



A reflectometric ion sensor for potassium based on acrylic microspheres[☆]

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ABSTRACT

A K⁺ ion-sensitive optical sensor based on Nile Blue (chromoionophore ETH5294) incorporated into poly(n-butyl acrylate) [poly(nBA)] microspheres was successfully developed. A combination of acrylic spheres and optical reflectometry had enhanced the sensing performance of the optical K⁺ ion sensor, as the former provides large surface area for better loading capacity and higher reaction rate, while the latter offers simple and rapid measurements. The sensing mechanism involves ion-exchange reaction, which occurs at the surface of the microspheres where influx of K⁺ ions and efflux of H⁺ ions take place. The sensor response was a result of the total deprotonation of chromoionophore immobilized within poly(nBA) microspheres in response to the formation of K⁺ ion-ionophore complex. The change of the response in the presence of K⁺ ions was studied via reflectance spectroscopy using an optical fibre probe. The K⁺ ion sensor based on these microspheres exhibited sensitivity slope of 43.15 Δ Intensity/decade ($R^2 = 0.961$) in a range 0.01–1000 mM of KCl with a lower detection limit of 0.01 mM. Sensor response time was 10 min with good reproducibility (relative standard deviation, %RSD = 5.71). Analysis of urine samples for K⁺ ions gave 87–115% recovery and the results were comparable with established method for K⁺ ions analysis. Leaching tests and life time study revealed that acrylic microspheres worked well as an immobilization matrix for hydrophobic chromoionophore.

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1. Introduction

Ionophore and chromoionophore are common sensing materials that frequently applied in the fabrication of ion-selective optode. Moreover, detection of clinical ions such as K⁺, Na⁺, Ca²⁺, Mg²⁺, Cl[−], PO₄^{3−}, CO₃^{2−} and SO₄^{2−} [1] via optode sensing has been widely explored. Cations and anions are essential electrolytes that built up living organisms and have significant effect in almost all kind of metabolic processes. A few major functions of these ions are (1) maintaining hydration and osmotic pressure, (2) acting as cofactor and redox agent respectively in enzymatic and electron-transfer reactions and (3) regulating membrane potential and well-being of muscular and nervous tissues. In addition, metabolic disorder often associated to electrolytes concentration imbalance. Therefore, ions detection in biological samples is important for human pathological states monitoring and diagnosis [2].

Majority of the reported optical K⁺ ion sensors are based on polymer matrix as the immobilization support for liquid membrane components, e.g. plasticizer and electroactive substance. Poly(vinyl chloride) (PVC) [1,3–10] is one of the most extensively used ion-selective membrane due to its properties of high tensile strength, chemical inert and plasticizer-friendly [11]. The purpose of plasticizer incorporated into the polymer matrix is to obtain a soft and elastic polymer that benefits ion complexation. This phenomenon can be explained as elastic polymer has lower glass transition temperature value (T_g) which contributes to lower resistance, hence allowing ion-transfer reaction to occur efficiently [12]. Simultaneously, plasticizer weakens the mechanical strength and adhesion of the polymer and further causing polymer membrane to be peel off easily from the transducer. Nevertheless, a number of soft polymers were available for membrane sensor, for instance, silicone rubber but the quantity of soft polymer that can be used without plasticizer is very limited [13].

Plasticized polymer matrix often has plasticizer leakage problem and resulted in major drift of sensor response [4]. As a result, lifetime of sensor is short and not appropriate for in vivo applications as plasticizer can cause severe inflammatory. Plasticized PVC based optode has also been reported lack of reproducibility [1,5]. All these weaknesses were associated with the loss of plasticizer from PVC matrix, but plasticizer addition is unavoidable

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since optode membrane needs to provide a high mobility phase for sensing components [11,14]. Thus, plasticizer-free methacrylate-acrylate is another alternative and has been successfully applied in sensor and biosensor development.

The use of plasticizer-free polymer can avoid the occurrence of plasticizer leakage problem and improve sensor performance, however long response time is inevitable if T_g value of polymer is high. As reported by Peper et al. [11,12], sensors developed from poly(dodecyl acrylate) which has T_g value of 15.88–20.03 °C exhibited long response time of up to 12 h. Therefore, most of the optode membranes are usually thin so as to achieve equilibrium response rapidly [15]. Furthermore, polymer matrix may be prepared as a three-dimensional spherical shape instead of two-dimensional film, which reduces the time required for diffusion of an analyte within the matrix [16]. Recently Ruedas-Rama and Hall [15] have demonstrated that optical sensors with shorter response time can be obtained when poly(nBA) nanospheres were used instead of microspheres where a smaller diffusion path provided by the nanospheres improve response time.

Optical transducer systems for K^+ ion sensors that are popularly used were absorbance [1,4,7,13,17–19] and fluorescence [3,8,9,11,12,15,20–23] measurements whilst few utilized reflectometry methods [24–26]. However the former has drawbacks that substantially affected the sensor response. Absorbance spectroscopy is difficult to use for analysis of nano/microspheres based sensors due to non-uniform absorption if spheres are heterogeneous. Chromoionophore is more stable with absorbance methods than fluorescent methods because high excitation energy in fluorescence causes photodecomposition of chromoionophore and hence shortens the sensor lifetime [10]. Fluorescent microspheres usually synthesized via sonic casting and has short lifetime of 2–4 weeks [23]. Further, dynamic linear range for most reported optode K^+ ion sensors were narrow within 2–4 decades of concentrations only.

