Potentiometric detection of ammonium in biological fluids: reducing the interference from potassium

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ABSTRACT:

A novel approach to detect ammonium in biological samples is introduced here. The strategy combines the extraction of the main interfering cation, i.e. potassium, by functionalized polystyrene microbeads and the potentiometric detection of ammonium. Indeed the detection of ammonium in real samples is typically hampered by the strong interference coming from potassium. The potentiometric detection of ammonium in artificial urine was first carried out. Then, the extraction of potassium was optimized by tuning the amount of components and polystyrene microbeads. We reported an extraction efficiency as high as 77 % which then reduced significantly the interference from potassium. Noteworthy, we hypothesized that the amount of microbeads has to be tuned to the amount of potassium present in the sample. A general strategy was therefore proposed: first potentiometric detection of potassium, second extraction of potassium by the microbeads and third potentiometric detection of ammonium. Several artificial samples were then measured and their corresponding recovery calculated. The present strategy present significant advantages although limitations could be suffered in particularly high content of potassium.

Ammonium is a biologically relevant cation and in the body, it results from the metabolism of amino acids.[1] Then, the liver convert ammonium into urea, as a major component of the urine. Ammonium is present in several body fluids: blood, urine, saliva and sweat. Malfunction of the liver or the kidney could lead to increased concentration of ammonium in blood and in urine. One example of such issue is the genetic disorder called Rey's syndrome in children.[2] In blood, the normal level is 30 µmol/L in adults.[3] Severe consequence could be detected with increase above 45 µmol/L: hyperammonia with encephalopathy and possible hepatic coma.[4] In Urine, ammonium is a marker of the metabolic acidosis coming from the impaired renal insufficiency. In addition, elevated concentration of salivary ammonium could be related to chronic kidney disease.[5] The secretion of ammonium in sweat is an indicator of metabolic breakdown of proteins, thus providing information during sport monitoring, i.e. from aerobic to anaerobic exercise.[6] Ammonium detection in biological fluids is therefore relevant to several health status, hepatic disorders and metabolic state.[7]

Despite the importance of ammonium detection, the available methods present several drawbacks. The most common methods are the

colorimetric chemical reaction and the enzymatic methodology.[3] First, the Berthelot reaction, which is the reaction of ammonia with phenol-hypochlorite to give indophenol with a blue color that is measured by absorption spectrophotometry, could suffer from the amino acids interference because they could form undesired complex.[8] In addition, the hydrolysis of proteins during the reaction process releases ammonium which could results in misleading values. Second, the enzymatic reaction is based on the reaction of ammonium with 2-oxoglutarate by the glutamate dehydrogenase. Then, the decrease of absorbance of nicotinamide adenine dinucleotide phosphate is measured by spectroscopy. As the enzyme is specific, the main drawbacks arise from the complexity and time of analysis.[3]

As an alternative, ion-selective electrodes (ISE) have been used in clinical analysis for decades. Detection of potassium, sodium and chloride are routinely performed by ISEs in hospitals and this is due to the simplicity of potentiometric sensors. Ion-selective membranes are composed of three elements: an ionophore, to afford the selectivity to the sensor, an ion-exchanger, to ensure the permselectivity of the membrane and, the polymeric matrix, commonly polyvinylchloride (PVC) with a plasticizer to solubilize all the components.[9] The difference of electrical potential at the interface between the ion-selective membrane and the solution generates the analytical signal which is measured against the electrode of reference.

Although many ion-selective electrodes afford suitable analytical performance, in some selected examples, they could lack selectivity in order to be applied in real matrix. This is mainly due to the ionophore. For ammonium, the most common ionophores are the natural antibiotics Nonactin and Monactin.[10] Whereas the ionophores offer good selectivity against many cations, the main problem comes from the poor selectivity against potassium. This issue is an inherent problem in the field of recognition of cation since ammonium and potassium are of similar size (1.43 Å and 1.33 Å respectively).[11, 12] Even if some alternative have been reported with improved selectivity coefficient against potassium,[9] their lower limit of detection hampered their suitability for detection of ammonium in biological matrix. This is a significant issue since potassium is the second cation more abundant in biological fluids (values of blood potassium are within the range of 3.5–5.1 mmol/L).

