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ORIGINAL PAPER

Fluorescent Recognition of Potassium and Calcium Ions Using Functionalised CdSe / ZnS Quantum Dots

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Abstract Schiff base receptor **1a** has been synthesised and attached to the surface of preformed CdSe/ZnS Quantum Dots (QDs) to form QD-conjugate **2a**. While **1a** was determined to be selective for Mg^{2+}, **2a** demonstrated selectivity for both K^+ and Ca^{2+} when tested against a range of physiologically and environmentally relevant cations by changes in the fluorescence spectra. Thus, the nanoparticle surface functions as a scaffold for the organisation of receptors enabling semi-selective binding. The fluorescence response was shown to be linear between 15–50 μM for K^+ and 2–35 μM for Ca^{2+}. It was also demonstrated that **2a** could measure both K^+ and / or Ca^{2+} in solutions containing both ions.

Keywords Fluorescence · Quantum dots · Sensor

Introduction

The development of new fluorescent probes incorporating Quantum Dots (QDs) as the signal transduction element is currently of great interest [1–3]. The unique optical properties associated with these nanoparticles make them attractive alternatives to traditional organic dyes for labelling and sensing applications [4–6]. For example, they absorb radiations across the UV and visible regions of the electromagnetic spectrum with large extinction co-efficients, have emission spectra that can be tuned to specific wavelengths, are more resistant to photobleaching than organic dyes and have long fluorescent lifetimes. In addition, we have also shown that QDs provide an useful framework for surface confinement of appended receptors resulting in dramatic changes in receptor binding affinity [7]. For example, when a Schiff base receptor was attached to the surface of a CdSe/ZnS QD, the resulting conjugate displayed selectivity for both Fe^{3+} and Cu^{2+}, through changes in the UV-Vis spectra, while the receptor alone displayed no selectivity. Furthermore, when the same receptor was incorporated into an all-organic tripodal framework the resulting probe was fully selective for Ag^+ [8–10]. Thus, the nanoparticle surface enabled an organisation of receptors that permits differential sensing behaviour.

Here, we extend this principle by adapting the receptor to include a fluorophore as fluorescence detection is usually considered to be a more sensitive technique than UV-Vis. In particular, we show that a CdSe/ZnS QD, surface functionalised with Schiff base receptor **1a**, is selective for both potassium and calcium ions through changes in the fluorescence spectra. The detection of these ions is of considerable importance due to their physiological relevance [11]. To the best of our knowledge this is the first reported example of a single sensor capable of measuring both potassium and calcium ions.

Experimental

Materials and reagents

Chemicals were purchased from Aldrich Co. and used as received without further purification. Green emitting CdSe-ZnS QD's were synthesised according to a literature method [12]. The synthesis of receptor **1a** and its reduced analogue **1b** have been reported elsewhere [13] (Scheme 1).

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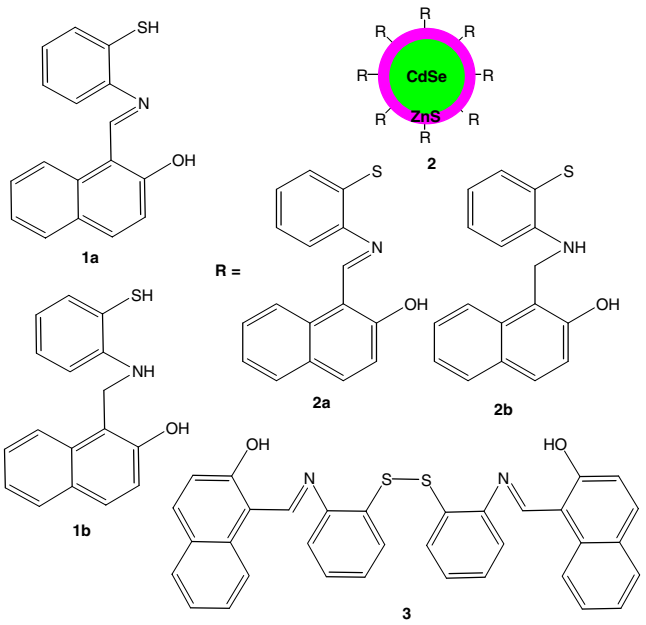