In the case of ETH5294 chromoionophore, ratiometric measurement can be applied to resolve the problem of non-uniform sphere sizes but it remains difficult to obtain good absorption data when the microsphere matrix does not transmit light sufficiently. Ratiometric method can also be used to reduce the effect from photodecomposition and light source instability for a more reliable response [23]. Transflectance, a combination of absorption and reflectance methods has been also proposed [5]. This method is more complicated as it is dependent on the membrane thickness and the radiation from the sample transmits through the membrane and then reflected back by a reflective material before transmitted through the membrane again to reach the detector.

Reflectance spectroscopy is a measure of the intensity change via the reflection of radiation from the sensing surface. Consequently, solid-state sensor that is opaque where absorption transduction is not possible and can employ reflectance mode for sensor response, especially colour change that resulted from interaction between analyte and sensing reagent at the sensing surface [27–30]. Another important advantage of using reflectance as a transduction method is to prevent photodecomposition of the sensing components when exposure to light source [29]. Thus, this stability of the sensing matrix contributes to good signal repeatability and eliminating drift in sensor response. The use of reflectance in conjunction with microsphere sensing matrix can contribute to the overall improved in sensor performance [16].

The intention of this study is to build an optical ion sensor by utilizing microspheres and reflectance spectroscopy. The advantage of using hydrophobic n-butyl acrylate (nBA) matrix is the formation of a non-plasticizer based immobilization matrix with low T_g value. Poly(nBA) microspheres provide large surface area, which enhances diffusion rate and loading capacity. Moreover, strong adhesive property of poly(nBA) favours robust adhesion to

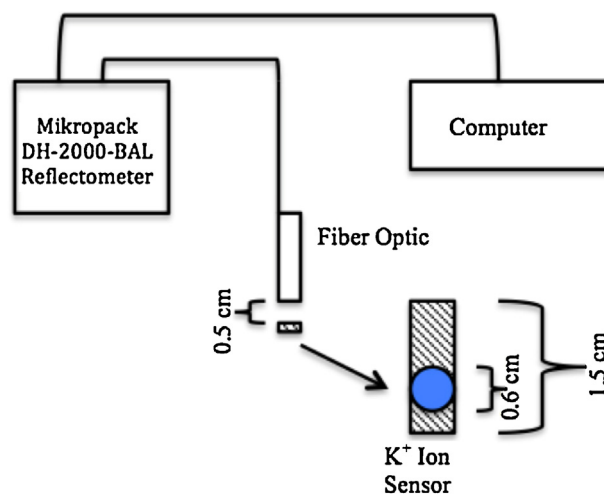


Fig. 1. Experimental setup of the reflectometric K^+ ion sensor.

the plastic support surface. Apart from the desirable properties of the nano/micro-sized poly(nBA) spheres, reflectance spectroscopy, which is important for the prevention of photobleaching of the chromoionophore is used as a transduction method.

2. Experimental methods

2.1. Chemicals

Chemicals used in this study included potassium chloride (KCl), sodium chloride (NaCl) and sodium dodecyl sulfate (SDS) from System; dimethoxy-2-phenylacetophenone (DMPP), magnesium chloride ($MgCl_2$), Nile Blue (chromoionophore ETH5294) and valinomycin (K^+ ionophore) from Fluka; 1,6-hexanedioldiacrylate (HDDA), potassium tetrakis (4-chlorophenyl) borate (KTICPB), nitric acid 69% (HNO_3) and tris-(hydroxymethyl) aminomethane (Tris-HCl) from Sigma Aldrich; n-butyl acrylate (nBA) from BDH; ammonium chloride (NH_4Cl) from Univar; calcium chloride ($CaCl_2$) from HmbG Chemicals; hydrochloric acid 37% (HCl) from Riedel-Haen; tetrahydrofuran (THF) from Scharlau. All chemicals were analytical grade and used as obtained. Deionized water was used for solutions preparation and cleaning glassware.

2.2. Instrumentation

Sonicator (Elmasonic), centrifuge (HERMLE Z230A, 5500 rpm) and ultraviolet (UV) exposure unit (R.S. Ltd) were used during the synthesis of acrylic spheres. The UV exposure unit radiated UV light (15 W) of 350 nm. The size and shape of poly(nBA) microspheres were examined using a scanning electron microscope (SEM, LEO 1450VP) with 20 kV acceleration voltage. A fibre optical reflectometer (Mikropack DH-2000-BAL) with UV-vis-NIR Lightsource, and built-in tungsten halogen lamp that operated at a wide spectral range of 200–1100 nm was used to measure the response of K^+ ion sensors (Fig. 1). The fibre optical probe is 0.6 cm in diameter and light guided by 0.15 cm optical fibre. For real sample analysis, inductively coupled plasma atomic-emission spectrometry (ICP-AES) from Perkin Elmer was used as a standard method. All apparatus involved in sample analysis were thoroughly washed with diluted HNO_3 .

2.3. Preparation of potassium cocktail and poly(nBA) microspheres solution

To prepare potassium cocktail, a mixture of 1 mol% K^+ ionophore, 0.5 mol% Nile Blue and 0.25 mol% KTICPB lipophilic salts were dissolved in 1 mL tetrahydrofuran. The cocktail was kept in dark at 25 °C. With the same preparation method, a blank cocktail without K^+ ionophore was also prepared. The poly(nBA) microspheres solution recipe was slightly altered from Ruedas-Rama and Hall [15] and Yew and Lee [31]. A mixture of 10 mg DMPP, 20 mg SDS, 480 μ L nBA monomers, 220 μ L HDDA and 5 mL deionized water were prepared in a sample bottle. Later, it was sonicated for 10 min so as to obtain a uniform milky white solution. Photocuring procedure was then carried out with the milky white solution exposed in a UV exposure unit under nitrogen gas for 10 min.

