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Detection and quantification of ppb level potassium in biological samples in the presence of high sodium by ion chromatographic method

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Abstract

Employing a silica gel column modified with carboxyl groups and an eluent consisting of 4.0 mM tartaric acid and 0.75 mM dipicolinic acid in 2% acetone, an otherwise difficult quantification of K^+ at ppb level in presence of 6000 ppm NaCl was achieved by incorporating 0.75 mM 18-crown-6 ether in the mobile phase and subtracting the blank NaCl signal from each chromatogram. Optimized analytical conditions were established in terms of relative standard deviation (%) of retention time, peak area and calibration equations, and also by peak asymmetry factor. The net efflux of K^+ into the gastric lumen under *in vitro* conditions of acid secretion was investigated in Ussing chamber model. The effects of the physiological secretagogue histamine and the antisecretory agents cimetidine, omeprazole and SCH28080 were studied. The decline of K^+ efflux in presence of cimetidine, and the rise of the same in the presence of omeprazole and SCH28080 were conspicuously discernible, thereby validating the usefulness of ion chromatography based K^+ quantification method under biological experimental conditions.

Keywords: Ion chromatography; Inorganic cations; Potassium efflux; 18-Crown-6 ether; Gastric antisecretory agents; Ussing chamber

1. Introduction

Potassium transport in biological systems is one of the key processes in many cellular functions [1]. The majority of the regulatory functions tend to maintain high K^+ and low Na^+ at intracellular level as opposed to low K^+ and high Na^+ extracellularly. We wanted to investigate the effects of different putative agents on the shuttle functioning of the K^+ flux through the gastric mucosa in regulating gastric acid secretion [2]. It was therefore of interest to estimate K^+ transport under *in vitro* conditions of gastric HCl secretion. Employing frog gastric mucosa in conventional Ussing chamber model [3,4], we were assessing the rate and extent of H^+ transport under different experimental conditions. It was therefore additionally necessary to monitor net K^+ efflux in experimental samples that contained high levels of Na^+.

Ion chromatography (IC) is a powerful technique for the determination of inorganic cations and anions. It has been successfully used in environmental sectors, and is also gaining grounds for wide ranging applications in food, clinical and pharmaceutical sciences [5,6]. The advantages of IC are many: it can separate and quantitate several cations and anions simultaneously, does not require extensive sample processing, can quantitate quite a large number of samples generated through different pharmacological experiments, needs only a small volume of sample, and is sensitive down to ppb level [7,8]. Moreover, its versatility in specific applications can be further expanded by selecting from a wide range of stationary and mobile phases [9,10].

Under the present biological experimental conditions where proton secretion through gastric mucosa is being monitored, it was necessary to quantitate ppb level of K^+ in the presence of about 6000 ppm of fixed concentrations of NaCl. This was required according to the experimental set up of Ussing chamber to monitor H^+ and/or K^+ secretion in isotonic NaCl solution. The advantages of using IC for such type of determination over conventional flame photometer and atomic absorption spectrometer as applied before [4] are as follows: with flame photometer the detection limit is very poor [7]. A particular problem that was faced is that during measurements, sodium chloride crystals tend to get deposited in the sample pathway producing interference as well as inaccuracy. It requires calibra-

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tion with standard solution several times during determinations. On the other hand, calibration in IC remains consistently stable and one can analyze samples continuously for 1 week without requiring to perform the calibration on regular basis. Further, as compared to atomic absorption spectrometry, IC is much more economic and efficient, particularly for the determination of K^+ [7] when large number of samples are required to be analyzed.

However, since both the peaks of high Na^+ and low K^+ tend to elute close to each other in different columns and eluents, it was difficult to achieve a quantitative estimation of K^+ [11]. Crown ethers of different types are known to influence retention and selectivity because of their different behaviour in forming complexes with alkali metal ions [12,13]. The addition of the crown ether 18-crown-6 to the eluent leads to a better separation of Na^+ and K^+. Since the increase in the retention time of K^+ is particularly dramatic, using appropriate column and 18-crown-6 ether as eluent modifier, we could optimize an ion chromatographic method with conductivity detection. Necessary conditions for the quantitative estimation of ppb-level K^+ were established followed by validation under conditions of gastric acid secretion and regulation, where expected changes of net K^+ efflux were found to correlate discernibly. Further, chromatographic separation of two biologically important divalent cations Ca^{2+} and Mg^{2+} (along with K^+) in the presence of high Na^+ was also demonstrated.

2. Experimental

2.1. Chromatographic system

A modular ion chromatographic system (Micro Devices Metrohm Limited, Herisau, Switzerland) comprising of Model 709 IC Pump, Model 733 IC Separation Center and Model 732 Conductivity Detector, was used. All system components were connected through Model 762 IC Interface to IC Net 2.1 workstation which performed system control, acquisition and analysis of the data.

Metrosep C2 250 analytical column (250 mm × 4 mm ID; particle size 7 μm; column body made of poly ethyl ether ketone; capacity 194 μmol (K^+)) and Metrosep C2 guard column (5 mm × 4 mm ID) were used for this study. Eluents were degassed for about 5 min and used without any further pH adjustment. Eluent flow rates were set at 1 ml/min with an injection volume of 100 μl, unless otherwise stated.

The detection of the peak was made by direct conductivity method and the data output was in terms of millivolt. Conductivity cell temperature was set at 40 °C and maintained thermostatically. The equivalent conductivity of monovalent cations like H^+, Na^+ and K^+ and that of divalent cations like $½Mg^{2+}$ and $½Ca^{2+}$ are 350, 50, 74, 53 and 60 S cm^2 Eq^{-1}, respectively. In the conductivity output, the signals obtained gave rise to negative peaks [(Λx) ∞ concentration (X-350)]. To display positive peaks, the 'conductivity polarity' in the detector screen was set to 'inverted'. The high background conductivity of the eluent was electronically suppressed to 'zero' level immediately before the injection of the sample in the column. Drift compensation of output signals was performed at the end of each chromatographic run.