Scheme 1 Structures of compounds 1a, 1b, 2, 2a, 2b and 3

Synthesis of 3

Compound **3** was prepared by the aerial oxidation of 2-Aminothiophenol (125 mg, 1.0 m mol) in methanol. The formation of disulfide bond was monitored with TLC and the product used in next step without purification. A solution of 2-hydroxy-1-naphthaldehyde (172 mg, 1.0 mmol) was prepared in methanol and this solution was mixed with the solution containing disulfide. The solution was stirred at room temperature for 8 h. The yellow precipitate was filtered, recrystalized with acetonitrile-methanol solvent mixture and dried under vacuum (495 mg, 89%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.17 (d, Ar, 2H, J=9.2 Hz), 7.29 (t, Ar, 2H, J=7.2 Hz), 7.38–7.44 (m, 4H, Ar), 7.58–7.65 (m, 4H, Ar), 7.82 (d, 2H, Ar, J=7.6 Hz), 7.89 (d, 2H, Ar, J=7.6 Hz), 8.04 (d, 2H, Ar, J=9.2 Hz), 8.62 (d, 2H, Ar, J=8.4 Hz), 10.82 (s, 2H, CH=N), 15.04 (s, 2H, OH); ^{13}C NMR (DMSO=d_6, 400 MHz) δ 109.5 (Ar), 119.2 (Ar), 120.0 (Ar), 120.9 (Ar), 123.8 (Ar), 127.3 (Ar), 127.5 (Ar), 127.7 (Ar), 128.1 (Ar), 128.7 (Ar), 128.9 (Ar), 129.4 (Ar), 132.7 (Ar), 136.3 (Ar), 145.2 (Ar), 158.9 (Ar), 164.9 (CH=N). Mass calculated for $C_{34}H_{25}N_2O_2S_2$ (M+H): 557.14, found 557.20.

Synthesis of 2a

The QD-Receptor conjugate (**2a**) was prepared with the ligand exchange reaction developed by Tomasulo et al. [2]. A solution of CdSe/ZnS (2 mL, 5.43×10^{-6} moles) core-shell QDs was added to receptor **1a** (2.8 g, 0.01 mol) in dry chloroform. The reaction was allowed to reflux for 15 h. Upon completion of reaction, the solvent was removed under reduced pressure. The crude mass was suspended in acetonitrile (5 ml) and centrifuged at 12,500 rpm for 5 min. The supernatant solution was decanted off and the solid was again suspended in fresh acetonitrile. This step was repeated twice and the product was vacuum dried to obtain pure **2a** as an orange coloured powder (0.07 g).

Synthesis of 2b

The QD-Receptor conjugate (**2b**) was prepared by using the same method as was adopted for the synthesis of QD-Receptor conjugate (**2a**), except the receptor **1b** (2.8 g, 0.01 mol) was added to the solution of CdSe/ZnS core-shell QDs instead of **1a**.

Recognition studies

The cation binding ability of **2(a,b)** were determined by preparing solutions containing 0.1 μM of receptor along with 50 μM of a particular metal salt in THF:H_2O (9:1, *v*/*v*) HEPES buffered solution (pH=7.0±0.1). For **3**, the cation binding ability was determined by preparing solutions containing 10 μM solution of receptor along with 50 μM of a particular metal salt in THF:H_2O (9:1, *v*/*v*) HEPES buffered solution (pH=7.0±0.1). The fluorescence spectrum of each solution was recorded with excitation at λ_{max}= 270 nm. The cation recognition behaviour of any receptor for the binding of a particular cation was evaluated from the changes in fluorescence spectrum of receptor upon addition of that metal salt. To find out the concentration range along which the receptor **2a** can operate for the metal ions, titrations were performed by taking volumetric flasks each containing 0.1 μM of **2a** along with varied amounts of a particular metal salt in THF:H_2O (9:1, *v*/*v*) HEPES buffered solution (pH=7.0±0.1). The solutions were shaken thoroughly and their fluorescence spectra were recorded with excitation at λ_{max}=270 nm. To evaluate any possible interference due to Ca^{2+} for the estimation of K^+ and vice versa, solutions were prepared containing receptor **1a** (0.1 μM in THF:H_2O (9:1, *v*/*v*) HEPES buffered solution (pH=7.0± 0.1)) along with either of (a) 40 μM K^+or (b) 40 μM K^+ along with 10 μM Ca^{2+}or (c) 10 μM Ca^{2+} or (d) 10 μM Ca^{2+} along with 40 μM K^+. The fluorescence intensity for solution (a) and (b) was measured at λ_{max}=320 nm, while for (c) and (d) it was measured at λ_{max}=370 nm.