2.3.1. Determination of morphology and size distribution of poly(nBA) microspheres

Dry poly(nBA) microspheres were deposited on a glass slide and followed by a thin coating of gold. The morphology and size distribution of poly(nBA) microspheres were determined based on a random selection of 264 microspheres that obtained from a scanning electron micrograph.

2.4. Synthesis of K^+ -poly(nBA) microspheres for K^+ ion sensor

Poly(nBA) microspheres from photocuring procedure were mixed and suspended in potassium cocktail, which then sonicated to form a uniform blue slurry. The blue slurry was incubated for at least 3 h under airtight condition in order to allow the absorption of sensing components into the spheres. The respective solution was then left overnight in open-air to remove the tetrahydrofuran via evaporation. Finally, the blue poly(nBA) microspheres slurry was washed with Tris–HCl buffer solution (1 mM, pH 7) several times and centrifuged (5500 rpm, 10 min) to remove excess unreacted chemicals. Clean K^+ -poly(nBA) microspheres were kept at 25 °C in dark when not used. In addition, blank poly(nBA) microspheres were prepared according to the above method by using a cocktail without ionophore. To fabricate K^+ ion sensor, approximately 4.3 mg K^+ -poly(nBA) microspheres were deposited on a plastic strip with a spherical well of 0.6 cm in diameter. A ready-to-use K^+ ion sensor was kept in Tris–HCl buffer solution (1 mM, pH 7) before reflectometric measurement was conducted. For comparison purpose, a blank sensor was also fabricated similarly as K^+ ion sensor with blank poly(nBA) microspheres.

2.4.1. Leaching of sensing components

Possible leakage of chromoionophore from the microspheres was examined by exposing the K^+ -poly(nBA) microspheres to Tris–HCl buffer solution (10 mM, pH 7) for a 5–120 min and measured the absorbance.

2.5. Evaluation of K^+ ion sensor response

K^+ ion sensor response was evaluated with a fibre optic reflectometer, which concurrently functioned as a light source and detector. Fibre optic probe was placed directly above the sensor and the height of probe from the sensor was adjusted to obtain a maximum intensity of light reflection. Response of K^+ ion sensor was measured before and after the exposure to KCl solutions (50 μ L) with interval time of 10 min. A series of KCl solutions with concentrations ranged from 10^{-9} –1 M was prepared in Tris–HCl buffer solution (10 mM, pH 7) for sensor testing. A blank sensor without the immobilization of K^+ ionophore was evaluated similarly.

2.5.1. Effect of KTICPB lipophilic salt

Four batches of K^+ ion sensors were fabricated using K^+ -poly(nBA) microspheres with different KTICPB compositions of 0.10, 0.25, 0.50 and 0.75 mol% to examine the effect of KTICPB on the sensor sensitivity and selectivity. The sensors were tested with several interfering cations (e.g. NH_4^+ , Ca^{2+} , Mg^{2+} and Na^+) by using their chloride salts (10^{-9} –1 M). All chloride salts solutions were prepared in Tris–HCl buffer solution (10 mM, pH 7).

2.5.2. Effect of pH and buffer concentration

To investigate the pH effect on responses of blank and K^+ ion sensors, Tris–HCl buffer solutions (10 mM) was prepared in a pH range 3–11. Buffer pH effect was evaluated by varying the buffer pH values from 6.5 to 8.0 at fixed concentration of Tris–HCl (10 mM). In contrast, buffer at various concentrations (5–15 mM) at fixed pH (pH 7) was also used to examine the buffer concentration effect.

2.5.3. Response time and reproducibility

To determine the response time of the K^+ ion sensor, sensors were tested with KCl solutions (10^{-9} –1 M) at time interval of 5, 10 and 30 min. Whereas reproducibility of sensor was studied based on three different batches of sensor responses. Each batch was measured repeatedly at least three times for all concentrations of standard KCl solutions.

2.6. Real sample analysis

Real sample analysis was conducted to verify the accuracy and validity of K^+ ion sensor based on reflectometric method. Urine samples were collected with sterilized plastic bottles and kept cool in the refrigerator before use. Urine collection started with first urination of the day before any food consumption, followed by several urinations, which collected after 1 h ingestion of food. Besides, first urination from another subject was also collected and these samples were used for both K^+ ion sensor and ICP-AES measurements. Urine samples for K^+ ion sensor were used first without any pre-treatment directly. In order to ensure similar matrix conditions as KCl standard solutions, Tris–HCl was later added into urine samples to obtain 10 mM concentration with pH adjusted to pH 7 before measurement.

For ICP-AES analysis, the urine samples were first digested via wet digestion. A mixture of 1 mL urine and 5 mL concentrated HNO_3 was heated to 90 °C until the total volume was reduced to less than 5 mL. After cooling to room temperature, a further of 5 mL concentrated HNO_3 was added and heating was resumed to 120 °C with a gentle reflux. Later, mild boiling until a total volume of less than 5 mL was reached and the solution turned colourless. This mixture was cooled to room temperature and a further of 10 mL diluted HCl (1:1) and 15 mL deionized water were added followed by 30 min heating. Finally, the solution was cooled, filtered through a 0.45 μ m membrane and diluted with deionized water until a total volume of 100 mL was achieved. This final mixture was used for ICP-AES analysis. Blank sample solution was also prepared using the same procedure except no urine was used.