As an alternative to ISEs, a new membrane-free optrodes were recently introduced. Traditionally, optrodes are comparable to ISEs because they are composed of an ionophore, an ion-exchanger and a polymeric matrix. However, optrodes transduction signal is afforded by a chromoionophore, which is a lipophilic dye.[9, 13] In their recent communication, Bakker et al. described the advantage of this new system as the improvement of some analytical features. For instance, the system was based on a mass extraction equilibrium compared to the phase equilibrium reported for the traditional sensors. This allowed to selectivity extract the cation of interest.[14, 15] Although Bakker et al. reported the system for potassium detection; we anticipated that this new approach could be of benefit for co-extracting interfering cations in real matrix. Indeed, here we introduce a simple and effective manner to reduce the interference of potassium for the detection of ammonium in biological samples. At first, optimization of the experimental conditions was performed, second the quantification of the extraction efficiency was determined and third, analysis of artificial and real samples was reported.

Experimental section

Reagents Latex beads (polystyrene, 0.8 µm mean particle size, without azide), nonactin (ammonium ionophore I), valinomycin (potassium ionophore I), sodium ionophore X, sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate, lithium tetrakis(pentafluorophenyl)-borate ethyl etherate , 3-Octadecanoylimino-7-(diethylamino)-1,2-benzophenoxazine, 9-(diethylamino)-5-(octadecanoylimino)-5*H*-benzo[*a*]phenoxazine, *N*-octadecanoyl-Nile blue, (chromoionophore I), 9-dimethylamino-5-[4-(16-butyl-2,14-dioxo-3,15-dioxaeicosyl)phenylimino]benzo[a]phenoxazine, (chromoionophore II), tetrahydrofuran (THF, anhydrous 99.9 %), magnesium acetate tetrahydrate (≥99% purity), ammonium chloride, potassium chloride, calcium chloride, magnesium chloride, sodium chloride, creatinine anhydrous (≥98%), were all purchased from Sigma-Aldrich (Spain). Doubly distilled deionized water (18.2 MΩ*cm^{-1} resistance) was produced by a Milli-Q water system (Millipore Corporation, Bedford, MA).

Polystyrene microspheres for extraction of K^+, and Na^+.

For potassium extraction: a solution of potassium ionophore I (V, 16.15 mM), chromoionophore I (Dye, 7.69 mM) and lithium tetrakis(pentafluorophenyl)-borate ethyl etherate (Li^+R^-, 10 mM) in 3 mL of THF was prepared. For sodium extraction: sodium ionophore X (16.15 mM) was used instead of potassium ionophore I.

1.1 mL of THF solution was added to 10 mL of a polystyrene microsphere suspension (20 µL of latex beads diluted with 40 mL of H_2O) on a vortex with a spinning speed of 1200 rpm. The resulting white suspension was blown with compressed N_2 for 20 minutes to remove THF. Then, the polystyrene microbeads were ready to use for extraction experiments and they are labeled PS beads in the following.

NH_4^+ and K^+ ion-selective membrane preparation.

The potassium-selective membrane contains 2.0 wt% of valinomycin, 0.5 wt% potassium tetrakis(4-chlorophenyl)borate, 64.7 wt% Bis(2-ethylhexyl)sebacate (DOS) and 32.8 wt% Poly(vinyl chloride) (PVC) high molecular weight.

The ammonium-selective membrane contains 0.2 wt% of nonactin, 69.0 wt% of 2-nitrophenyl octyl ether (*o*-NPOE) and 30.8 wt% of poly(vinyl chloride).

