addition to the elevated selectivity, neutral ionophores used in ISE are not subjected to protonation and consequently, the equilibrium constants are not affected by the pH value, as the case for nonactin [21], ionophore used in the present work.

This paper describes a FIA system developed to determine K^+ in pharmaceutical samples, using potentiometric detection with a flow-through electrode [22,23] containing the nonactin neutral ionophore occluded in a poly(ethylene-*co*-vinyl acetate) (EVA) polymeric membrane. This copolymer has a low glass transition temperature ($T_g = -50$ °C) [24] and can be used as an alternative to poly(vinyl chloride) (PVC, $T_g = 81$ °C) in electrodes [25] without the need of a plasticizing solvent, thus minimizing possible interferences or loss of ionophore from the polymeric matrix.

These new kinds of membrane have solved many problems with ISEs where there are increases in the stability and selectivity of electrode. Also, it is possible to dissolve the EVA copolymer in six different solvents, as opposed to the tetrahydrofuran and cyclohexanone commonly used with PVC. The solvents are carbon tetrachloride, chloroform, dichloromethane, *p*-xylene, toluene and tetrahydrofuran. Other polymers, such as poly(methyl methacrylate) ($T_g = 105$ °C) and poly(styrene) ($T_g = 100$ °C), have been used in ISEs with less success, because of their higher glass transition temperatures [26].

2. Experimental

2.1. Reagents and standards

The standard solutions of potassium chloride from Merck (Darmstadt-Germany), with concentrations in a range of 5.0×10^{-2}–1.0×10^{-5} M, were prepared by weight (±0.01 mg) and diluted in Tris–HCl (tris–hydroxymethyl aminomethane) buffer (pH 7.0; 0.1 M) from Aldrich (Milwaukee–USA) using deionized water (>18 MΩ cm) obtained from a Millipore Milli-Q system. For the interference measurements, chloride salts (from Merck) of ammonium, sodium, lithium, calcium and magnesium at 10^{-3} M were prepared in Tris–HCl buffer. The poly(ethylene-*co*-vinyl acetate) copolymer (40% w/w in vinyl acetate), was acquired from Aldrich. All reagents used to prepare the solutions were of analytical grade.

2.2. Apparatus

The potential difference measurements were obtained with a pH/ion analyzer OP-271 (±0.1 mV), a double junction electrode OP-0820P of Ag|AgCl with $Ca(NO_3)_2$ (from Merck) 1 M in the exterior compartment and a temperature detector OP-0003P. For pH measurements, an OP-808P

glass electrode was used. All these components are manufactured by Radelkis (Hungary). A XY recorder (model RB-101) was also employed. The FIA manifold included a peristaltic pump (Ismatec model IPC-8).

2.3. Preparation of the potentiometric sensitive membrane for K^+

To prepare the potentiometric electrode with the sensitive membrane for K^+, a solution containing EVA copolymer in tetrahydrofuran (THF) at 5.0% (w/v) was made. Then, the nonactin ionophore from Fluka (Buchs–Switzerland) was added to the EVA solution at 1.0% (w/v). The resulting solution was dropped into the tubular graphite-epoxy electrode (Fig. 1). This membrane was let to dry for 30 min and the electrode was stored at ambient. The all-solid-state selective electrodes were made in accordance with a technique described elsewhere [27], using a mixture of epoxy resin (Araldite M and HR Hardener, both from Ciba-Geigy, Buchs–Switzerland) and graphite powder (20 μm granulometry, Fluka) in a 20:80% (w/w) proportion, respectively.