Results and discussion

Synthesis and characterisation

The Schiff base **1a** was synthesised in one step by stirring 2-aminothiophenol and 2-hydroxy-1-naphthaldehyde in dry

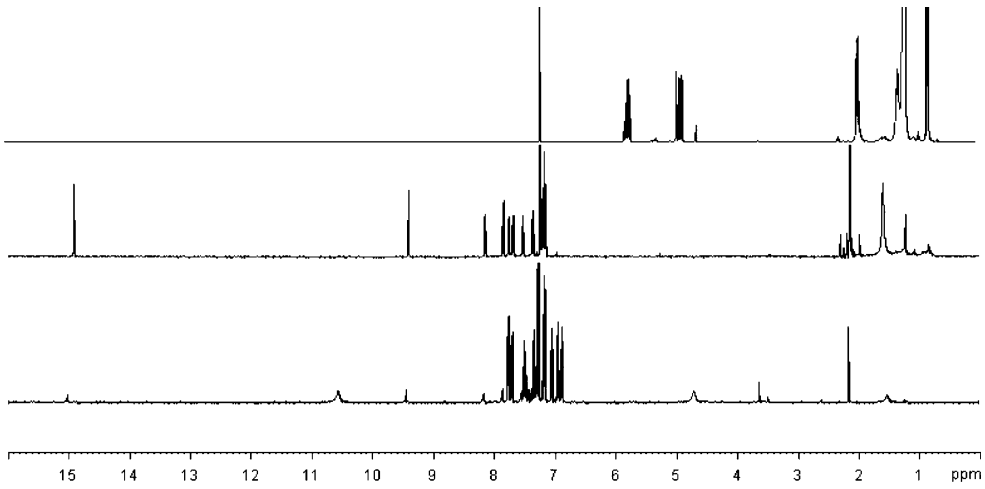

Fig. 1 Stacked ^{1}H NMR spectra of CdSe / ZnS QD's (*top*) **2a** (*middle*) and **1a** (*bottom*) recorded in $CDCl_3$

methanol for 0.5 h [13]. Control compound **1b** was prepared by reduction of **1a** by treatment with sodium borohydride in a THF/Methanol solvent system [13]. Compound **3** was prepared by first making the disulfide bond through aerial oxidation of 2-aminothiophenol in methanol. This disulfide product was subjected to a condensation reaction with 2-hydroxy-1-naphthaldehyde to obtain compound **3**. CdSe / ZnS QDs were prepared following a known procedure [12]. The CdSe /ZnS QD's were surface functionalised with **1a** after refluxing in chloroform for 18 h. Following removal of the solvent by rotary evaporation, the product was precipitated from acetonitrile and centrifuged to yield **2a** as a yellow solid. A similar procedure was followed for the preparation of **2b**. Surface functionalisation was confirmed by ^{1}H NMR spectroscopy with Fig. 1 showing the stacked NMR spectra