3. Results and discussion

3.1. Poly(nBA) microspheres as immobilization matrix

The morphology of poly(nBA) microspheres that synthesized via photopolymerization were presented in Fig. 2. The size distribution was 87.12% of spheres with diameter range 0.8–3.0 μ m. Narrow size distribution of the poly(nBA) microspheres enable a uniform loading of chromoionophores. Poly(nBA) microspheres containing the cross-linker 1,6-hexanediol diacrylate (HDDA) provides softness, elasticity and flexibility to the poly(nBA) matrix

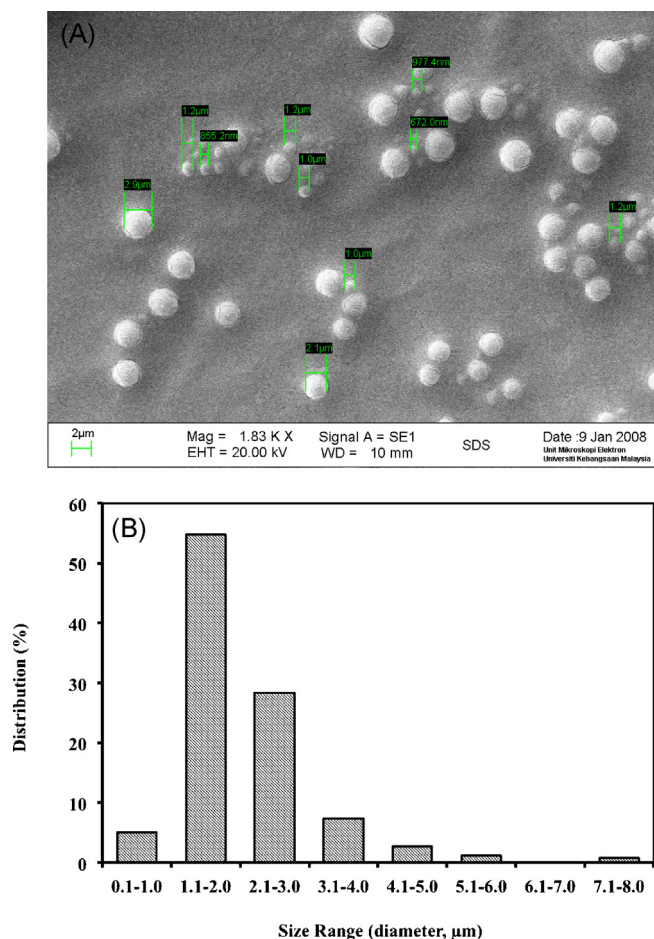


Fig. 2. (A) Scanning electron micrograph of the poly(nBA) spheres and (B) its size distribution pattern.

where the movement of ionophore and chromoionophore within the matrix has enhanced ion-exchange reactions at the surface of the microspheres. However, leakage of sensing materials from polymer matrix is still a major problem for most reported polymer matrix based sensors [17,20,24].

Leakage can lead to drift in response [4] and change of response behaviour [7]. In order to resolve such problem, approach such as attaching sensing components covalently or grafting to polymer has been proposed. Nonetheless, grafting method is useful only if the sensing components are modified in a manner to avoid reaction by-products that need tedious isolation [16]. On the contrary, hydrophobic poly(nBA) provides the benefits of proper immobilization of water-insoluble components via simple absorption and entrapment methods. As expected, there is no obvious fluctuation in intensity of K^+ ion sensor ($0.04 \pm 0.01 \Delta\text{Intensity/decade}$) with poly(nBA) microspheres when exposed to Tris–HCl buffer for 2 h. This implies a good capability of poly(nBA) microspheres as an immobilization matrix for K^+ ion sensing components where leakage of these components is unlikely.

3.2. Response of K^+ ion sensor from poly(nBA) microspheres

The total reflectance yield is affected by the angle of light radiation, refractive index, surface roughness and absorption property of the sample [5]. Hence, the colour change and intensity of the K^+ -poly(nBA) microspheres can cause variation in refractive index, so it is important to measure the sensor response before and after reacting with sample solutions. The response of the K^+ ion sensor

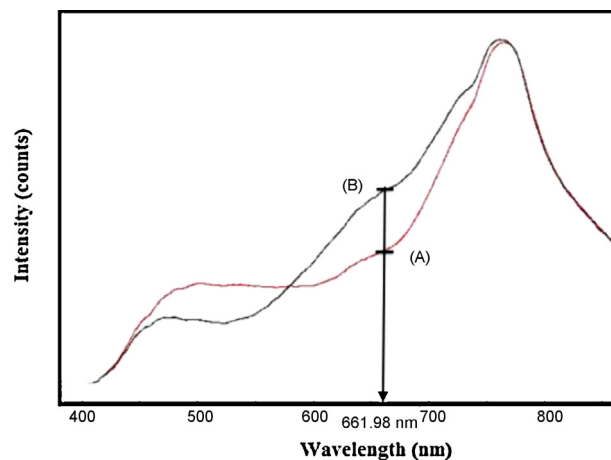
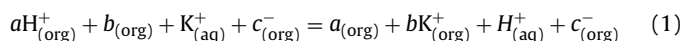


Fig. 3. Reflectance spectra of K^+ ion sensor obtained (A) before and (B) after tested with 1 M KCl solution.

is based on the difference of the reflectance observed at 661.98 nm (Fig. 3). The sensor response was measured at 661.98 nm because the maximum intensity difference occurred at this wavelength although there are other reflectance peaks found in the spectrum. This wavelength was denoted as the working wavelength and applied throughout for the evaluation of K^+ ion sensor response.