All the membranes were prepared by dissolving 100 mg of the mixture in 1 mL of THF and stirring for 30 min in an ultrasonic bath. After the stirring, 50 µL of the ion-selective membrane was drop cast on top of glassy carbon, and was left to dry for one day at room temperature. These cocktails were stored in the fridge at 4 °C and they remained stable for 2 weeks.

Measurements

Electromotive force (EMF) was measured with a high input impedance (1015 Ω) EMF16 multichannel data acquisition device (Lawson Laboratories, Inc. Malvern) at room temperature (22 °C). A double junction Ag/AgCl/KCl 3 M reference electrode (type 6.0726.100, Metrohm AG). Calibration curves were obtained by successive addition of the chloride salt (ammonium, potassium, sodium) to a 5 mL cell containing initially Milli-Q water.

Table 3. **(a), (b) experiments preparation:**

(2-a) Measured ammonia in 10 µL of urine sample treated with 800 µL of beads containing dye. (3-a) Measured ammonia in 10 µL of urine sample treated with 800 µ L of beads with no dye. (2-b) Measured ammonia in 10 µL of urine sample treated with 1500 µL of beads with dye. (3-b) Measured ammonia in 10 µL of urine sample treated with 1500 µL of beads with no dye.

Extraction efficiency:

Ratio [Dye]/ [K^+] is calculated in (10 µL of solution of potassium + 1500 µL of PS beads solution), the concentration of [Li^+R^-] = 0.99 mM, [V] = 1.61 mM, [Dye] = 0.76 mM in µL PS beads.

As a matter of example, ratio [Dye]/ [K^+]:

In 50 mM of potassium a (10 µL of 50 mM [K^+]) + (1500 µL of PS beads contain [Dye] =0.76 mM) and results as a ratio [Dye]/ [K^+] =2.30.

Where V: valinomycin, Li^+R^-: lithium tetrakis(pentafluorophenyl)-borate ethyl etherate and Dye: chromoionophore I.

Ratio E = ([K^+] remaining after extraction)/ ([NH_4^+] in the sample) = (0.5 to 1.9). In the following, we calculate the [K^+] remaining after extraction for the upper and lower limit, i.e. 0.5 and 1.9:

[K^+] remaining after extraction for [NH_4^+] = 20 mM: (0.5 x 20 =10 and 1.9 x 20 =38),

[K^+] remaining after extraction for [NH_4^+] = 40 mM: (0.5 x 40 =20 and 1.9 x 40 =76).

Blind samples in figure 6.

Sample	[K^+] (mM)	[NH_4^+] (mM)
1	50	25
2	75	20
3	60	0
4	0	35

25 % extraction of K^+ for sample 1 means that the ratio [Dye]/ [K^+] is 1.5.

50 % extraction of K^+ for sample 2 and 3 means that the ratio [Dye]/ [K^+] is 2.2.

RESULTS AND DISCUSSION

The detection of ammonium was carried out by potentiometry using a conventional cell with a working and a reference electrodes. Time response of the ammonium selective electrode was recorded in order to obtain a calibration curve and the corresponding analytical parameters. In order to characterize the ISEs for real sample measurements, we used two different media: first Milli-Q water in order to confirm the analytical performance of the ISEs and second artificial urine without sodium to assess the interference coming from potassium only (Figure 1). The performance are summarized in table 1.

Table 1 Sensitivity (slope), linear range (LR), limit of detection in Milli-Q water and artificial urine without sodium (n.d.: not detected).

	H_2O	Artificial Urine
Slope (mV/ decade)	57.2 ± 0.7	49.2 ± 4.1
LR (M)	10^{-2} - 10^{-5}	10^{-2} -10^{-3}
LOD (M)	$10^{-5.7}$	n.d.