2.4. FIA manifold

The FIA system employed (Fig. 2) utilized a carrier solution, B (Tris–HCl buffer, pH 7.0; 0.1 M), conducted by a peristaltic pump, PP, to the tubular electrode, ISE. A manual injector, I, is used to insert K^+ solutions, S, into the carrier solution. The potential measurements were obtained with a (±0.1 mV) potentiometer, P. An Ag|AgCl double junction electrode was used as reference, RE. A recorder, R, was employed to register the potentiometric signals. The optimized conditions of system were: recorder sensitivity of

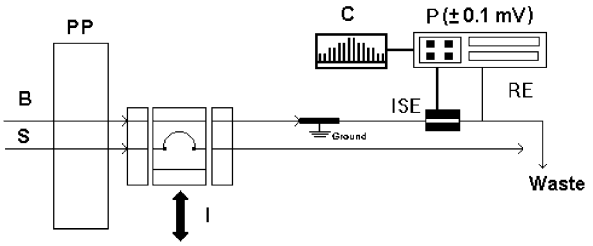

Fig. 2. Flow injection manifold for K^+ determination in pharmaceutical samples by potentiometric detection using tubular electrode based on nonactin ionophore occluded in EVA membrane.

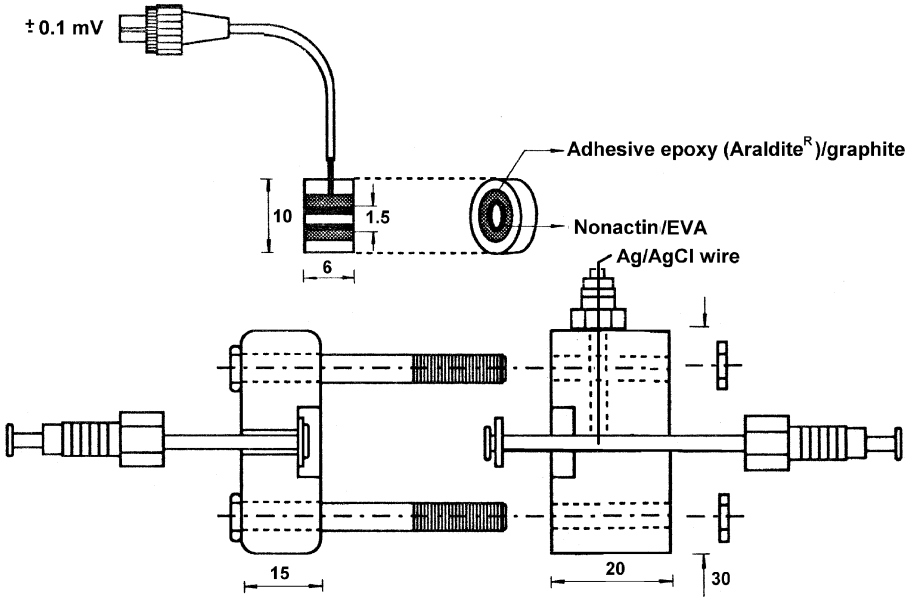

Fig. 1. Scheme of the cell and tubular electrode used in flow system for the K^+ determination.

500 mV; Tygon™ and polyethylene tubes for other connections with 0.80 mm internal diameter and sample loop of 50 μl. To minimize noise, a stainless steel tube was used (0.5 mm i.d.; 20 mm length). The pump uses two channels through which solutions are inserted. A channel contains K^+ solutions and the other channel is assigned to the loading sample solutions, both with a flow rate of 1.8 ml min^{-1}.

2.5. Sample preparation

The K^+ samples (all purchased at local pharmacies) were prepared by directly dissolving the sample in Tris–HCl buffer (pH 7.0; 0.1 M). The pharmaceutical samples containing K^+ are administrated via oral like tablets ($KCl/KHCO_3$) and cough syrups (KCl or KI), which contain 12.0 mEq in terms of K^+. The solutions prepared in this way present concentrations of approximately 5.0×10^{-3} M. These solutions were injected into the FIA system after obtaining calibration curves with standard K^+ solutions.

3. Results and discussion

3.1. Potentiometric response characteristics of the flow-through electrode

All-solid-state potassium-selective electrodes with plasticizer-free membranes have been developed in recent years [28,29] associated to the suitable supporting polymers to ensure compatibility with the active component and deter leaching. An alternative to improve the potentiometric sensor performance is employing membranes that have a glass transition temperature below room temperature to eliminate the need of the solvent mediators, which normally have plasticizing properties and are effective in reducing the glass temperature. In this manner, it has been suggested that polymers such as poly(ethylene-co-vinyl acetate) (EVA) can be used as the matrix membrane for the construction of ISE's especially useful as tools in the analysis and monitoring of analytes of biological [30] and pharmaceutical [31] importance.

