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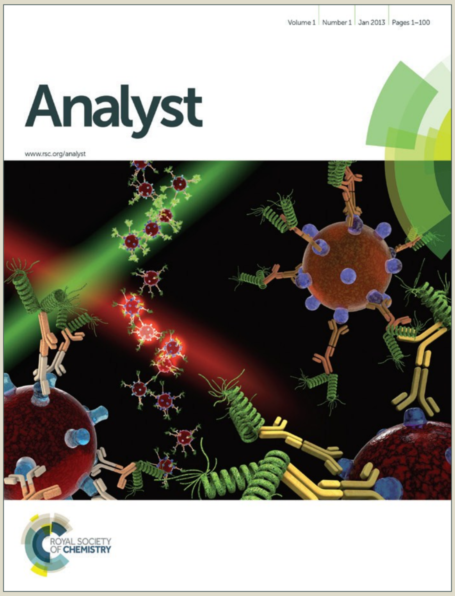

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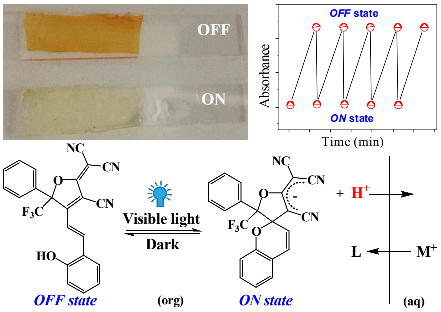
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COMMUNICATION

Visible Light-Induced Ion-Selective Optodes based on Metastable Photoacid for Cation Detection

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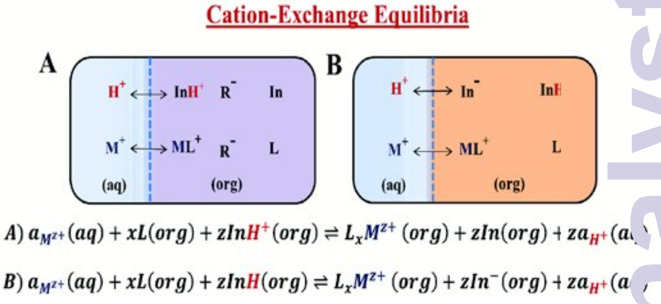
A new platform of ion-selective optode is presented here to detect cations under thermodynamic equilibrium via ratiometric analysis. This novel platform utilizes a 'one of a kind' visibile light-induced metastable photoacid as a reference ion indicator to achieve activatable and controllable sensors. These ion-selective optodes were studied in terms of its stability, sensitivity, selectivity, and theoretical aspects.

Ion selective optodes (ISOs) have been extensively studied to monitor different cations for biomedical and environmental applications.[1-11] In recent years, there has been considerable interest to study ISOs that are activatable, reversible and controllable for their use in ion sensing applications that require localized detection of free ions without perturbing its surrounding.[12-15] Attempts have been made to convert the traditional passive mode ISOs into active mode by the use of photoactive compounds such as spiropyrans and photoacid generators.[16-19] However, the use of spiropyran in an ISO requires UV light to activate the sensor, which can result in cellular damage when applied in biomedical applications.[20] In addition, UV light photodegrades the active compound in the ISO, shortening the sensor's lifetime.[13-17] Moreover, the use of photoacid generators in an ISO undergoes photolysis, making the sensor irreversible.[18,19] To overcome photodegration and irreversibility for such ISOs, we propose to use a 'one of a kind' visible light activatable and controllable ISO based on metastable photoacid (*m*PAH) to detect cations under equilibrium conditions by ratiometric analysis.