2.2. Chemicals

All reagents were of analytical grade. Pyridine-2-6-dicarboxylic acid (PDCA, dipicolinic acid, >98% pure), tartaric acid (>99%) and 18-crown-6 ether (CE, >99%) were purchased from E. Merck, Qualigen and Acros Organics respectively. Histamine, cimetidine and chloride salts of sodium, potassium, calcium and magnesium were obtained from Sigma Chemical Co. Other inorganic salts were purchased from E. Merck. All reagents and eluents were prepared in Milli-Q (Millipore) deionized water (resistance >18 MΩ cm). Omeprazole and SCH28080 (2-methyl-8-[phenylmethoxy]imidazo[1-2-α] pyridine-3-acetonitrile) were kindly supplied by Dr. H. Mattsson, Astra Hassle AB, Sweden and Dr. J.J. Kaminski, Schering Corporation, New Jersey, USA, respectively.

2.3. Standards and sample processing

Stock solutions of the cations were prepared by dissolving appropriate amounts of different chloride salts in filtered (0.22 μm membrane filter) 6000 ppm NaCl solution at a concentration of 100 ppm. The K^+ standard was prepared by diluting 100 ppm stock solution of K^+ in 6000 ppm NaCl to obtain a wide range of standard solutions (0.1–10 ppm K^+) as required. Also, multi cation standards were prepared by diluting and mixing the stock solutions in 6000 ppm NaCl solution. Since gastric secretory fluids would be expected to have different pH values ranging ∼6.5–4.0 under different experimental conditions, all the samples, standard as well as experimental, were adjusted to pH ∼2.5–3.5 with 2 M nitric acid (2 μl in 2 ml sample) prior to injecting into the column to obtain reproducible results with the divalent cations as well as to maintain the mobile phase pH uniformly stable [14,15].

2.4. K^+ transport in chambered frog gastric mucosa

The rate and extent of K^+ and H^+ transport across frog gastric mucosa were monitored essentially according to Ref. [4]. Frogs (*Rana hexadaectila*) were obtained from a local dealer and kept in a tank at ambient temperature. The fundic mucosa from freshly pithed frog was carefully separated from the submucosa and mounted over one end of a plastic tube (14 mm × 100 mm) with the mucosal surface facing out as shown in Fig. 1. The area of the mounted mucosa actively secreting acid was 1.5 cm^2. The mounted tissue was placed vertically inside a 25-ml container.

The compositions of the bathing solutions were as follows. The nutrient solution contained (mM): Na^+ 102; K^+ 4; Ca^{2+} 1; Mg^{2+} 0.8; Cl^- 82.6; HCO_3^- 25; PO_4^{3-} 1; and glucose 11 (pH adjusted to ∼7.0 prior to experiment); the luminal side was bathed in an unbuffered 104 mM NaCl solution (∼6000 ppm NaCl). The volumes of nutrient and mucosal solutions were 2.5 ml and 14.5 ml, respectively. Both solutions were bubbled slowly with 95% O_2/5% CO_2. The mucosal solutions (secretory side, lumen-directed) were stirred slowly and continuously, and

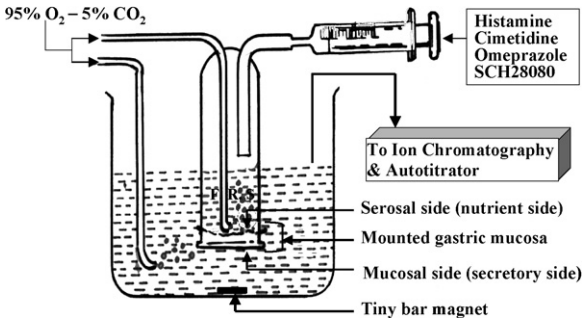

Fig. 1. Experimental set-up of Ussing chamber to follow K^+ and H^+ secretion through gastric mucosa. Freshly isolated frog gastric mucosa (*Rana hexadaectila*) was mounted over one end of a plastic tube inside a 25-ml beaker, wherein the mucosal side (secretory side) was bathed in unbuffered NaCl solution (104 mM) and the serosal side (nutrient side) in nutrient medium (FRS—frog ringer's solution). The volumes of nutrient and mucosal solutions were adjusted in a way so as to maintain constant hydrostatic pressure on the mucosa from both the sides throughout the duration of the experiment. Histamine, cimetidine, omeprazole or SCH 28080 were added from the nutrient side. Basal acid secretion was continued between 0 and 1 h (with FRS in the serosal side). Secretagogue-stimulated acid secretion was continued for next 60 min by adding histamine (0.1 mM) from the nutrient side during 1–2 h. Antisecretory agents were added from the same serosal side in histamine containing nutrient medium after 2 h, and the experiment was continued for another 2 h. The secretory solutions were collected at 15 min interval. For the quantification of K^+ by ion chromatography, 100 μl of secretory solution was used while the quantification of H^+ by autotitration in pH stat mode, 12.5 ml of the same was employed.

collected at 15-min intervals after an initial stabilization period of 1 h, in thoroughly washed plastic vials. The basal secretion was continued for 1 h followed by stimulation of the gastric mucosa from the nutrient side (serosal side, blood-directed) by histamine (0.1 mM) for another hour. At the end of 2 h, antisecretory agents like cimetidine, omeprazole or SCH28080 were added from the nutrient side and the experiment continued for another 2 h.

For the determination of K^+, 2 ml each of the mucosal samples (secretory solution) collected at designated time points were filtered through 0.22 μm membrane filter, 2 μl of 2.0 M HNO_3 added and 100 μl of the solution was injected onto the column. The H^+ secretion was quantitated with the residual amount (12.5 ml) of the samples by pH-stat titration with 0.1 mM NaOH to pH 6.5 while gassing the solutions with 100% N_2 employing Model 751 GPD Tritino Autoburette and Model 730 Sample Changer (Metrohm). The data obtained with experimental samples for both K^+ and H^+ are expressed in terms of μEq/h, i.e., the rate of K^+ or H^+ transport.

Student's unpaired *t*-test was used to compare secretory data with initial steady-state values, and the differences were regarded as statistically significant when $p < 0.05$. The results are expressed as the mean ± SEM.