The mechanism of K^+ ions sensing with poly(nBA) microspheres involves an ion-exchange process, which occurs at the interphase of each microsphere and the sample solution. The ion-exchange reaction suggested is [10,23]:



where a is chromoionophore, b is ionophore (i.e. valinomycin) and c^- is KTICPB lipophilic salt. The K^+ ion is complexed in the poly(nBA) microspheres solid phase (org). As well, chromoionophore, a fluorescent dye plays a major role in the ion-exchange process, which influences the behaviour of the sensor response. Response of the K^+ ion sensor was a result of ion equilibrium related to the ratio of deprotonated/protonated form of chromoionophore. Under neutral condition, deprotonated and protonated chromoionophores are at equilibrium (Eq. (2)).



Initially, K^+ ions from sample solution were attracted to the surface of microspheres by the effect of anionic sites. Ionophores were then selectively form complexes with K^+ ions after diffusing to surface of the poly(nBA) microspheres. Dissociation of H^+ ions from H^+ -chromoionophore complexes is to compensate for the incoming K^+ ions, which leads to deprotonation of the chromoionophore. At constant pH, total influx of K^+ ions into the microspheres was equivalent to the total efflux of H^+ ions into the sample solution [16]. So the degree of protonation and deprotonation were proportional to the K^+/H^+ ions exchange activity, which was meant to achieve ionic equilibrium and maintain electroneutrality [11,15].

In this work, the blue coloured K^+ -poly(nBA) microspheres (protonated chromoionophore) changed into red (deprotonated chromoionophore) gradually with increasing concentration of K^+ ions. Based on reflectance measurement, the response of the sensor to K^+ ions yielded sensitivity slope of $43.15 \Delta\text{Intensity/decade}$ ($R^2 = 0.960$) over a wide range of K^+ ions concentrations (0.01–1000 mM) (Fig. 4).

3.2.1. Effect of K^+ ionophore on sensor response

Referring to Fig. 5A, a blank sensor with no K^+ ionophore in the microspheres did not response to K^+ ions ($0.23 \pm 0.03 \Delta\text{Intensity/decade}$) indicating an important role of

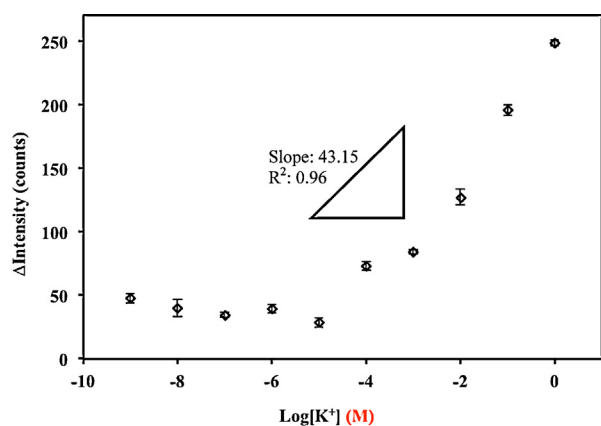


Fig. 4. The response of K^+ ion sensor towards KCl solutions that prepared in Tris–HCl buffer solution (10 mM, pH 7).

K^+ ionophores as the specific receptor of K^+ ions via efficient ion-exchange reaction that led to sensor selectivity. Besides, the examination of the possible interfering cations e.g. NH_4^+ , Ca^{2+} , Mg^{2+} , Na^+ on the K^+ ion sensor response showed no clear effect as demonstrated in Fig. 5 B. The use of valinomycin as K^+ ionophore is known to be very selective towards K^+ ions because of its cyclic dodecadepsipeptide structure and formation of a complex with stability constant of 10^6 [15,32]. Hence, the fabricated reflectometric K^+ ion-sensitive sensor is suitable for K^+ ions analysis.