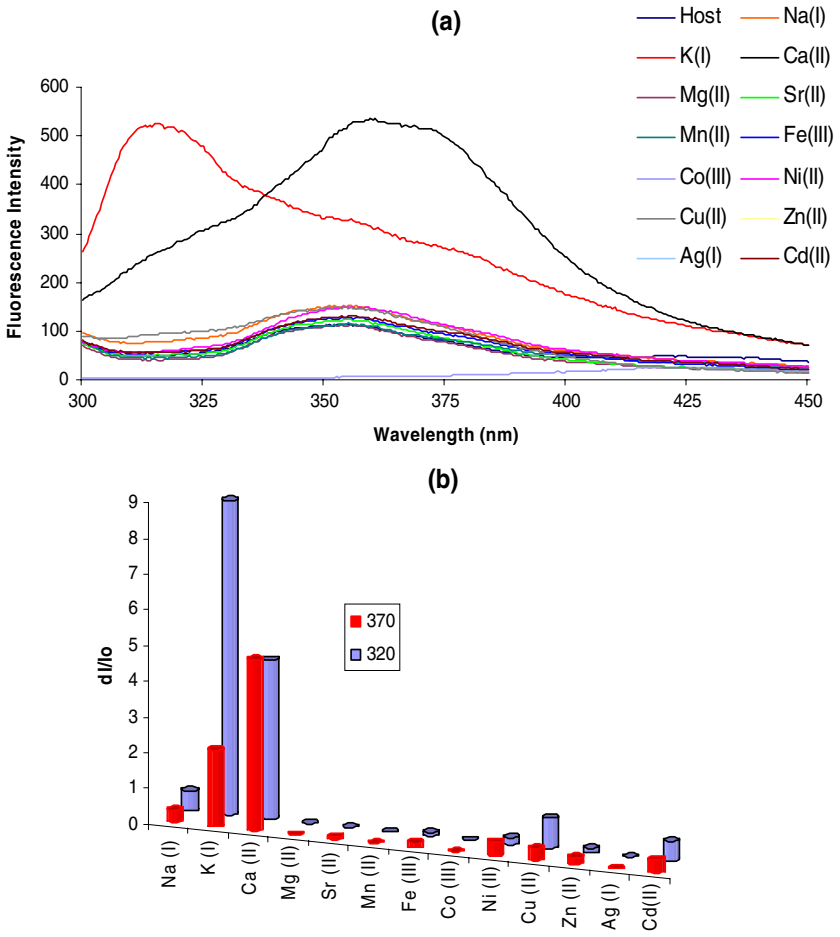

Fig. 2 **a** Changes in fluorescent intensity of receptor **2a**; **b** Fluorescence ratio (dI/I_o) of receptor **2a** upon addition of a particular metal salt in THF:H_2O (9:1,*v*/*v*) HEPES buffer solution (pH= 7.0±0.1). [**2a**]=0.1 μM. λ_{EX}= 270 nm. (Where I_o is the fluorescence intensity of pure receptor)

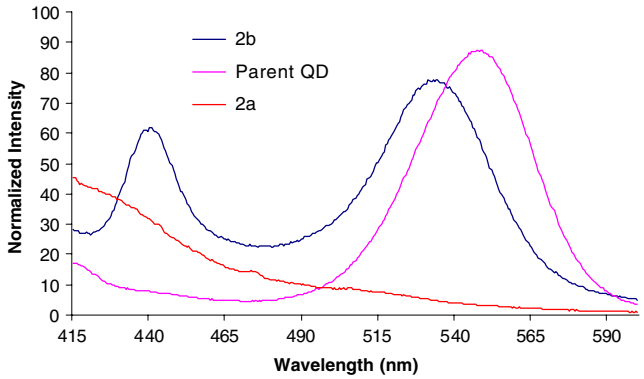

Fig. 3 Normalized fluorescence spectra for the parent QDs recorded in acetonitrile, **2a** and **2b** recorded in THF : HEPES buffer (9:1). [parent QD's]=[**2a**]=[**2b**]=0.1 μM. λ_{EX}=350 nm

for the parent QDs, **2a** and **1a** in descending order. The resonances present at 5.10 and 5.80 ppm in the spectrum of the parent QDs reflect the olefinic peaks of octadecene, a non-co-ordinating, high boiling point solvent used in the synthesis of the core QDs, while the upfield resonances (0–2.50 ppm) represent the methyl and methylene signals from octadecene and the co-ordinating ligand trioctylphosphine oxide (TOPO). The signals due to octadecene were not present in the spectrum of **2a** however, with only minor evidence of the signals from the TOPO groups. In addition, the thiol proton observed in the spectrum of **1a** as a broad resonance centred at 4.80 ppm was absent in the spectrum of **2a.** This suggests an almost complete exchange of the TOPO ligands for **1a** and that **1a** attaches to the QD through the thiol group as expected. Furthermore, there were significant differences in chemical shifts for the receptor protons before and after conjugation with the nanoparticle surface. For instance, the resonance at 7.85 ppm in the spectrum of **1a** and representing the imine proton, shifts significantly downfield to 9.50 ppm in **2a**. There was also a substantial downfield shift for the hydroxyl proton from 10.50 ppm in **1a** to 15.00 in **2a**. This is consistent with a hydrogen bonding interaction of hydroxyl groups between adjacent receptors on the nanoparticle surface [14]. The hydrodynamic diameter of **2a**, as determined by dynamic light scattering, was 10.9±2.1 nm which was larger than for the parent QDs alone (7.1±1.1 nm) (not shown). This is most likely due to presence of the rigid Schiff base receptor on the surface of **2a** compared to the more flexible TOPO groups of the parent QD's.