In Mili-Q water, the ISEs reported a Nernstian sensitivity with a slope of 57.2 ± 0.7 mV/dec in a linear range from 10^{-5} up to 10^{-2} M with a limit of detection of $10^{-5.7}$ M. However, in artificial urine without sodium, the ISE strongly suffered interference from potassium. The linear range was therefore reduced to only one order of magnitude (from 10^{-2} M to 10^{-3} M) and the slope to 49.2 ± 4.1 mV/dec. The very narrow linear range of detection clearly hindered the detection of ammonium in real samples (Figure 1).

This first experiment confirms the interference from one of the most abundant cation in biological fluids. This behavior is in good agreement with the selectivity coefficient for the ammonium selective electrode (log $K_{(NH_4^+)/(K^+)}$ = -1 and log $K_{(NH_4^+)/(Na^+)}$ = -2.6).[11] From this first experiment, we concluded that we cannot use the conventional ISE for the direct detection of ammonium in biological samples. To overcome this issue, several researchers have introduced improvements. For instance, Chin et al. have introduced a novel ionophore based on tris(pyrazol-1-ylmethyl)benzene with improved selectivity (log $K_{(NH_4^+)/(K^+)}$ = −2.6).[11] However, the low binding constant between the ammonium and the synthetic

receptor resulted as a lower limit of detection (LOD = 2.5 x 10^{-5} M).[18] As an alternative, Georges has proposed to increase the pH of the solution in order to detect the ammonium in gas phase.[16] Although this method affords suitable detection, it could suffer interferences from volatile products.

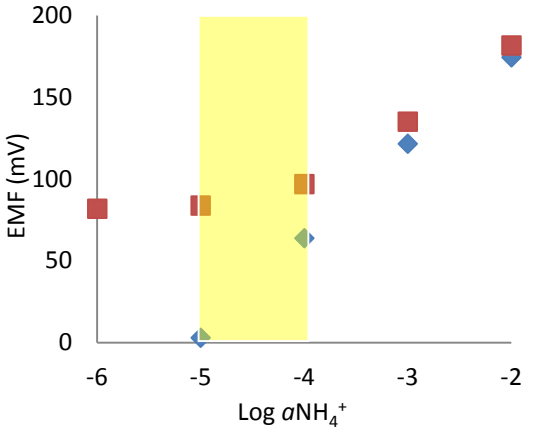


Fig. 1 calibration plot for NH_4^+ sensing in Milli-Q water (blue) and in artificial urine without sodium (red). Normal [NH_4^+] range is represented in yellow.

Recently, a new approach was proposed to detect potassium in solution. Xie and co-workers reported an ion-selective microsensor based on the modification of polystyrene microbeads. The systems is composed of the chromoionophore I (Ind), the cation exchanger sodium tetrakis(pentafluorophenyl)-borate (Na^+R^-) and the potassium ionophore I (valinomycin, V). All the components are dissolved in tetrahydrofuran (THF). Then THF cocktail was injected into an aqueous PS microsphere suspension, finally the THF was removed using compressed N_2 on the surface of the suspension (Figure 2).[17]

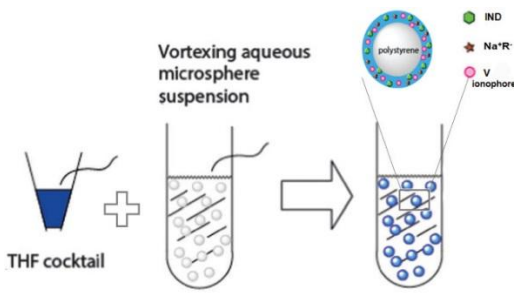


Fig. 2 General scheme of PS beads preparation with K^+ ionophore.

The mechanism works on a mass extraction equilibrium principle so that it offers attractive analytical performance such as higher sensitivity compare to conventional optrodes. The mechanism of the detection can be expressed by the following equation:

$$HInd^+(s) + R^-(s) + V(s) + K^+(a) \rightleftharpoons Ind(s) + R^-(s) + V(K)^+(s) + H^+(a)$$

Where (a) is aqueous phase, (s) PS beads surface, V valinomycin, R ion exchanger and ind the dye.