3. Results and discussion

3.1. Effect of mobile phase on quantification of K^+ in presence of high Na^+

The Metrosep C2 250 analytical column contains silica gel modified with carboxyl groups. An isocratic elution with 5 mM tartaric acid and 0.09 mM PDCA in 2% acetone produces simultaneous separation of zinc, lithium, cobalt, sodium, ammonium, monoethanolamine, potassium, manganese, magnesium and calcium in a single run in 35 min [14,15]. Acetone was added in the mobile phase to remove organic contaminants that might arise from the biological sample. Employing an eluent consisting of 4 mM tartaric acid and 0.75 mM PDCA in 2% acetone, and injection volume of 100 μl, the transitional metal–PDCA complex gets eluted at the solvent front, and the peaks for Na^+, K^+, Ca^{2+} and Mg^{2+} appeared at 7.5, 11, 19 and 25 min, respectively (data not shown). Using this eluent composition and 20 μl injection volume, the detection limit of K^+ in water at S/N ratio of 3:1 was observed to be 1 ng/ml (≡1 ppb). However, the detection limit of K^+ in 6000 ppm NaCl was observed to be ≥500 ng/ml (≡500 ppb), and also necessitated the use of 100 μl loop. Since targeted biological experiments of gastric acid secretion and regulation under the influence of putative antisecretory agents were required to be carried out in isotonic NaCl solutions (~6000 ppm NaCl) to assess alteration in ppb level of K^+, it was of interest to see whether the experimental crisis could be overcome using crown ether. Macrocyclic crown ethers like 18-crown-6 ether (1,4,7,10,13,16-hexaoxacyclooctadecane) are expected to increase the retention time of K^+ [8,11] and thus help separate the small peak of K^+ from the broad peak of Na^+ at high concentrations.

3.2. Effect of addition of 18-crown-6 ether in the mobile phase on the elution of K^+

The incorporation of 18-crown-6 ether (CE) in the mobile phase provided good peak resolution of K^+ at lower ppb range. To exclude the effect of high concentration (6000 ppm) of NaCl, each chromatographic signal was required to be subtracted from the corresponding blank NaCl signal. The chromatogram thus obtained showed single peak of K^+ and was used for calibration and subsequent experimental purposes. Fig. 2 shows how the chromatograms of 0.5 and 5 ppm K^+ appeared when the eluent contained 4 mM tartaric acid, 0.75 mM PDCA with or without 0.75 mM CE in 2% acetone (see panels A–E and F–J). Upon subtracting the blank chromatogram of 3000 ppm Na^+ (panel B) from the standards 0.5 and 5 ppm K^+ chromatograms taken in 6000 ppm NaCl (panels C and G, respectively), the final chromatograms of K^+ only (panels D and I, respectively) were obtained. Chromatogram H represents the intermediate step, after blank subtraction but prior to baseline drift compensation of the 5 ppm K^+ system (the corresponding chromatogram with 0.5 ppm K^+ was not shown). It is to be borne in mind that such blank subtraction method would not be of any use for chromatographic run in mobile phase devoid of CE, since peak for K^+ below 0.5 ppm is not detectable particularly due to system noise appearing in the 10–11 min region. Moreover, without CE and blank subtraction, the peak for K^+ below 0.5 ppm is not quantifiable even if detectable, and at a wide range of 0.5–10 ppm precise calibration cannot be made (RSD% indicating poor goodness-of-fit).

The elution profile of K^+ as a function of CE concentrations in such high NaCl medium was then evaluated. Fig. 3