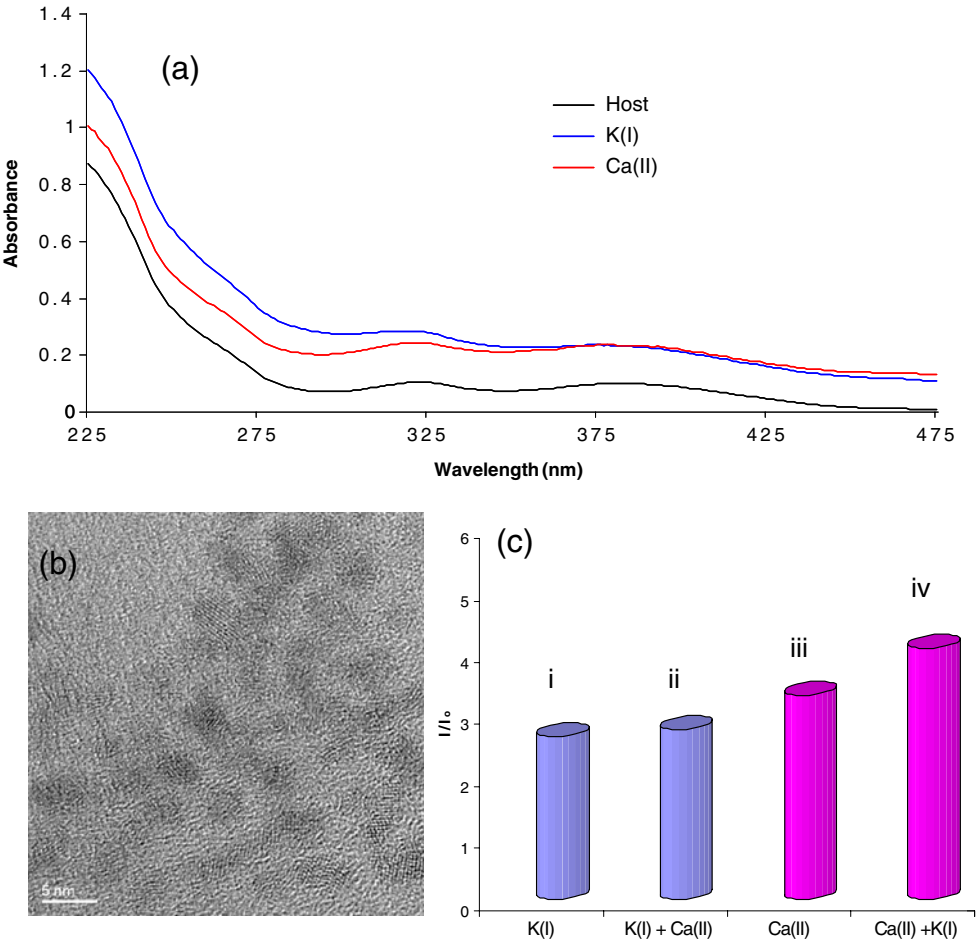

Fig. 4 **a** UV-Vis spectra for **2a**, **2a** + K^+ (50 μM) and **2a** + Ca^{2+} (50 μM). **b** TEM image of **2a** and **c** plot of relative fluorescence intensity for **2a** in the presence of (i) 40 μM K^+, (ii) 40 μM K^+ in the presence of 10 μM Ca^{2+}, (iii) 10 μM Ca^{2+} and (iv) 10 μM Ca^{2+} in the presence of 40 μM K^+. [**2a**]=1.5 μM for (**a**) and 0.1 μM for (**c**), λ_{EX}= 270 nm. λ_{EM}=320 and 370 nm for K^+ and Ca^{2+} respectively. (Where I_o is the fluorescence intensity of pure receptor and I represents the fluorescence intensity of receptor in the presence of cation/cations)