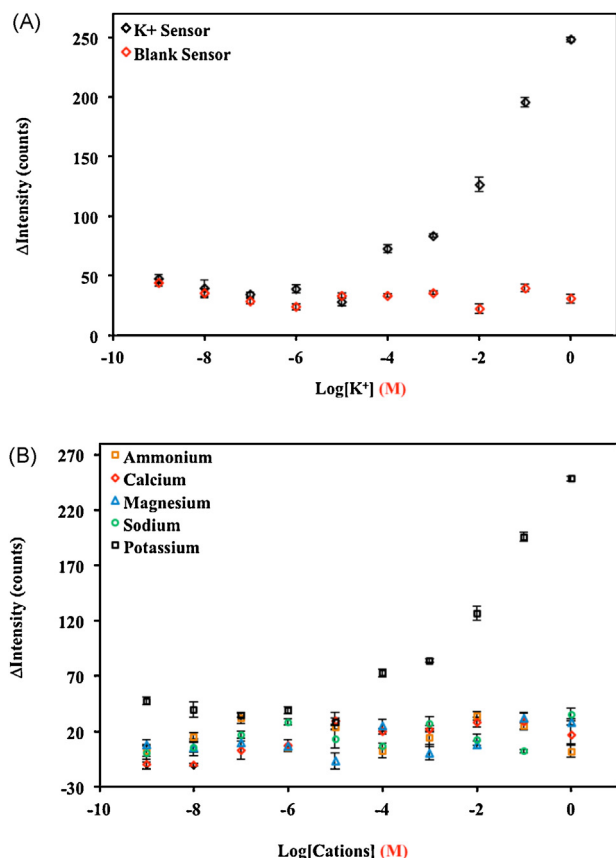


Fig. 5. (A) Responses of sensors with ionophore (K^+ ion sensor) and without K^+ ionophore (blank sensor) towards KCl solutions at various concentrations. (B) Behaviour of K^+ ion sensor when tested in several chloride salt solutions. All chloride salt solutions were prepared in Tris–HCl buffer solution (10 mM, pH 7).

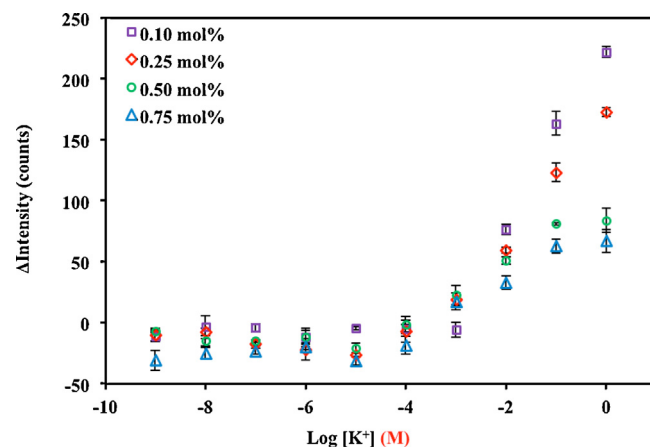


Fig. 6. The effect of various KTICPB lipophilic salts compositions in poly(nBA) microspheres towards the response of K^+ ion sensors.

3.2.2. Effect of KTICPB lipophilic salts

Potassium tetrakis (4-chlorophenyl) borate (KTICPB) was incorporated into poly(nBA) microspheres in order to increase the anionic charge of the microspheres. The presence of anionic sites is important for the sensor to have better sensitivity and shorter response time by attracting cations effectively to the matrix surface for ion-exchange process [15]. The high lipophilicity of these lipophilic salts is able to minimize leakage and reduce drifting in sensor response [4]. Nevertheless, too much of anionic sites can cause sharp decrease in sensor selectivity due to the dominant effect of the cation exchange reaction by anionic sites over that of the K^+ ion selective reaction by the ionophore.

Reflectance measurements showed that as the amount of KTICPB increased (from 0.10 mol% to 0.75 mol%) in the microspheres, the K^+ ion sensor's sensitivity deteriorated (from 77.01 Δ Intensity/decade to 23.77 Δ Intensity/decade) but the linear response range improved (from 1–1000 mM to 0.01–1000 mM) (Fig. 6). Increase in the KTICPB content also improved sensor selectivity because it is now less sensitive to other cations (Fig. 7). This is because the reflectance intensity differences for the sensor with higher KTICPB content is smaller attributed to the darker colour of sensor matrix. Since KTICPB affects the sensor response, 0.25 mol% of KTICPB was chosen to compromise between a balance of both sensitivity and selectivity.

3.2.3. Effect of buffer pH and concentration

pH has significant effect on the optical K^+ ion sensor, which contained pH-sensitive chromoionophore [4,24]. Both sensors of K^+ and blank (without valinomycin) show similar responses when

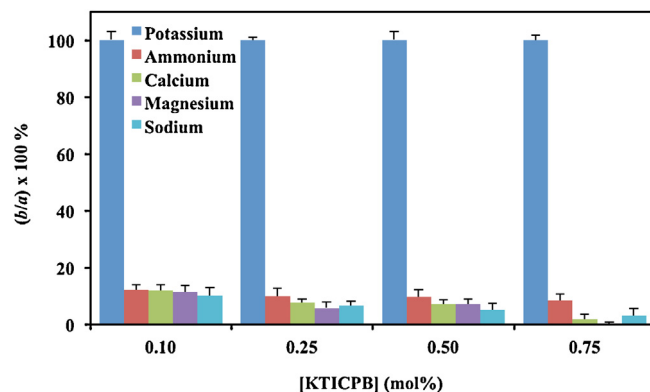


Fig. 7. The effect of the KTICPB compositions on the selectivity of K^+ ion sensors towards several cations at the concentration of 100 mM.

Table 1

Performance of K⁺ ion sensor in KCl solutions prepared with Tris–HCl buffer (10 mM) of different pH values.

pH of Tris–HCl buffer	Sensitivity [$\Delta(\text{Intensity})/\text{decade}$]	R ²	Dynamic linear range (mM)
6.5	58.04	0.982	1–1000
7.0	40.78	0.960	0.01–1000
7.4	110.80	0.981	1–1000
8.0	136.50	0.971	1–1000