A typical ISO consists of a neutral basic indicator (In) that is selective to a reference ion (H^+), an ionophore (L) that is selective to the cation of interest, and anionic additives (R^-) that maintain electroneutrality in the plasticized polymer matrix (Scheme 1A).[21-23] The response mechanism of these ISOs is dictated by mass transfer equilibria (cation-exchange process) between an organic (ISO) and aqueous phases.[24,25] Consequently, the detection of the cation of interest, after achieving thermodynamic equilibrium, is made by measuring the changes in the indicators' optical property, either by absorption or fluorescence spectroscopy.[26] Nevertheless, prior to the cation detection, these ISOs needs an external source of protons for the cation-exchange process to proceed, resulting in a response mechanism with multiple steps. In contrast, the proposed tricyanofuran based *m*PAH (CF_3PhTCF-PAH) acts as a neutral acidic indicator (InH), providing protons for the cation-exchange process. Considering that *m*PAH is a photoactive compound, it photodissociates its proton upon irradiation and thermally undergoes proton reassociation with a dissociated state that is sufficiently long-lived.[27,28] Thus eliminating the need for an external source of protons, while the dissociated state of the *m*PAH acts as an anionic additive to maintain electroneutrality within the ISO as shown in Scheme 1B.[illegible] Hence, an ISO based on the proposed *m*PAH exhibits a one-step response mechanism.

We have recently shown that merocyanine based *m*PAH linked to an acrylate polymer backbone can be utilized in ISO to detect calcium ions.[29] This merocyanine based *m*PAH is a neutrally charged acidic indicator which exhibits longer equilibrium response time (in the order of hours). Consequently, the detection of calcium ions was performed



Scheme 1. Cation-exchange process of ISOs that utilize a (A) neutral basic indicator, and (B) neutral acidic indicator. (In: neutral basic

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indicator; L: cation selective ionophore; R^-: anionic additive; InH: neutral acidic indicator).

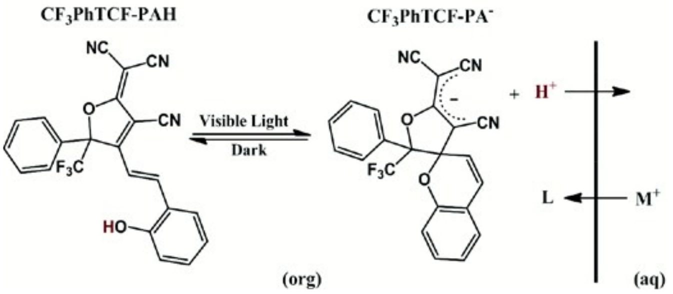
under non-equilibrium conditions.[29] Moreover, we have shown that by modifying the merocyanine based *m*PAH with an appropriate functional group (electron donating group), the equilibrium response time can be shortened to the order of minutes.[30] However, the charged nature of the merocyanine based *m*PAH was optimal only with polar plasticizer, such as 2-nitrophenyl octyl ether (*o*-NPOE), to reduce ion-pair formation that affects selectivity.[29] *o*-NPOE absorbs under 400 nm, interfering with the absorbance peak of the deprotonated state merocyanine based *m*PAH, and inhibited ratiometric analysis. In contrast, the non-charged CF_3PhTCF-PAH has shown ideal compatibility with non-polar plasticizer, such as bis(2-ethylhexyl) sebacate (DOS) which does not interfere optically, allowing ratiometric analysis to further increase sensor sensitivity and signal reproducibility.[31]

As shown in Scheme 2, it is expected that CF_3PhTCF-PAH within the ISO would undergo oxidative photoreaction under visible light irradiation. Resulting in a stable carbanion state (CF_3PhTCF-PA^-) of the *m*PAH along with its photodissociated proton.[32] Likewise, photodissociated protons would be exchanged when exposed to cation of interest, as the CF_3PhTCF-PA^- state is sufficiently long-lived to allow diffusion mediated cation-exchange process.

CF_3PhTCF-PAH was synthesized according to literature procedure.[32-34] The calcium and sodium ISOs proposed here contains CF_3PhTCF-PAH (7.5 mmol/kg), and calcium ionophore IV (22.5 mmol/kg) or sodium ionophore X (7.5 mmol/kg) within poly(vinyl chloride) (33 wt%) and DOS (66 wt%).

At first, these ISOs were exposed to their respective buffer solution without any cation of interest. As shown in Fig. 1, the ISOs based on CF_3PhTCF-PAH were stable over repeated activation cycles between *ON* (deprotonated form) and *OFF* state (protonated form) without any loss of absorbance signal, indicating no observable photodegradation.