Photophysical properties

The fluorescence recognition properties of **1a** have been detailed by us in an earlier communication [12]. **1a** was found to display selectivity for Mg^{2+} through an Excited State Intramolecular Proton Transfer (ESIPT) mechanism. Upon excitation, the enol form of **1a** was converted to its keto tautomer which was responsible for Mg^{2+} binding. The presence of a thiol group, an imine linkage and a hydroxyl group were found to be mandatory for the selectivity of **1a**.

QD-receptor conjugate **2a** was characterised by a weak napthalenic emission with λ_{max}=360 nm when excited at 270 nm (Fig. 2a). In contrast to **1a**, there was no evidence of dual emission for **2a** suggesting that the ESIPT process and resulting keto-enol tautomerisation does not occur when the same receptor is anchored onto the nanoparticle surface. The reason for this is most likely due to the aforementioned hydrogen bonding interaction between neighbouring hydroxyl groups. The expected emission from the QD itself at λ_{max}=~ 540 nm was not observed. This is most likely due to a photoinduced electron transfer (PET) from the electron rich receptor to the excited QD that quenches fluorescence, as has been observed before when electron rich analytes were added to solutions of QD's [1, 15, 16]. This was confirmed by the formation of control compound **2b** which contained the reduced Schiff base (**1b**) as receptor. This receptor is less electron rich due to the presence of a sp^3 hybridised carbon that disrupts the conjugation present in **1a**. The fluorescence spectra of the parent QD, **2a**, and **2b** are shown in Fig. 3. The presence of a QD emission at λ_{max}=535 nm for **2b**, which was not evident for **2a**, suggests the quenching of QD fluorescence is indeed due to PET.

The cation recognition properties of **2a** were investigated in a THF : H_2O (9:1) HEPES buffered solution at pH7.0. Unfortunately, none of the ions tested (Fig. 3) led to a restoration in the QD fluorescence, suggesting that if ion binding occurs it does not modulate the oxidation potential of the receptor sufficiently to cancel the PET process. However, there were changes in the napthalenic emission upon additions of certain ions. Large enhancements in fluorescence were observed for both K^+ and Ca^{2+}, accompanied by a blue shift for K^+ (from λ_{max}=360 nm to λ_{max}=320 nm) and a slight red shift for Ca^{2+} (from λ_{max}= 360 nm to λ_{max}=370 nm)(Fig. 2a). No other ions had a major effect on the intensity of **2a** except Co(II) which resulted in a quenching of the fluorescence. In contrast, neither the disulfide compound **3** or control compound **2b** showed selectivity for any metal highlighting the necessity of the imine linkage and anchorage to the nanoparticle surface for semi-selective binding. One possible explanation for the behaviour of K^+ and Ca^{2+} is as follows: it is known that Schiff base containing fluorescent sensors are weakly emissive due to *cis-trans* isomerisation about the C=N bond that quenches fluorescence by non-radiatve decay, and that a metal ion binding event that prevents this rotation leads to an enhancement in fluorescence [17, 18]. It may be possible that either K^+ or Ca^{2+} upon binding, locks a particular form (i.e. either *cis or trans*) in place, resulting in the observed fluorescence enhancements. The UV-Vis spectra of **2a** in the presence of K^+ and Ca^{2+} are shown in Fig. 4. Both ions caused changes in the UV profile of **2a** illustrating that these ions also bind **2a** in the ground state. However, their absorption spectra were slightly different. Both caused increases in absorbance of the peaks with $\lambda_{max's}$ 320 and 390 nm, with the latter region being almost identical for both K^+ and Ca^{2+}. However, there was a slight increase in the absorbance at λ_{max}=320 nm for K^+ compared to Ca^{2+}. These absorbances can be attributed to the Schiff base chromophore [19, 20] and suggest that K^+ and Ca^{2+} bind to this region of **2a** in a different manner, perhaps by preferentially binding to either the *cis* or *trans* forms.