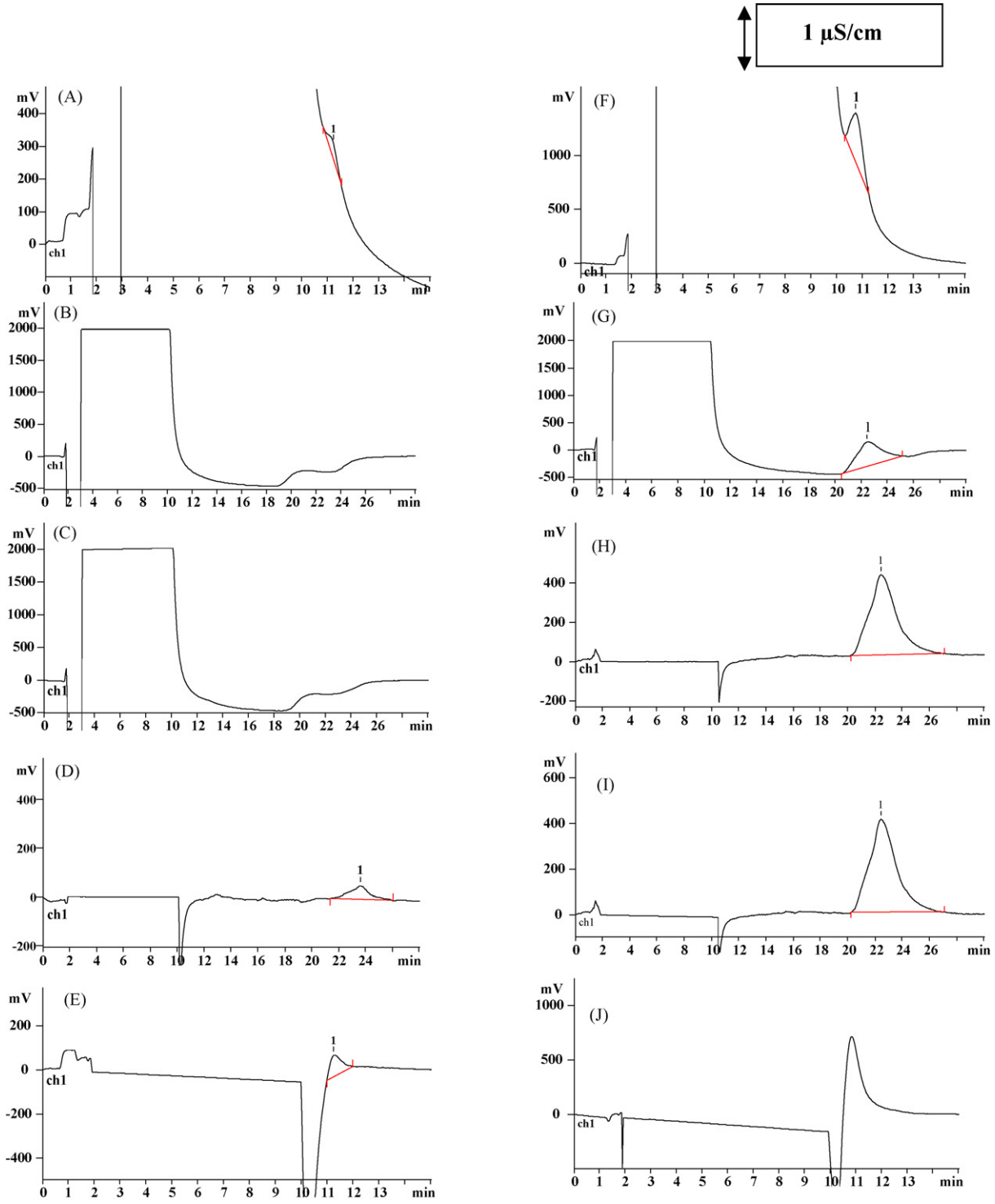

Fig. 2. Separation of 0.5 and 5 ppm K^+ in 6000 ppm NaCl. Eluent contains 4 mM tartaric acid and 0.75 mM PDCA in 2% acetone in the absence (panels A, E, F and J) or the presence (panels B–D and G–I) of 18-crown-6 ether (0.75 mM). Left panels (A, C–E) are for 0.5 ppm and the right panels (F–J) for 5 ppm K^+. Chromatogram B represents the elution profile of only NaCl (6000 ppm) in the presence of 0.75 mM CE. Panels C and G represent the standard 0.5 and 5 ppm K^+ chromatograms taken in 6000 ppm NaCl, respectively. Chromatograms D and I represent blank-subtracted K^+ elution profiles (0.5 and 5 ppm, respectively) after drift compensation, obtained by subtracting chromatogram B from chromatograms C and G, respectively. Tracings E and J represent blank (6000 ppm NaCl without crown ether) subtraction of chromatograms A and F, respectively. Panel H represents an intermediate step after blank subtraction and before drift compensation in the case of 5 ppm K^+. Flow rate 1 ml/min; injection volume 100 µl; detection by direct conductivity measurement.

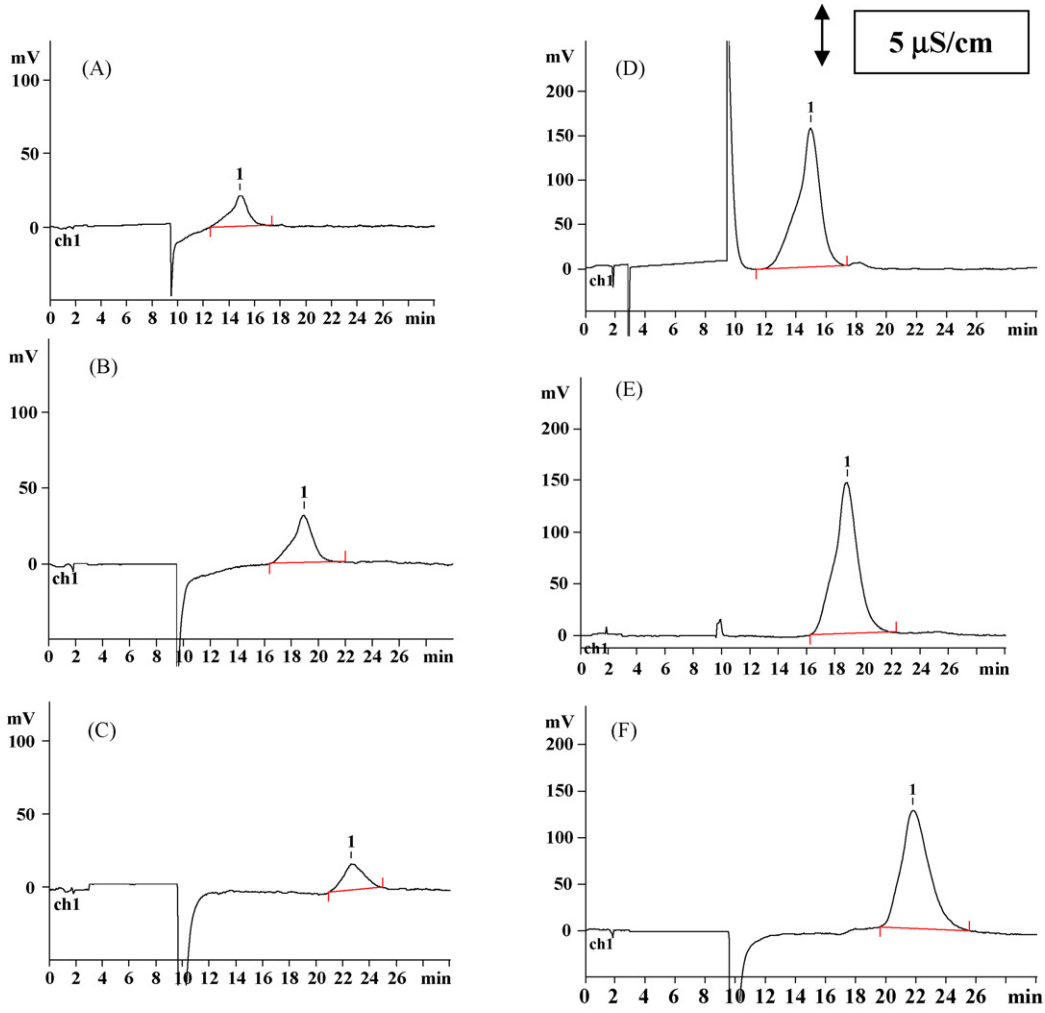

Fig. 3. Separation of peak for 0.5 ppm and 5 ppm K^+ in 6000 ppm NaCl in eluent containing different concentrations of 18-crown-6 ether. Left panels (A–C) are for 0.5 ppm K^+ and the right panels (D–F) are for 5 ppm K^+. The basic eluent composition is 4 mM tartaric acid and 0.75 mM PDCA in 2% acetone. Panels A and D: 0.25 mM CE, B and E: 0.45 mM CE, C and F: 0.75 mM CE. Chromatographic conditions are same as in Fig. 2.