The nonactin ionophore must be able to bind a metal ion selectively and tightly while at the same time be flexible enough to release the ion fairly rapidly. Furthermore, to be compatible with a biomembrane, the ionophore must have a hydrophobic exterior while having a hydrophilic interior capable of binding an ion. Nonactin meets these requirements by having a balled hydrophobic exterior in the complexed form and showing conformational flexibility. Thus, the nonactin immobilization into an EVA matrix exhibited good operational characteristics for the K^+ flow-through ISE.

The sample volume and flow-rate were studied to verify the effects of these parameters on the potentiometric signal (Fig. 3). The signal increases with the sample volume and it trends to a constant for volumes higher than 50 μl. The flow-rate causes significant influence in the signal, where it is observed an increase in the signal for flow-rates lower than 1.8 ml min^{-1}, but the regeneration times are higher than 3 min. In contrast, the regeneration time and the signal are kept almost constant with flow-rate higher than 1.8 ml min^{-1}, showing a high analytical frequency.

In this sense, it is possible to observe the potential dependence on the flow-rate, indicating a kinetically controlled system. The K^+ molecular

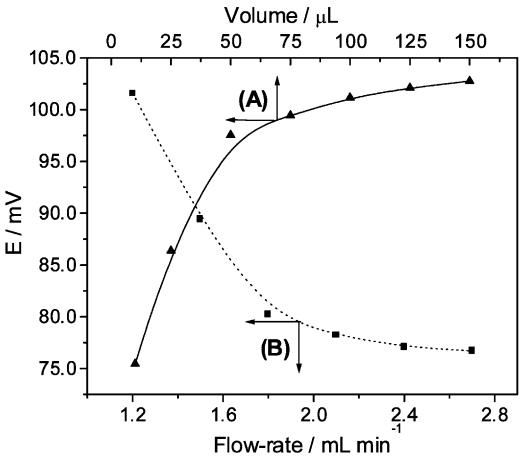

Fig. 3. Influence of the injected sample volume (A) and flow-rate (B) in the signal of the proposed potentiometric-FIA system for K^+ determination. Conditions: $[K^+]$, 5.0×10^{-4} M; (A) flow-rate, 1.8 ml min^{-1}; (B) sample volume, 50 μl.

recognition by the naturally-occurring macrotetralide nonactin becomes almost constant for flow-rate higher than 1.8 ml min^{-1} (Fig. 3(B)). For higher than 1.8 ml min^{-1}, the kinetic control is predominant in the FIA-system, presenting a constant response. Thus, the best conditions to obtain good sensitivity and rapidity were 1.8 ml min^{-1} and 50 μl for flow rate and sample volume, respectively. The carrier solution employed in all studies for K^+ determination was Tris–HCl buffer (0.1 M; pH 7.0).

3.2. Selectivity of K^+-sensitive membrane and effect of pH

The potentiometric selectivity coefficients ($K^{pot}_{i,j}$) of K^+ nonactin-EVA sensor were evaluated using the mixed solution method [32] with 10^{-3} M concentration of ammonium, alkali and alkaline earth ions. The obtained results (Table 1) show that the selectivity of the sensor with regard to interfering ions is in the order: $NH_4^+ > Na^+ > Mg^{2+} > Li^+ \gg Ca^{2+}$. The similarity in ionic radii [25] of K^+ ($r_i = 1.33$ Å) and NH_4^+ ($r_i = 1.48$ Å) is responsible for the distinctly low discrimination between these two cations by the carrier antibiotic nonactin compared with the good selectivity for the other physiologically important cations like Na^+, Ca^{2+} and Mg^{2+}.

The most interesting feature of EVA-nonactin based potassium ion sensor compared with those incorporating crown ethers and nonactin in PVC membranes (Table 2) are the better potentiometric selectivity for ammonium ions and similar selectivity for sodium ions, characteristic that make the use of the proposed sensor useful and convenient when the sample contains significant quantities of these ions.