To determine the sensitivity of **2a** as a sensor for both K^+ and Ca^{2+} titrations were performed. Upon continuous addition of Ca^{2+} to **2a**, the λ_{max} showed a red shift, while upon addition of K^+ the λ_{max} showed blue shift. The Ca^{2+} and K^+ specific enhancements in fluorescence intensity were separated by a $\Delta\lambda$=50 nm. This large separation in wavelength and substantial ion specific enhancement of

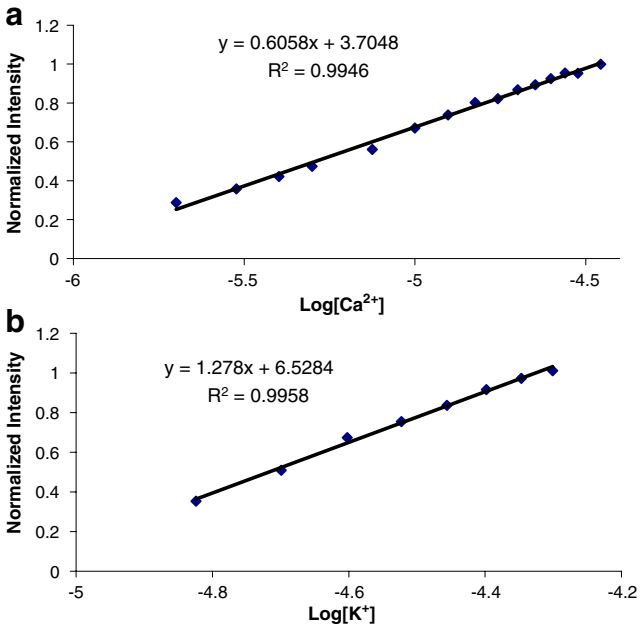

Fig. 5 Plot of relative fluorescent intensity against concentration for **2a** upon addition of **a** Ca^{2+} measured at 370 nm and **b** K^+ measured at 320 nm. [**2a**]=0.1μM, solvent = THF: H_2O (9:1,*v*/*v*) HEPES buffer solution (pH=7.0±0.1). λ_{EX}=270 nm. (Where I_o is the fluorescence intensity of pure receptor and I represents the fluorescence intensity of receptor in the presence of cation)

fluorescence intensity offers an opportunity for the simultaneous estimation of these two metal ions. Figure 5 shows that good linearity was observed for both ions in the range 15–50 μM for K^+ and 2–35 μM for Ca^{2+}. Unfortunately, due to the inability to determine the stoichiometry of the complexes formed between **2a** and K^+ or Ca^{2+} binding constants could not be determined.

Figure 2b shows that only Ca^{2+} causes any significant interference in the measurement of K^+ and vice versa. Therefore, we attempted a series of competitive titrations to determine the limit at which K^+ and Ca^{2+} could be measured in the presence of each other as competing ions. Figure 4c shows that Ca^{2+} could be measured accurately by **2a** in the presence of up to four equivalents of K^+ whereas interference was observed in the measurement of K^+ when greater than 0.25 equivalents of Ca^{2+} was added. Hence the receptor **2a** can be effectively used for solutions where K^+: Ca^{2+} exists in 4:1 ratio. Under normal physiological conditions Ca^{2+} is considerably less abundant than K^+ in blood plasma, [11] thus **2a** could potentially be used to measure the concentration of these ions in plasma samples.

In conclusion, we have prepared a QD-Schiff base conjugate capable of recognising and estimating the concentations of both K^+ and Ca^{2+} ions in semi aqueous solution through changes in their fluorescence spectra. However, the Schiff base receptor itself demonstrated selectivity for Mg^{2+}. Thus, although the general selectivity for alkali / alkali earth metal ions remains, the attachment of the receptor onto the nanoparticle surface alters the binding properties of the receptor dramatically. These results complement those obtained by us in a previous study and confirm the ability of QDs to act as a framework for the self organisation of receptors. This could provide a method of improving / altering the selectivity of known receptors that could potentially lead to a new class of optical sensors.

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