demonstrates the chromatograms of 0.5 and 5 ppm K^+ (panels A–C for 0.5 mM and D–F for 5 mM K^+) in 3000 ppm Na^+ after blank subtraction at 0.25, 0.45 and 0.75 mM CE concentrations. Upon increasing the concentration of CE in the mobile phase, a gradual increase in retention time of K^+ was observed. A system stabilization with fresh mobile phase for about 1(½) h was required for reproducible data. At each concentration of CE, the calibration curve was generated by running a series of standard solutions of K^+ (6-point calibration: 0.5, 1.0, 1.5, 2.0, 5.0 and 10.0 ppm) in 6000 ppm NaCl solution. The peak areas obtained after blank subtraction followed by drift compensation at different concentrations of K^+ were used for the calibration. The results were analyzed statistically in terms of RSD (%) of the calibration equation, peak asymmetry factor, slope and correlation coefficient (Table 1). Without CE, poor linearity was observed (RSD% 7.279 ± 0.532). Gradual increase in CE concentration improved both RSD (%) and peak symmetry. The calibration obtained with 0.75 mM CE (%RSD 0.7896 ± 0.384, asymmetry 0.8–1.0) was considered to be optimal for performing experiment in the concentration range of 0.1–10 ppm K^+

Table 1
Elution characteristics of K^+ in high Na^+ concentrations in the presence of 18-crown-6 ether

Crown ether concentration (mM)	Retention time (min)	Peak asymmetry factor	RSD (%) of calibration equation	Slope ($\times 10^{-3}$)	Correlation coefficient
0 ($n = 5$)	10.77 ± 0.212	0.60–0.90	7.279 ± 0.5319	1.288 ± 0.003	0.9987 ± 0.0003
0.25 ($n = 3$)	14.88 ± 0.125	0.40–0.60	9.642 ± 0.1505	0.28 ± 0.0005	0.9968 ± 0.0001
0.45 ($n = 3$)	18.94 ± 0.101	0.60–0.80	2.004 ± 0.6863	0.29 ± 0.009	0.9997 ± 0.0001
0.75 ($n = 6$)	23.38 ± 0.225	0.85–1.00	0.7896 ± 0.384	0.078 ± 0.007	0.9999 ± 0.0001

Statistical parameters of the elution profiles of the system that did not contain crown ether (first row) were generated without blank subtraction (operating conditions are same as in Fig. 2A). For each determination, statistical evaluations were made as mean ± SD (n denotes the number of determinations in each case).

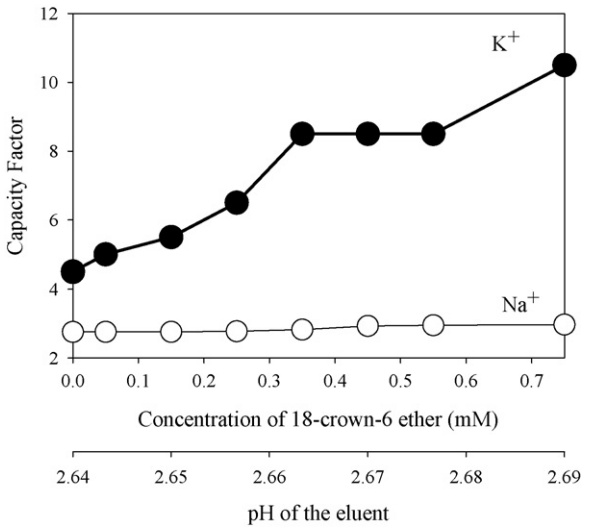

Fig. 4. Effect of 18-crown-6 ether on the capacity factor of potassium and sodium. Eluent: 4 mM tartaric acid and 0.75 mM PDCA in 2% acetone with different concentrations of crown ether. The retention time of K^+ was determined quantitatively as detailed in Fig. 2 panels F–I, while the same for sodium by running 1 ppm sodium in water under otherwise same experimental condition. Chromatographic conditions are same as in Figs. 2 and 3. Capacity factor: [(retention time − dead time)/dead time]. Dead time 120 s.

in 6000 ppm NaCl. The calibration plots were linear over two orders of magnitude (correlation coefficient 0.9999).

It was also of interest to see whether higher crown ether concentrations could help avoid the necessity of blank subtraction. Even at 1.00 mM, it did not help to overcome the requirement of blank subtraction for accurate quantification of K^+, particularly at low ppb level. Neither it improves RSD%, correlation coefficient and peak symmetry profile; it only prolonged the retention time to about 25 min (data not shown). The shift in retention time of Na^+ as a function of increasing concentrations of CE, however, remained only marginal (∼7.5–7.9 min). This was further evident when the effect of varying concentrations of CE (0.00–0.75 mM) on the capacity factor of K^+ and Na^+ vis-à-vis change of pH of the eluent was evaluated (Fig. 4). Significant increase in capacity factor of K^+ and not of Na^+ was evident because of considerably higher affinity of K^+ as compared with Na^+ manifested differential effect on retention time and hence the capacity factor [8]. There was, however, a small increase in the observed pH of the mobile phase from 2.64 to 2.70 with increase of CE concentration, perhaps because of complexation of hydronium ion.

Thus, the caging effect of 18-crown-6 ether was successfully used here to obtain better resolution of potassium from sodium. Several theories could postulate the mechanism of the influence of 18-crown-6 ether. First, as reported by Ohta et al. in 2002 the 18-crown-6 in the eluent can adsorb onto the stationary phase forming additional retention sites. Second, there is an ion-pairing mechanism between the eluent ion and the analytes. Third, 18-crown-6 ether can form aqueous complexes with K^+ in the eluent, affecting its association with the stationary phase. It is apparent from this study that the concentration range of 18-crown-6 ether chosen for the present purpose preferably favours the adsorption of the mobile phase 18-crown-6 ether-K^+ complex towards stationary phase, resulting in gradual increase in the retention time, and thus the capacity factor.