The equation is based on mass extraction equilibrium and suggests that the released amount of H^+ to aqueous phase is equal to K^+ extracted into PS beads surface.

We hypothesized that the system described by Xie al. could work either for detection or for extraction so that it could extract K^+ in a sample where it exhibit interference. We then prepared K^+ microbeads as described Xie et al. except for the cation exchanger, we herein used the lithium tetrakis(pentafluorophenyl)-borate ethyl etherate (Li^+R^-) in order to reduce the interference of sodium.

To gain further insights on the extraction method, we have performed a first experiment: ammonium was measured in artificial urine sample, and then in two samples of artificial urine, the first one was treated by K^+PS microsphere to extract K^+, and the second treated with Na^+PS microsphere to extract Na^+. The results are summarized in table 2. The recovery was calculated in each case in order to assess the improvement given by the method introduced herein. As anticipated, the higher amount of ammonium was detected in artificial urine, as it is actually the sum of several cations (ammonium, potassium and sodium). Then, after extraction of sodium, the amount detected was not significantly decreased which confirm the lower interference form sodium. Eventually, after

extraction of potassium, the concentration of ammonium was decreased by more than 10 mM, which ensure the high interference from potassium. Figure 3 displays the trend observed in the measurement of table 2. Although it is clear that the extraction affords a possible improvement in the detection of ammonium, the components and conditions require further optimization to give acceptable results.

Table 2 Measured NH_4^+ in urine samples (before and after Extraction K^+, Na^+)

NH_4^+ detection (mM)		Recovery %
Artificial urine	47.4 ± 4.6	158
Urine after Na^+ extraction	44.3 ± 5.3	148
Urine after K^+ extraction	38.6 ± 3.7	129
30 mM NH_4^+ (Reference)	28.2 ± 2.9	94

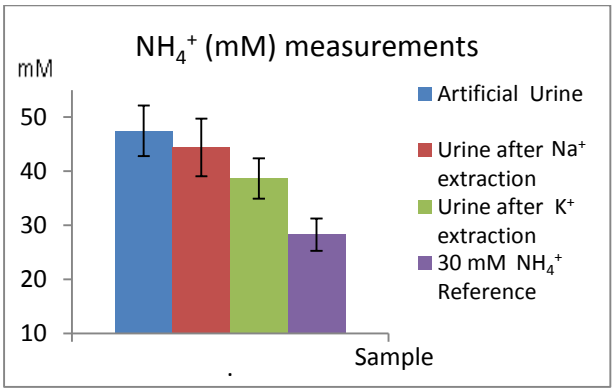


Fig. 3 Ammonium concentration in urine samples (before and after extraction of K^+, Na^+).

As we do not require a transducer here (the dye for the optical detection), we decided to optimize the system of extraction by using two different conditions: one with the dye as reported by Xie et al. and one without the dye. Therefore the same samples were treated with the two conditions and the results were compared (Table 3). In table 3a, the recovery calculated significantly decreased from 220% down to 136% (without and with dye respectively). Although, the result is still not acceptable in this first approach (more than 110% recovery), a clear improvement was detected. To confirm this issue, we performed a second experiment by adding a two-fold amount of beads. The results are presented in Table 3b. A similar trend is displayed and a better recovery was reached (112%) in the case of the beads containing the dye. As a control experiment, a reference value was given to compare the measurements done by the ISEs.

Table 3. **(a)** Measured ammonia in artificial urine samples and **(b)** with double amount of PS microspheres.