Fig. 2 shows the absorption spectra of the ISOs based on CF_3PhTCF-PAH towards different concentrations of calcium (Fig. 2A) and sodium (Fig. 2B) ions at thermodynamic equilibrium (25 minutes in the dark) once the ISOs were activated by visible light (470 nm) for 1 minute. From both absorption spectra, as the concentration of the cation of interest increases there is a gradual decrease in the CF_3PhTCF-PAH peak (470 nm) and gradual increase in the CF_3PhTCF-PA^- peak (318 nm). These absorbance changes allows for ratiometric analysis (protonated and deprotonated absorbance peaks of the CF_3PhTCF-PAH) to indirectly correlate the activity of the cation of interest.



Scheme 2. Photoresponsive behaviour of CF_3PhTCF-PAH when incorporated in ISO.

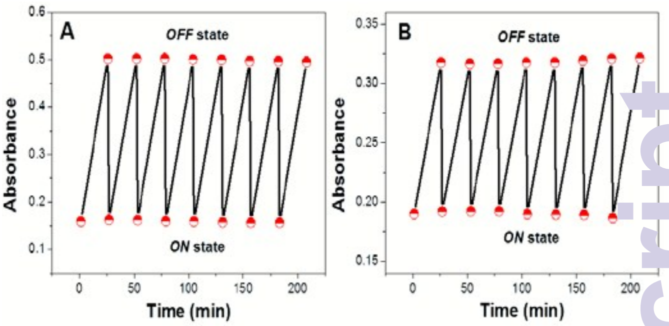


Fig. 1. Stability of CF_3PhTCF-PAH in ISOs for (A) calcium sensor in 0.5 M formate buffer at pH 4.5, and (B) sodium sensor in 0.3 M magnesium acetate buffer at pH 5.5. Absorbance recorded at 470 nm. (*ON* state: after 1 minute irradiation with 470 nm; *OFF* state: after 25 minutes in the dark).

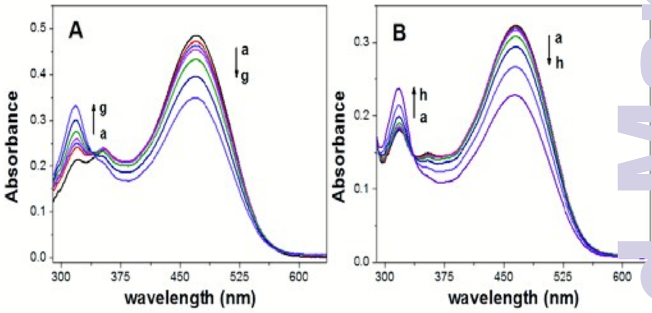


Fig. 2. Absorption spectra of ISOs at 25 minutes in the dark after activation for different concentrations of (A) calcium ion in 0.5 M formate buffer at pH 4.5; concentrations a) 0, b) 1.0x10[illegible], c) 1.0x10^{-7}, d) 1.0x10^{-6}, e) 1.0x10^{-5}, f) 1.0x10^{-4}, g) 1.0x10^{-3} M. (B) sodium ion in 0.3 M magnesium acetate buffer at pH 5[illegible] concentrations a) 0, b) 1.0x10^{-6}, c) 1.0x10^{-5}, d) 1.0x10^{-4}, e) 1.0x10^{-3}, f) 1.0x10^{-2}, g) 1.0x10^{-1}, h) 1.0 M.

The experimental data obtained was then compared to the theoretical response function. The theoretical response function (Equation 1), in terms of the activity of protons and the cation of interest, was generated from the ion-exchange equilibria (Scheme 1B). This was derived by utilizing mass balance (Equation 2), charge balance (Equation 3), degree of deprotonation using ratio of absorbance at 470 nm and 318 nm (Equation 4), and the ion-exchange equilibrium constant (Equation 5) equations. This is analogous to that of traditional ISO theory which was established in the early 1990's.[21-23]

Equation 1: Theoretical response function for cation.

$$a_{M^{z+}} = (K_{exch})^{-1}\cdot\left(\frac{(1\text{-}\alpha)a_{H^+}}{\alpha}\right)^{z}\frac{[InH]_T(1\text{-}\alpha)}{z\left([L]_T\text{-}\frac{x}{z}[InH]_T(1\text{-}\alpha)\right)^{x}}$$

Equation 2: Mass balance equations.

$$[InH]_T=[InH]+[In^-]$$
$$[L]_T=[L]+x[L_xM^{z+}]$$

Equation 3: Charge balance equation.