3.3. Sensitivity, precision and accuracy

Suitability of the mobile phase (4 mM tartaric acid, 0.75 mM PDCA, 0.75 mM CE in 2% acetone) for quantitative analysis of low level of K^+ in presence of high NaCl was examined in terms of reproducibility of the retention time and peak area as well as the limit of detection (LOD), over a long period of time. Employing two concentrations of K^+, 0.5 and 1.0 ppm, precision and accuracy of retention time and peak area data from 10 replicate runs carried out on the same day or on different days were analyzed (Table 2). Small relative standard deviations only were observed in retention times (RSD% < 0.5) and peak areas (intra-day RSD 0.5% and inter-day RSD ∼5%). The percent accuracy, evaluated with 0.5 and 1.0 ppm K^+, appeared to be 99.56 and 100.15 respectively with respect to retention time. In terms of peak area, intra-day accuracy with replicate samples was 98.04 and 100.89 for 0.5 ppm K^+ and 1.0 ppm K^+, respectively. The inter-day accuracy of peak area, on the other hand, has had somewhat more fluctuations (see Table 2). A set of validation experiments using spiked samples of known concentrations were also carried out to further evaluate the accuracy of the results. Since there was no sample processing step prior to conductivity measurement, the issue of recovery was not considered. However, using a few experimental samples that putatively contained different amount of K^+ covering the experimental range of K^+, we have added spiked samples of known concentrations to assess the accuracy of determinations. A blank NaCl (104 mM) solution and two experimental samples containing about 0.25 and 1.0 ppm K^+ was spiked with known amount of analyte (0.5, 1 and 4 ppm K^+), and then quantitated to see accuracy of deter-

Table 2
Reproducibility of retention time and peak area

K^+ concentration (ppm)	Relative standard deviation (%) of			%Accuracy of		
	Retention time	Peak area		Retention time	Peak area	
		Intra-day	Inter-day		Intra-day	Inter-day
0.5	0.395	0.5	2–3	99.56	98.04	96.33
1.0	0.319	0.5	5–7	100.15	100.89	103.01

Operating conditions are as in Fig. 1. The intra-day performance was evaluated by repeated run of 10-replicate samples whereas the inter-day data was obtained by repeat analysis over a period of 1 month. Accuracy is expressed as [(mean observed concentration)/(nominal concentration)] × 100.

mination. Three replicate samples were run at 3 days interval. The recovery was found to range between 96 and 102% in the case of blank sample while in case of experimental samples the same was 92–107% (data not shown). The limit of detection calculated at an S/N ratio of 3:1 (noise determined at peak-to-peak envelop of the baseline for 100 μl injection volume) was determined to be 0.10 ppm.

This system worked for nearly 3 years without any significant drift of peak area; however the retention time gradually shifted from 23 to 21 min with increase in column pressure at the end of 3 years, reflecting the aging of the column to some extent. Nevertheless, the suitability of the above chromatographic conditions for the quantitative analysis of low level of K^+ in biological samples under different experimental conditions was amply evident. Further, usefulness of 18-crown-6 ether in achieving baseline separation of K^+ from Na^+, as suggested by others [16–18], is also demonstrated. The eluent containing 0.75 mM CE is considered optimum for analyzing K^+ content