NH_4^+ detection (mM)			Recovery %
1-a	Artificial Urine	86.8 ± 14.6	289
2-a	After K^+ extraction, PS beads without dye	65.9 ± 9.3	220
3-a	After K^+ extraction, PS beads with dye	41.0 ± 5.0	136
4-a	30 mM NH_4^+ (reference)	32.0 ± 2.7	106
1-b	Artificial Urine	68.4 ± 25.1	228
2-b	After K^+ extraction, PS beads without dye	55.2 ± 13.5	184
3-b	After K^+ extraction, PS beads with dye	33.6 ± 5.3	112
4-b	30 mM NH_4^+ (reference)	29.4 ± 4.2	98

From the experiments carried out here, the polystyrene beads containing the dye afford a higher extraction capacity. Those preliminary results could point to the importance of the confinement of the charged species onto the surface of the beads for enhanced extraction efficiency. Although the role of the positive charge on the surface of the beads is not clear, the more lipophilic the better the extraction (Lithium is more

hydrophilic than the charged dye). After this first screening, our aim was to quantify the extraction efficiency in order to define the amount of beads which are required for potassium extraction. Indeed, it is reasonable to think that a higher amount of beads could also extract ammonium and afford misleading results (the selectivity coefficients of the K^+ ionophore are log $K_{(NH_4^+)/(K^+)}$ = -1.2)[6]. To do so, we prepared K^+ ISEs and several solution of known amount in order to assess the quantity of beads required. Three solutions containing different concentration of K^+ were prepared (65 mM, 50 mM and 20 mM respectively). Then, 10 µL of each sample was treated with 1500 µL of K^+PS microbeads. The concentration of potassium was measured before and after extraction and the percentage of extraction was calculated for each concentration (Table 4 and Figure. 4).

Table 4.
(a) Extraction of K^+ percentage in three different concentrations

[K^+] (mM)	20	50	65
Ratio [Dye]/[K^+]	5.7	2.3	1.7
Extraction %	77.8 %	51.4%	36.1 %

(b) shows the ratio [Dye]/[K^+] in volume of (10 µL of sample treated with 1500 µL of PS beads).

[K^+] (mM)	200	100	85	65	50	20
Ratio [Dye] /[K^+]	0.5	1.1	1.3	1.7	2.3	5.7

The Figure 4 shows increase of potassium extraction as the ratio [Dye]/ [k^+] is increased. For instance, for 65 mM concentration of potassium the extraction was 36% and for 50 mM it was 51%. In the case of the concentration is inferior to 20 mM, the detection by ISEs could not be performed since the resulting concentration was below the lower limit of the linear range (below 10^{-5} M). In the case of the concentration was superior to 10 µM, a high amount of microspheres was required and it was therefore challenging to perform. From the experiments, we can tune the amount of beads required depending on the amount of potassium present in the sample to analyze. A maximum extraction efficiency was reported as 78% for a ratio of [Dye]/ [K^+] equal to 5.7.

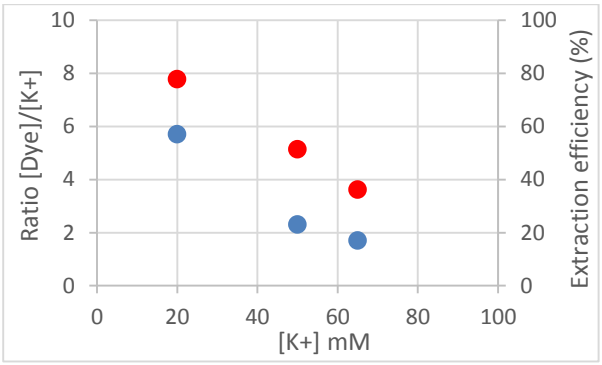


Fig. 4 Potassium extraction percentage *vs* K^+ concentration (red). The ratio [Dye]/[K^+] is represented on the right axis (blue).

Table 5. Six different samples from (K^+, NH_4^+)

Sample	[K^+] (mM)	[NH_4^+] (mM)
1	10	10
2	30	10
3	30	20
4	30	40
5	125	20
6	125	40

With these results in hands, we wanted to assess the performance of the approach in several artificial samples. To do so, we have to first detect the concentration of potassium and then to tune the amount of beads to extract it so that detection of ammonium could be feasible. We prepared samples containing both K^+ and NH_4^+ with concentration that falls within the clinical range in real urine and saliva samples (table 5). We then performed two experiments; first tuning amount of beads to extract 30% of K^+ from this samples and

second tune the beads to extract 50%. After the extraction, we measured the levels of ammonium, and the results are summarized in (table 6).