$$[In^-]=z[L_xM^{z+}]$$

Equation 4: Degree of deprotonation (α).

$$\alpha_{(470nm/318nm)} = \frac{[InH]}{[InH]_T} = \frac{A\text{-}A_D}{A_P\text{-}A_D}$$

Equation 5: Cation-exchange equilibrium constant (K_{exch}) for neutral acidic indicator.

$$K_{exch} = \left(\frac{[In^-]a_{H^+}}{[InH]}\right)^z \cdot \frac{[L_xM^{z+}]}{a_{M^{z+}}[L]^x}$$

The subscript "T" indicates total concentration of CF_3PhTCF-PAH ($[InH]_T$) and the ionophore ($[L]_T$). The activity of the cation of interest and proton is denoted as $a_{M^{z+}}$ and a_{H^+}. Also, "x" and "z" denotes for the value of the ionophore chelating with the cation of interest and charge of the cation of interest, respectively. The ratio of absorbance for CF_3PhTCF-PAH is denoted as A, protonated state of CF_3PhTCF-PAH in 1 M hydrochloric acid as A_P, and deprotonated state of CF_3PhTCF-PAH in 1 M sodium hydroxide as A_D; for 25 minutes in the dark after activation.

As shown in Fig. 3, the experimental data presents a strong correlation with the theoretical response curve. From the resulting cation response curves, the experimental limit of detection (LOD) for calcium and sodium ions were $2.6x10^{-6}$ M and $2.3x10^{-3}$ M, respectively. These values were obtained in by intersecting two extrapolated segments of the response curve as indicated in the literature.[35] Furthermore, the cation-exchange constant ($logK_{exch}$) for calcium and sodium ISOs were -9.3 ± 0.3 and -5.3 ± 0.1, respectively. The ionophore-cation complex ratio for calcium ISBO was 3 to 1 and for sodium ISOs was 1 to 1.[36,37]

It was hypothesized that ISOs which contains no anionic additive, cannot maintain constant ionic strength within the ISO.[23] As a result, the cation-exchange constant cannot be retained, due to each change in the activity of protons and the activity of the cation of interest.[23] However, the changes in the ionic strength within the ISO based on CF_3PhTCF-PAH, containing no additional anionic additive, were negligible as observed in the kinetic data for different concentrations of sodium ions (Fig. 4), where stable responses over time were obtained at thermodynamic equilibrium. It is noteworthy that interactions between the negatively and positively charged components for such ISO do occur, after a certain threshold (1-α > 0.5), as the ionic strength within the ISBO changes drastically. Accordingly, higher concentrations of cations were not shown in Fig. 3 because the thermodynamic equilibrium was not maintained, and thus the upper detection limit were $6.3x10^{-4}$ M and 0.6 M for calcium and sodium ions, respectively, (at α = 0.5).

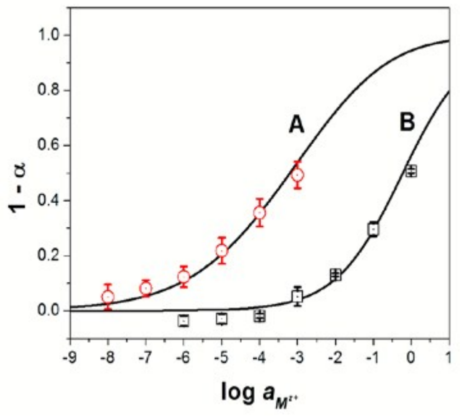


Fig. 3. Experimental (○ and □) and theoretical (lines) response of ISOs based on CF_3PhTCF-PAH for the detection of (A) calcium, and (B) sodium ions at 25 minutes in the dark after activation (n = 3).

Furthermore, these ISOs based on CF_3PhTCF-PAH were studied in terms of selectivity towards interfering cations (J) by separate solution method.[25,38] As shown in Fig. 5, all interfering ions were highly discriminated for both ISOs, calcium (Fig. 5A) and sodium (Fig. 5B). Thus, the selectivity coefficient values for calcium ISO towards magnesium, sodium and potassium ions were -3.8, -3.1 and -3.3, respectively. When compared to other ISOs containing same ionophore and spiropyran as the reference indicator, the selectivity coefficient were -2.9 (magnesium ion) -6.6 (sodium ion) and -8.9 (potassium ion).[16] The $logK_{M,J}$ values for sodium ISO towards potassium, calcium and magnesium ions were -4.5, -6.6 and -2.7, respectively. These values are comparable to the selectivity coefficient of sodium against potassium ion (-2.4), calcium ion (-4.0) and magnesium ion (-4.1) containing spiropyran as reference indicator.[16] The selectivity coefficient ($logK_{M,J}$) values were calculated using Equation 6, and is analogous to that of ISO theory at α = 0.5.