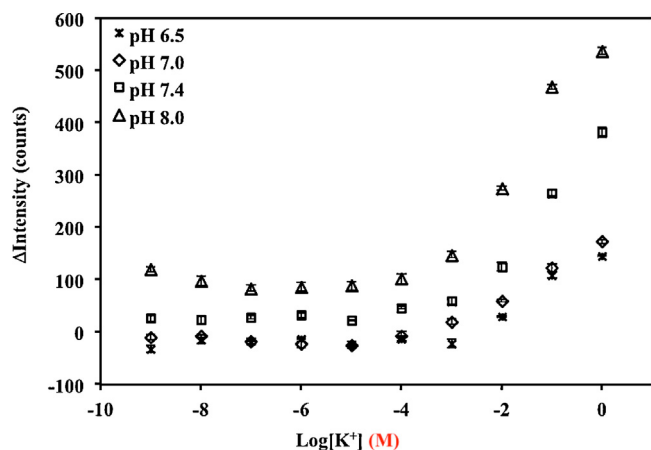


Fig. 8. K⁺ ion sensor responses in various KCl concentrations under varying pH conditions (Tris–HCl buffer, 10 mM).

tested in a range of Tris–HCl buffer solutions with pH varies from 3 to 11 ($\sim 87.71 \pm 2.77$ $\Delta\text{Intensity}/\text{decade}$). The presence of K⁺ ionophores in poly(nBA) microspheres does not disturb the formation and decomplexation of H⁺–chromoionophore complex. Hence, the determination of K⁺ ions concentration based on K⁺ ion sensor is reliable. To minimize interference from some other ionic species, Tris–HCl buffer was used to prepare KCl solutions throughout the K⁺ ion sensor evaluation.

From Table 1, the sensitivity of K⁺ ion sensor increases as the pH of buffer increases. Besides, Fig. 8 showed that the intensity values of the K⁺ ion sensor response also increase simultaneously. Buffer pH affects the equilibrium constant and H⁺ ions activity according to Hisamoto et al. [1]. At higher pH values, tendency of H⁺ ions extracted from H⁺–chromoionophore in the organic phase is higher and thus promotes the complexation of K⁺ ion–valinomycin. Additionally, K⁺ ion sensor exhibits declined sensitivity as buffer concentration increased (Table 2). It is because buffer of higher concentration and capacity has better ability to buffer off H⁺ ions causing the sensor to be insensitive towards the change of pH.

3.3. Performance of K⁺ ion sensor

Excess or insufficient amount of K⁺ ions in blood are symptoms of hyperkalemia and hypokalemia respectively, which very much related to kidney failure, and if it is severe, it may cause death [22]. Fluctuation of K⁺ ions concentration in patients particularly those recovering from operation or diabetic coma, continuous monitoring

Table 2

The effect of Tris–HCl buffer (pH 7) of various concentrations on the response of K⁺ ion sensor.

Molarity of Tris–HCl buffer (mM)	Sensitivity [$\Delta(\text{Intensity})/\text{decade}$]	R ²	Dynamic linear range (mM)
5	41.82	0.973	0.05–1000
10	40.78	0.960	0.01–1000
15	29.60	0.953	0.01–100

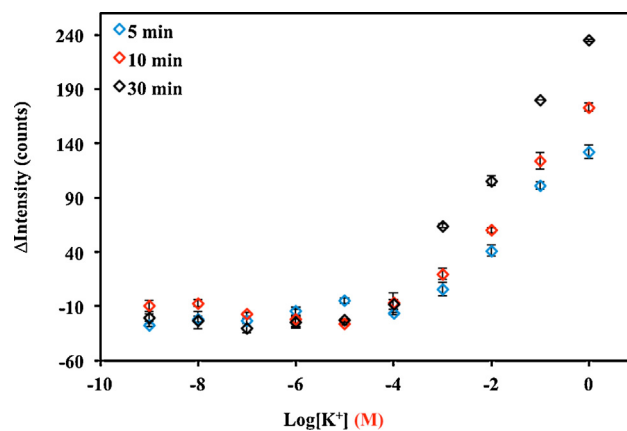


Fig. 9. The response profile of the K⁺ ion sensor at several response times.

Table 3

Analytical performance of reflectometric poly(nBA) microspheres based K⁺ ion sensor.

Parameters	K ⁺ ion sensor
Sensitivity [$\Delta(\text{Intensity})/\text{decade}$]	43.15
R ²	0.961
Dynamic linear range (mM)	0.01–1000
Lower detection limit (mM)	0.01
Repeatability (%RSD)	0.14
Reproducibility (%RSD)	5.71
Response time (min)	10

Table 4

K⁺ ions analysis in real samples and comparison between K⁺ ion sensor ($n=3$) and ICP–AES standard method.

Urine sample	ICP–AES (mM)	K ⁺ ion sensor (mM)	% Recovery
Subject 1–0 ^a	24.76	26.70 \pm 1.74	101–115
Subject 1–1 ^a	29.17	24.61 \pm 1.36	89–97
Subject 1–2 ^a	29.81	31.07 \pm 3.23	93–115
Subject 1–3 ^a	72.79	72.77 \pm 4.58	94–106
Subject 2–0	39.45	36.83 \pm 2.41	87–99

^a Subject 1–0 to 1–3: Urine collection from individual 1 from 1st urination followed by several urinations after food ingestion.

of K⁺ ions in blood is crucial [24]. Hence the reflectometric K⁺ ion sensor developed here was used to analyze urine samples and the overall performance of the K⁺ ion sensor in the analysis of urine samples is presented in Table 3.