Table 6.Measuring NH_4^+ after extraction 30% of K^+ where ratio [Dye]/ [K^+] equals to 1.6, 50% of K^+ where ratio [Dye] / [K^+] equals to 2.3. (Ratio E: K^+ remains after extracted/NH_4^+ exist in sample)

Sample	Extraction	[NH_4^+] (mM)	Recovery %	Ratio E
1	30%	10.8	108	0.7
	50%	9.4	95	0.5
2	30%	14.2	141	2.1
	50%	9.6	96	1.5
3	30%	22.2	110	1
4	30%	40.5	101	0.5
	50%	32.6	81	0.4
5	30%	33.9	169	4.3
6	30%	57.8	144	2.1
	50%	38.8	97	1.6

In table 6, we can notice that the accepted NH_4^+ values were measured in samples (1, 3, 4) for (30 % of K^+ extraction) and (1, 2, 6) for (50 % of K^+ extraction). In order to deep into these results, we defined a new ratio: Ratio E. The ratio E is the amount of K^+ remaining after extraction divided by amount of ammonium exists in the sample.

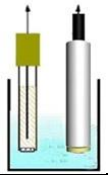
The acceptable values were obtained when ratio E was in the range (0.5 - 1.9), while the rejected ones gave false high ammonium values when ratio E was above 2 and gave false low ammonium values when Ratio E was less than 0.5.

From this experiment, we can assume that the accepted NH_4^+ measurements could be related to ratio E. When ratio E is in between 0.5 and 1.9, the ammonium prediction is acceptable.

After improving the extraction system, we have to confirm that this approach can be applied to artificial samples. For instance, we suggest that the extraction procedure of urine samples can be done through the following steps: first, measuring K^+ in our sample by ISEs electrodes. Second, calculate the beads amount based on ratio E. Since we know that the typical NH_4^+ values range is 20-40 mM in urine sample, we calculated the accepted K^+ after extraction by using ratio E. The results was (10-38 mM K^+) for 20 mM NH_4^+ and (20-76 mM K^+) for 40 mM NH_4^+. The remaining amount of potassium after extraction must be between (20 and 38 mM K^+) to give a ratio E in the range 0.5-1.9, so that it makes the extraction successful. Finally, by converting the percentage to [Dye]/ [K^+] ratio we can tune the volume of K^+PS microspheres. The figure 5 summarizes the general procedure of extraction of measurement of ammonium in urine.

Fig 5. Extraction procedure steps in urine sample.

(A) [K^+] detection in the sample by ISEs



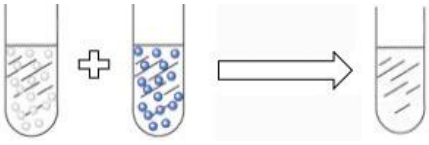
(B) Tune the amount of PS beads required to extract K^+

Ratio E = $\frac{\text{Remaining } K^+ \text{ after extraction}}{NH_4^+ \text{ In the sample}}$

Ratio E = **0.5 - 1.9** , NH_4^+ = **20 - 40** mM

Remaining K^+ after extraction = **20 – 38** mM

(C) Mix urine sample with PS beads, centrifuge and remove the precipitate



(D) [NH_4^+] detection by ISEs after removing K^+

To see if the extraction procedure is working in artificial urine samples, blind samples were prepared. After measuring the potassium, we performed the extraction experiments and the detection of ammonium. The results are shown in Fig 6. (See measurement for details)

Fig 6. [K^+] in blind artificial urine samples, blue columns shows the measured [K^+] by ISEs, red columns the real [K^+]. Sample 4 contains no NH_4^+.