Equation 6: Selectivity coefficient for interfering cations.

$$K_{MJ}^{opt} = \frac{K^J_{exch}}{K^M_{exch}}\left[\frac{(1\text{-}\alpha)a_{H^+}}{\alpha}\right]^{z\text{-}w} \frac{w\left([L]_T\text{-}\frac{p}{w}[InH]_T(1\text{-}\alpha)\right)^w}{z\left([L]_T\text{-}\frac{x}{z}[InH]_T(1\text{-}\alpha)\right)^x}$$

The high discrimination observed may be due to the proton affinity towards the CF_3PhTCF-PA^- (deprotonated form) to be greater than the binding energy of interfering cations by the respective ionophores. Consequently, these ISOs inhibit the competitive behaviour between the interfering cations and protons during the cation-exchange process, even at high concentrations. As a result, ISO based on CF_3PhTCF-PAH may also be utilized for practical purposes which demands negligible interactions towards interfering cations.

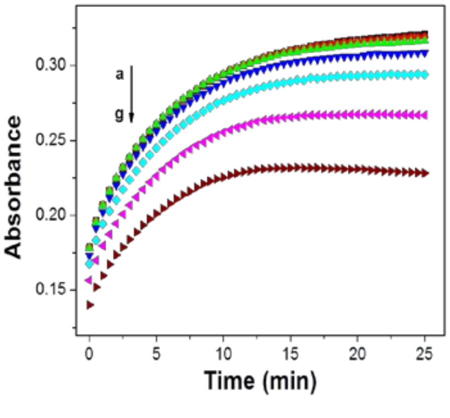
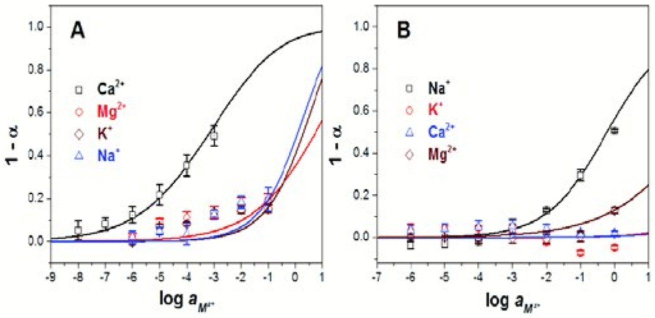


Fig. 4. Kinetic study for ISO based of CF_3PhTCF-PAH at different concentrations of sodium ions in 0.3 M magnesium acetate buffer at pH 5.5. Scans every 30 second after activation. Concentrations a) $1.0x10^{-6}$, b) $1.0x10^{-5}$, c) $1.0x10^{-4}$, d) $1.0x10^{-3}$, e) $1.0x10^{-2}$, f) $1.0x10^{-1}$, g) 1.0 M.

Fig. 5. Selectivity response for (A) calcium (Buffer: 0.5 M formate at pH 4.5), and (B) sodium (Buffer: 0.3 M magnesium



acetate at pH 5.5) ISOs towards interfering cations at 25 minutes in the dark after activation (n = 3).

In conclusion, this novel visible light activatable and controllable ISO based on CF_3PhTCF-PAH that does not exhibit photodegradation, showed good stability, selectivity and reproducibility. Furthermore, the responses of these ISO towards calcium and sodium ions were standardized following the cation-exchange equilibria. Similarly, other cations can be detected using this platform by interchanging the ionophore within the polymer matrix. Also, we aim to optimize this type of ISO in terms of size reduction and sensitivity. Likewise, we expect that this novel *m*PAH (CF_3PhTCF-PAH) may act as a substitute to neutral basic indicators for their use in cation sensing applications that provide control using visible light as needed.

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