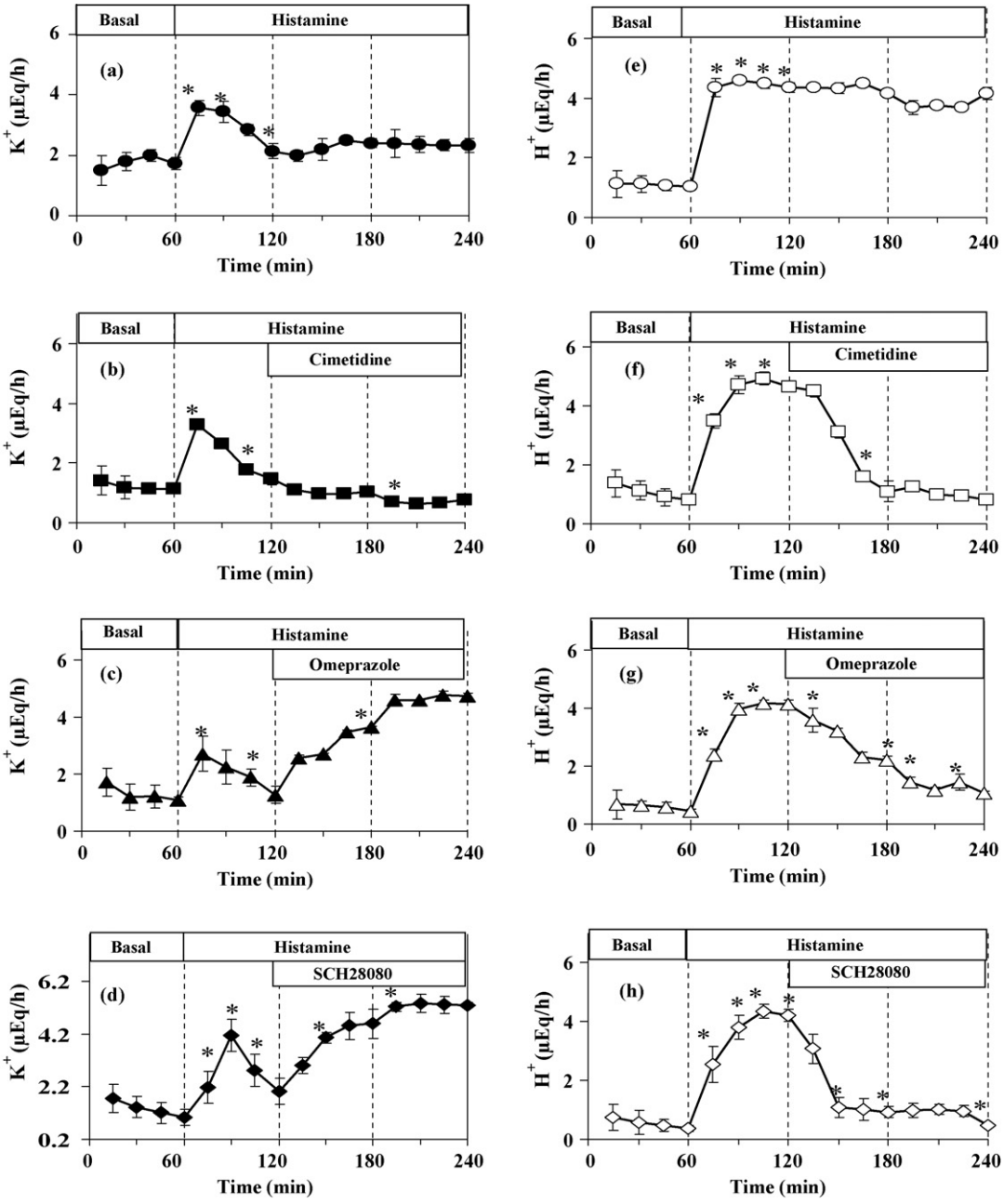

Fig. 5. Rate and extent of K^+ and H^+ efflux under influence of cimetidine, omeprazole and SCH28080 in histamine stimulated frog gastric mucosa. Experimental details are as in Section 2.4. Panels a–d describe net K^+ efflux data and panels e–h H^+ transport data for control (a and d), cimetidine (b and f), omeprazole (c and g) and SCH28080 (d and h), respectively. For each compound, the experimental results were plotted using same symbols for K^+ (closed) and H^+ values (open). Cimetidine was given at a single dose of 1 mM at 120 min with a change of nutrient side after 1 h. Both omeprazole and SCH28080 were given at a graded dose of 0.01 and 0.02 mM at 120 and 180 min, respectively. Data for control, cimetidine, omeprazole and SCH28080 are mean ± SEM ($n = 3$–4). An asterisk (*) indicates a statistically significant difference (at least $p < 0.05$) from the prior steady-state values.

in our biological experiments where the latter is expected to range between 0.1 and 10 ppm. To perform routine and accurate analysis of samples of gastric secretions, the detector range was set at 1 mS/cm (background eluent conductivity 592 μS/cm), and the full scale at 1 μS/cm. Further increase of sensitivity at full scale (0.5 μS/cm) hinders accurate quantification of higher concentration range (10 ppm and above).

3.4. Validation with biological samples in Ussing chamber experiments

To determine whether such ion chromatographic method with conductivity detection can be applied to quantitatively monitor the net K^+ efflux through the gastric mucosal membrane under conditions of acid secretion into the gastric lumen, we wanted to validate such method in Ussing chamber model where frog gastric mucosa is allowed to secrete HCl under the influence of the physiological secretagogue histamine to stimulate the final component of the signal transduction cascade, the pump H^+,K^+-ATPase [4,19–21]. This in turn would trigger K^+ recycling pathway [2,4,19,20]. Employing a few designed experiments where net K^+ efflux is expected to get characteristically altered under the influence of different types of gastric antisecretory agents, we have assessed K^+ secretion over time in secretory solution (luminal side) containing 104 mM NaCl solution (~6000 ppm NaCl). A histamine H_2 receptor blocker (cimetidine), a suicide inhibitors of gastric H^+,K^+-ATPase (omeprazole) and a K^+-competitive reversible inhibitor of H^+,K^+-ATPase (SCH28080) were used as probes in experimental systems to demonstrate diagnostic changes in net K^+ efflux [4,19–22]. The net efflux of K^+ under basal secretion followed by histamine-stimulated secretion, and the effects of additions of such modifiers in the presence of histamine were estimated. We have also simultaneously quantitated H^+ transport under identical conditions by pH-stat titration. The results are summarized in Fig. 5.

Following histamine (0.1 mM) stimulation, a transient spike of net K^+ efflux was observed which declined with time (panels a–d, 60–120 min). This surge in K^+ transport over basal secretion was found to be statistically significant ($p < 0.05$). Such characteristic phenomenon as was noted by others [4,19,20,22] lends support in favour of the precision and accuracy of this ion chromatographic method. Panels f–h further describe the H^+ efflux data under the influence of antisecretory agents like cimetidine, omeprazole and SCH28080. Panels a and e served as control for panels b–d and f–h, respectively. Cimetidine (1.0 mM) lowered the K^+ efflux rate significantly ($p < 0.05$) and, with time, the value went even lower than basal secretion level (panel b) producing resting mucosa. On the other hand, both omeprazole and SCH28080 increased the net K^+ efflux over time (panels c and d, respectively). The effects were dose-dependent and statistically significant ($p < 0.05$). Simultaneous measurements of H^+ secretion in all such samples (panels e–h) validated the experimental frame that under conditions of histamine-stimulated HCl secretion all three antisecretory agents, though working through different mechanisms, are capable of reducing the rate and extent of H^+ secretion (see panels e–h 3rd and 4th hour data).

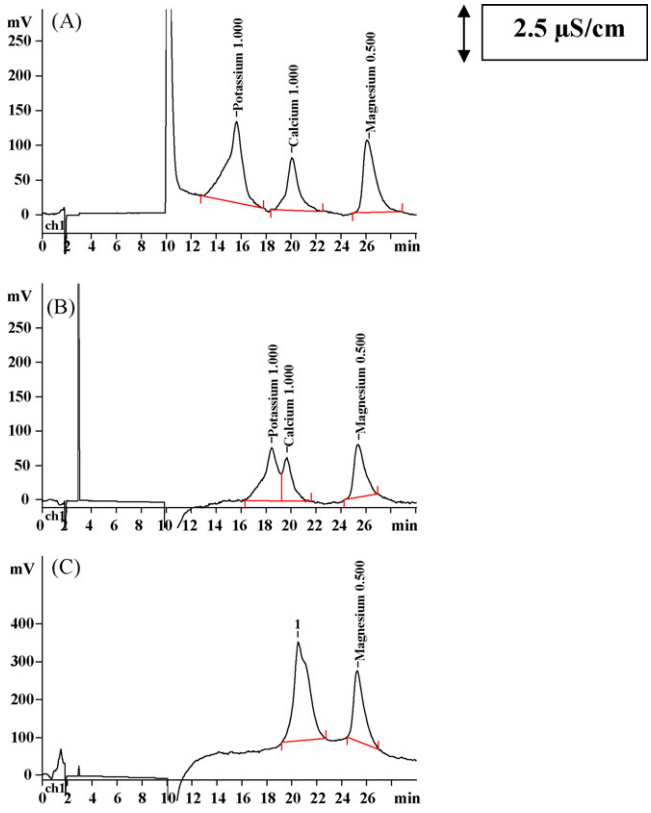

Fig. 6. Separation of 1 ppm each of K^+, Ca^{2+} and 0.5 ppm Mg^{2+} in high Na^+ with eluent containing different concentrations of 18-crown-6 ether. Eluent contains 4 mM tartaric acid, 0.75 mM PDCA, 2% acetone and 0.25 mM (A), 0.45 mM (B) or 0.75 mM (C) 18-crown-6 ether. The chromatograms were obtained after appropriate blank subtraction of 6000 ppm sodium chloride solution. Peak 1 in (C) indicated the merged peak of calcium and potassium. Operating conditions are as in Fig. 2.