Nevertheless, the obtained selectivity coefficients depend on the absolute values of activity of interfering and primary ions. Because the above used levels were extremely high compared with pharmaceutical concentrations for these interfering compounds, the influence of the cations, mainly ammonium ions, did not affect the electrode performance as can be seen in the K^+ determination of real samples.

The pH influence on the flow-through electrode response was studied at 10^{-3} and 10^{-4} M K^+ concentrations (Fig. 4). The pH dependence of the electrode potential was tested over the range 2–10, adjusting the pH with dilute lithium hydroxide and hydrochloric acid solutions. The plots in Fig. 3 indicate that at pH 3–8, the potentials are constant due to the nonactin neutral behavior.

Table 1
Potentiometric selectivity coefficients*, log $K^{pot}_{i,j}$, for the K^+-sensitive tubular electrode, using the mixed solution method with interfering cations fixed at 10^{-3} M

Interfering cation	$-\log K^{pot}_{i,j}$
NH_4^+	0.23
Na^+	1.30
Mg^{+2}	1.33
Li^+	1.84
Ca^{+2}	2.95

* i, potassium and j, interfering cation.

Table 2
Comparison of some potassium sensors

Ionophore/membrane	$-\log K^{pot}_{i,j}$		References
	NH_4^+	Na^+	
Nonactin/PVC	−0.42	2.18	[33]
Dibenzo-18-crown-6/PVC	−0.06	0.35	[34,35]
Nonactin/EVA	0.23	1.30	Present work

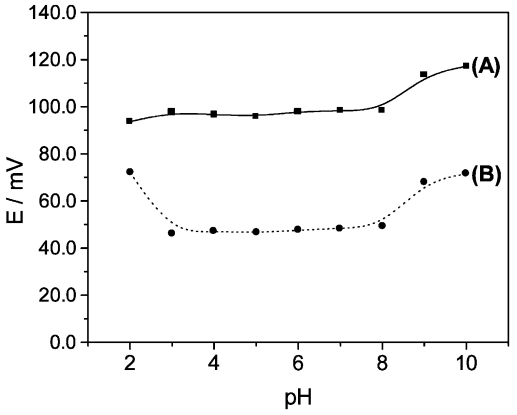

Fig. 4. The influence of the pH on the electrode response for the nonactin/EVA tubular electrode: (A) [K^+], 10^{-3} M; (B) [K^+], 10^{-4} M (Reilley diagrams).

3.3. Analytical performance of the system

Fig. 5 shows the potentiometric signals of the standard solutions to obtain calibration curve for K^+ with the proposed potentiometric-FIA system. The sensor presented near-Nernstian behavior from 5.0×10^{-5} up to 5.0×10^{-2} M concentration range for K^+ with a detection limit of 1.3×10^{-5} M in the optimized conditions. The equation that describes the analytical performance of the sensor is given by:

$$\Delta E = (249.3 \pm 0.5) - (51.5 \pm 0.8) \log [K^+]$$

The correlation coefficient (r) was 0.9995 for $n = 6$.

The system allows an analytical frequency of 120 samples per hour with precision of 3.6% (relative standard deviation (R.S.D.) for repeatability of 15 injections). It was tested during three months without change in the signal produced. Its applicability is presented on Table 3 for K^+ determination in pharmaceuticals samples. The analyzed drugs contain several compounds in their formulations, as sweeteners, salts and preservatives, but no significant interference was observed.

The flame photometry has found widespread application in elemental analysis overall in the sodium, potassium and lithium determinations. Because of its convenience, flame emission spectroscopy has become the method of choice for several elements, which are otherwise difficult to determine. In this sense, the flame photometry was employed in this work just like a reference method to evaluate the potentiometric sensor developed. In despite of this spectrochemical method to be used to compare the obtained results for potassium determination, can occur chemical interferences mainly by anions that form compounds of low volatility with the analyte and thus decrease the rate at which it is atomized. This fact can be minimized by a suitable choice of protective agents to prevent interference when necessary, increasing the time of analysis. Nevertheless, the analyzed samples by both methods did not exhibit interference problems like that mentioned previously.