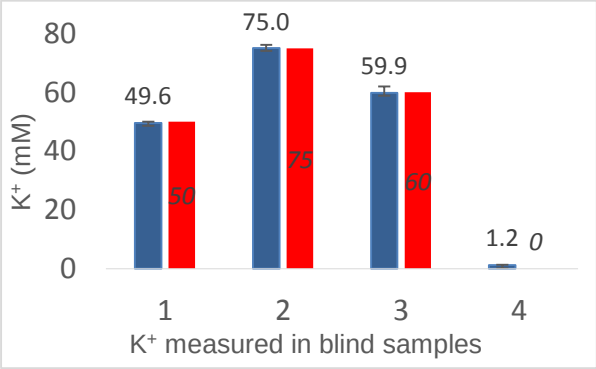


Figure 6 shows the measurements of K^+ using ISEs electrodes in four different artificial urine samples. The values were (49, 75, 59, 1 mM) respectively.

Potassium percentage of the extraction must afford a K^+ concentration value between (20-38 mM) otherwise the ammonium would be extracted too or we will not be able to eliminate the potassium interference.

By removing 25 % from the K^+ of sample (1), 50 % from sample (2) and (3) and there was no extraction from sample (4) because we did not obtain a potassium in it (See measurements for details). After treating blind samples with tuned beads, we centrifuge, remove the precipitate, and measure ammonia. The results obtained are summarized in (table 8).

From the results we can conclude that the extraction worked in sample (1) and did not work in sample (2) and (3). On the other hand the sample (3) does not contain any ammonium and gave a false result related to the remaining potassium after extraction. This is a drawback in the extraction system approach and it seems that we extract all the amount of potassium from sample (2). By repeating the extraction of K^+ from sample (2) with a percentage of 30 %(that equals to ratio 1.6 of [Dye]/ [K^+]) instead of 50 %. Then we measured the K^+ to assess the amount we extracted and the results showed concentration of 42.6 mM. This means that the extraction was 43 % instead of 30 %. It could explain the very low value of ammonia when we extracted 50 % of K^+ in the previous experiment.

Table 8. Measured NH_4^+ in blind artificial urine samples after extraction K^+

Sample (mM)	Expected NH_4^+ (mM)	Measured NH_4^+ (mM)	Recovery %
40 NH_4^+	40	43.6	109
20 NH_4^+	20	21.1	105
1	25	22.6	90
2	20	1.1	5
3	0	14.5	∞
4	35	37.4	107

After the last extraction from sample (2) we added tuned beads again to extract 12 % from it and then we measured the remaining NH_4^+ and it was 21.4 mM with recovery of 107 %.

It seems that using the PS beads to extract 50% of potassium would extract ammonium also giving misleading results. However, dividing the PS solution into two parts and using each part in different step make the extraction successfully done.

Also in sample (1), although the real extraction should be 35 % instead of 25 % in first experiment, the extraction worked because the remaining K^+ after extraction is still in the accepted range (20 and 38 mM). Anyway although the proof of concept was demonstrated here, it still has some complications and needs further studies to solve these issues. The extraction process still requires three steps,

measuring the [K^+], preparing the PS beads amount and then measuring the ammonia. Misleading results could be given when co-extraction of ammonium together with potassium is obtained.

CONCLUSIONS

We have reported a novel approach to measure ammonium in biological samples. The approach is based on potentiometric detection of ammonium and extraction of the main interfering cation, potassium. The method used modified polystyrene beads: optimization and quantification of the extraction were reported. Extraction efficiency could be improved to 77% although further experiments are required to understand the driving force of the extraction when the microbeads are used with or without dye. We have therefore proposed a general strategy to detect ammonium in biological samples and several artificial samples were measured with acceptable recovery. However, we assumed that this approach could hardly be applied to samples containing elevated amount of potassium. Although extraction of the interfering cations could be carried out, cross-extraction could be also a significant limitation. Further experiments are required in order to define the suitable extraction percentage in different kinds of artificial samples.

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