Under such conditions, net changes in K^+ efflux are expected to decrease in the presence of cimetidine and increase in the presence of omeprazole and SCH28080, as observed by others [19–21]. Such changes of net K^+ efflux are conspicuously discernible and statistically significant. Thus the validity of the present quantification method is established.

3.5. Separation of K^+, Ca^{2+} and Mg^{2+} in presence of high Na^+

Additionally, this procedure and protocol provided another consistent method for the simultaneous determination of ppb levels of K^+, Ca^{2+} and Mg^{2+} in the presence of high NaCl at a single run. Fig. 6 presents the chromatographic elution profile, after blank subtraction, of a mixed standard of K^+, Ca^{2+} (1 ppm each) and Mg^{2+} (0.5 ppm) in high NaCl. Employing the mobile phase 4 mM tartaric acid and 0.75 mM PDCA in 2% acetone containing 0.75 mM crown ether, the peaks of Ca^{2+} and K^+ were merged (panel C). However, with 0.45 mM CE, K^+ eluted just before Ca^{2+} (panel B), and at 0.25 mM CE, complete separation of all three ions with smooth background was achieved (panel A; retention time of K^+, Ca^{2+} and Mg^{2+} being 15.5, 20.0 and 26.2 min, respectively).

Table 3
Calibration equation characteristics of the separation of K^+, Ca^{2+} and Mg^{2+} in presence of 6000 ppm NaCl using mobile phase containing 0.25 mM 18-crown-6 ether

Ion	Retention time (min)	Peak asymmetry factor	RSD (%) of Calibration equation	Slope ($\times 10^{-3}$)	Correlation coefficient
K^+	15.65 ± 0.339	0.51–0.64	3.549 ± 0.867	0.144 ± 0.004	0.9997 ± 0.0002
Ca^{2+}	20.53 ± 0.343	1.0–1.62	3.461 ± 0.942	0.260 ± 0.029	0.9997 ± 0.0001
Mg^{2+}	26.27 ± 0.216	1.74–3.62	5.01 ± 0.3912	0.142 ± 0.002	0.9995 ± 0.0001

Three-point calibration of the mixed standards containing 0.1, 1.0 and 5.0 ppm of each of the three ions, K^+, Ca^{2+} and Mg^{2+} was carried out ($n = 3$ in each cases). Eluent used was 4 mM tartaric acid, 0.75 mM PDCA and 0.25 mM 18-crown-6 ether in 2% acetone. Detector range was set at 5 mS/cm and full scale at 2.5 μS/cm. Data represent mean ± SD. The calibration equation was obtained after appropriate blank subtraction (6000 ppm NaCl solution).

One might argue whether Ca^{2+} could interfere with the potassium ion determination in the experimental samples. Using both 0.25 mM and 0.75 mM CE, chromatographic analyses of the pharmacological experiments related to changes in K^+ concentrations, were therefore simultaneously carried out during the initial stages of standardization. Only a small Ca^{2+} peak, almost below detection limit and tentatively estimated to be about 10–50 ppb, was found to appear with samples of basal collections under both the elution conditions, and such peaks were always distinguishable from the large peaks of K^+ in experimental samples. The central objective of this piece of investigation being quantifying and validating the fluxes of K^+ expected to change dramatically under the present experimental conditions and in systems that contained very high Na^+, the interference of low Ca^{2+} was rather ignored. Since 0.75 mM CE is considerably better as compared with 0.25 mM CE considering the risky retention time zone (14.9 min) and low RSD% of 9.64 for potassium with the latter eluent, we have performed all the experiments using the former eluent.

Changing the concentration of either tartaric acid or PDCA produced dramatic change in the chromatogram (data not shown). The LODs at S/N ratio 3:1 were found to be 0.1 ppm for each individual ion. Table 3 summarizes the calibration equation for K^+, Ca^{2+} and Mg^{2+}. The calibration plots of K^+, Ca^{2+} and Mg^{2+} were found to be linear over two orders of magnitude with excellent correlation coefficient and reasonably low RSD (%). The importance of such divalent cations in gastric HCl secretion and regulation is well known [22,23]; however, validation of the method with appropriate experiments is awaited.

4. Conclusion

An ion chromatographic method has been developed for quantification of ppb-level potassium in biological samples in the presence of 6000 ppm NaCl. The objective of this work has been to investigate whether the net efflux of K^+ through the gastric mucosal membranes into the gastric lumen under conditions of acid secretion could be quantitatively monitored by IC method for assessing the role of receptors and intracellular messengers in signal transduction cascade. With a view to investigating the effects of different putative agents on the shuttle functioning of K^+ recycling pathways, we have quantitated net K^+ efflux over time in secretory solutions. Employing C2 250 silica gel column with carboxyl groups as stationary phase, an otherwise difficult quantification of ppb level K^+ in the presence of 6000 ppm NaCl was achieved by incorporating 18-crown-6 ether in the mobile phase consisting of 4.0 mM tartaric acid, 0.75 mM PDCA in 2% acetone. A six-point calibration performed over a concentration range of 0.5–10 ppm K^+ after appropriate blank subtraction (~6000 ppm NaCl) indicated that an eluent containing 0.75 mM 18-crown-6 ether is optimum in terms of RSD (%) of retention time, peak area and calibration equations. The characteristic decline of K^+ efflux in presence of H_2 receptor blocker cimetidine, and the rise of the same in presence of H^+,K^+-ATPase inhibitor omeprazole (suicide inhibitor type) and SCH 28080 (K^+ competitive type) were conspicuously discernible, thereby validating the usefulness of this method. Additionally, the eluent containing 0.25 mM crown ether was found to be useful for simultaneous quantification of K^+, Mg^{2+} and Ca^{2+} in high NaCl solution in a single run. This later development was intended to permit further probing of such metabolically important divalent cations in gastric acid secretion and regulation [22,23] under different experimental conditions. We felt, such ion chromatographic method with conductivity detection can be employed to screen putative antisecretory agents *in vitro* that interfere with K^+ transport pathways.

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