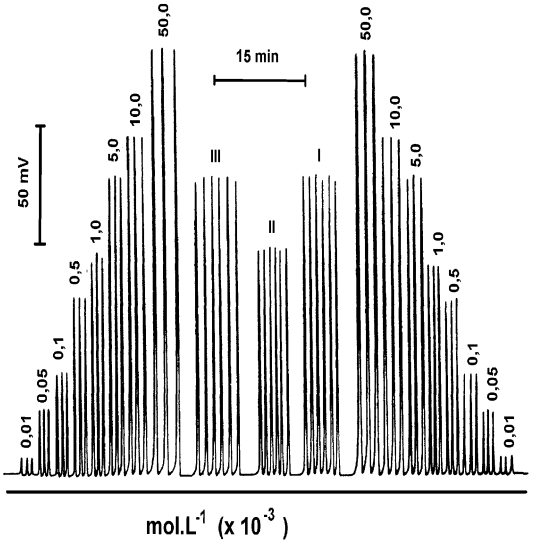

Fig. 5. Diagram showing the typical signal obtained with the proposed FIA system for the K^+ determination using a potentiometric sensor based on nonactin occluded in EVA membrane. From left to right: ascending and descending calibration curves (1.0×10^{-5}–5.0×10^{-2} M) intercalated with injections of three types of commercial samples (I-tablets; II and III-syrups). Injected volume, 50 μl; flow-rate, 1.8 ml min^{-1}. All solutions were freshly prepared ($n = 6$ determinations).

4. Conclusions

Few FIA systems for K^+ potentiometric determination are described in the literature, being in its majority associated to the spectrochemical detection. The K^+ determination using a tubular ion-selective electrode based on ionophore nonactin is particularly useful in analysis of real samples with good operational characteristics, particularly considering the linear range, the selectivity and the stability.

Association of the electrode with a FIA system enhances the use of this sensor, when repeated analyses are required as, for instance, in routine procedures. FIA systems coupled with these sensors present advantages such as continuous washing of the detector, small sample volumes and the possibility of system automation.

The use of the EVA copolymer offers a promising alternative to other polymer-based membrane electrodes. This copolymer has been successfully applied to the construction of tubular electrodes without a plasticizing solvent, eliminating pro-

Table 3
Results obtained in the K^+ determination for the pharmaceuticals samples with the proposed potentiometric-FIA system and flame photometry

Samples	Nominal K^+ content (M)	Found K^+ content (M)		Relative deviation [b] (%)
		FIA-potentiometry	Flame photometry	
#I	1.20	(1.23 ± 0.06 [a])	(1.25 ± 0.03)	1.6
#II	0.12	(0.11 ± 0.01)	(0.11 ± 0.02)	–
#III	0.80	(0.78 ± 0.05)	(0.75 ± 0.04)	3.8

Containing: #I, 0.60 g of KCl and 0.40 g of $KHCO_3$ per tablet, dissolved in 10.0 ml of Tris–HCl (tablets for hypopotassemy prophylaxis); #II, 0.02 g ml^{-1} of KI, 0.003 g ml^{-1} of *lobélia inflata* and 0.003 g ml^{-1} of *hyoscyamus niger* extracts (syrup for bronchus dilatation); #III, 0.06 g ml^{-1} of KCl (syrup for hypopotassemy prophylaxis).

[a] Standard deviation estimates for six determinations.

[b] Relative deviations between potentiometric and spectrophotometric methods.

blems such as interference and leaching out of active material from the polymeric matrix.

The tubular electrode exhibits a good lifetime with great reproducibility of potentiometric signals, providing a rapid, sensitive and accurate method for the K^+ determination in pharmaceutical analysis with minimal sample pre-treatment. This methodology can be applied with good precision in the quality control of pharmaceuticals containing K^+ utilized in hypopotassemy prophylaxis.

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