The sensor demonstrated relatively wide dynamic linear range (0.01–1000 mM) with lower detection limit of 0.01 mM. Based on this performance, the sensor may be usable as a monitoring device in the clinical field as the K⁺ ions concentration in human serum and urine ranged 3.5–5 mEq/L and 25–125 mEq/L respectively. Therefore, K⁺ ion sensor was tested for real sample analysis and being compared to standard ICP–AES method. Recoveries of K⁺ ions found in urine samples via K⁺ ion sensor were 87–115% (Table 4) and do not differ significantly from ICP–AES standard method (t -test, $p > 0.05$). In short, K⁺ ions concentration in urine differs among individuals, which greatly affected by food intake, lifestyle and health condition.

Table 5

Performance of K⁺ ion sensor at chosen response time of 5–30 min.

Time (min)	Sensitivity [$\Delta(\text{Intensity})/\text{decade}$]	R ²	Dynamic linear range (mM)
5	39.31	0.977	0.1–1000
10	40.78	0.960	0.01–1000
30	54.05	0.979	0.01–1000

Table 6The performance of reported K⁺ ion sensors based on polymer matrix (spheres and membrane) and spectrophotometry measurements.

References	Sensing components (ionophore and chromophore)	Immobilization matrix	Detection methods	Thickness/ diameter (μm)	Dynamic linear range (mM)	Limit of detection (mM)	Response time (s)
Spheres							
This study	Nile Blue (ETH5294), valinomycin	Poly(n-butyl acrylate) (nBA) microspheres	Reflectometry	0.8–3.0	0.01–1000.00	0.01	600
[8]	Nile Blue (ETH5294; ETH5418), potassium ionophore III	Plasticized poly(vinyl chloride) (PVC)	Ratiometric fluorescence analysis	10.0	0.32–316.00	–	60–120
[10]	Nile Blue (ETH5294), potassium ionophore III	Plasticized PVC	Ratiometric fluorescence analysis, UV–vis spectrophotome- try	20.0	0.06–10.00	0.06	900
[11]	Nile Blue (ETH5294), potassium ionophore III	Poly(methyl methacrylate- decyl methacrylate) (MMA-DMA)	Ratiometric fluorescence analysis	10.0	0.10–100.00	–	43200
[15]	Nile Blue (ETH5294), valinomycin	Poly(nBA)	Spectrofluorimetry	>3.0 (micro), <0.2 (nano)	3.16–316.20	–	1800 (micro), <300 (nano)
Membrane							
[4]	Nile Blue (ETH5350), valinomycin	Plasticized PVC	Flow-injection analysis system and UV–vis spec- trophotometry	4.0	1.00–10.00	–	60

Besides, the responses of K⁺ ion sensors were repeatable and reproducible with 0.14%RSD and 5.71%RSD values respectively with average sensitivity of close to $40.81 \pm 2.33 \Delta \text{Intensity/decade}$. K⁺ ion sensor also performs well after three months storage with 3.62%RSD. Good repeatability, reproducibility and storage stability were attributed to well incorporation of sensing ingredients into the poly(nBA) microspheres. Inert, hydrophobic, elastic and low polarity of poly(nBA) microspheres make excellent immobilization matrix for the anionic KTICPB, neutral K⁺ ionophore and chromoionophore. In addition, the preparation of K⁺-poly(nBA) microspheres was simple and do not involve any complicated chemical reaction. Negligible leakage was observed and the sensor is reusable.

Response time of the K⁺ ion sensor was determined by exposing the sensor to various concentrations of KCl at a fixed time of 5, 10 and 30 min before measurements. The exposure time that yielded the best overall performance in sensor response was considered as the sensor response time. The response intensity and sensitivity of K⁺ ion sensor increased accordingly with the response time (Fig. 9, Table 5). It is because the total formation of K⁺ ion–ionophore complexes increased and leading to the increasing amount of free chromoionophore in the polymer matrix. Hence, the colour of K⁺-poly(nBA) microspheres changed greatly and resulted in a larger intensity change. Also, lower detection limit can be achieved at response times of 10 and 30 min compared with 5 min.

3.4. Comparison with reported optical K⁺ ion sensor

The performance of K⁺ ion sensor in this study was briefly compared with several reported optical sensors as presented in Table 6. Most of the optical sensors utilized plasticized PVC matrix and almost all measurements were based on fluorescence and absorbance spectrophotometry. Reflectometric K⁺ ion sensor demonstrated better performance in terms of a wide linear response range (0.01–1000 mM) span over five decades of K⁺ ions concentrations compared with other reported sensors of limited linear range between 1 and 4 decades. Besides, detection limit of K⁺ ion sensor was lower if not comparable with most of the optical

sensors. Response time of K⁺ ion sensor in this work was relatively short (10 min) as some reported to have response time up to 12 h [11]. Repeatability and reproducibility of K⁺ ion sensor were considerably good. Therefore, the analytical performance of K⁺ ion sensor was basically enhanced and improved due to the advantages from using micro-sized plasticizer free acrylic spheres coupled with reflectance measurements. Furthermore, the developed sensor benefits in wide applications from clinical to environmental analysis.

4. Conclusions

A reflectance based K⁺ ion sensor utilizing poly(nBA) microspheres was successfully developed. Poly(nBA) microspheres were shown to be an immobilization matrix compatible with the sensing components such as KTICPB, valinomycin and Nile Blue. The overall satisfactory analytical performance of the sensor was contributed by (1) the homogeneity of the spheres and (2) negligible leakage of sensing materials. It is also shown that anionic sites created by KTICPB have strong influence towards the response of the K⁺ ion sensor. In conclusion, K⁺ ion sensor with improved analytical performance has been developed based on the use of non-plasticized acrylic microspheres coupled with relectometric transduction.

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