ChemComm

RSC Publishing

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2013, **49**, 9818

Received 5th July 2013,
Accepted 27th August 2013

DOI: 10.1039/c3cc45051a

www.rsc.org/chemcomm

A new receptor with a FRET based fluorescence response for selective recognition of fumaric and maleic acids in aqueous medium†

Upendar Reddy G.,[a] Rabindranath Lo,[b] Sovan Roy,[a] Tanmay Banerjee,[a] Bishwajit Ganguly*[b] and Amitava Das*[a]

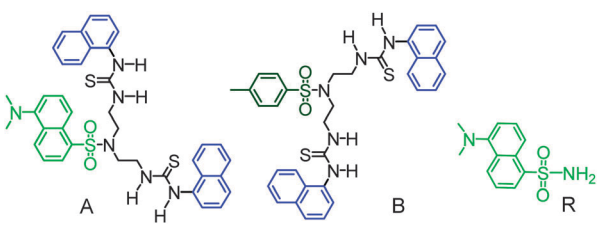
Preferential binding of a new reagent to fumaric acid could be utilized for its estimation in aqueous medium and in commercial fruit juice.

Molecular recognition studies of dicarboxylic acids have gained importance because of their presence as key structural moieties in many bioactive molecules, their role in various metabolic processes and involvement in the biosynthesis of some important intermediates.[1] Fumaric (Fum) and maleic (Mal) acids find a broad range of uses in medicine, food and polymer industries.[1] More recently, fumaric acid derivatives have been tested for treatment of multiple sclerosis and patients with psoriasis.[2] Maleic acid is known for its role as an inhibitor of the Krebs cycle and in different kidney diseases.[2] Due to the widespread use of these two acids as ingredients in food as well as beverages and their possible adverse influences on human health upon prolonged exposure, it is important to develop an efficient reagent for their recognition and quantitative estimation in aqueous medium. In the recent past, considerable progress has been made in the recognition of various dicarboxylic acids through hydrogen bonded adduct formation using hydrogen bond donor units like urea/thiourea/amide, guanidium groups, and allosteric phosphate-based receptors.[3–6] Among these, the only report that reveals the recognition of certain dicarboxylate ions in pure aqueous tris buffer medium of pH 7.5 shows the lack of the desired specificity towards maleate or fumarate.[4a] Alternative approaches to investigate the coordinative interactions of these dicarboxylate ions with certain metal ions or Lewis acid based receptors for recognition studies are truly limited and in these examples the key issue of specificity remains unaddressed.[4,7] A chemodosimetric reagent was reported to be able to discriminate between maleate and fumarate ions.[8] However, this reagent was also found to bind to *ortho*- and *meta*-dibenzoic acids in mixed aq. buffer medium at nearly neutral pH. A synthetic molecular probe was reported for estimation of citrate or tartrate–malate in mixed aq. buffer medium.[4b] Thus, the issue of developing a reversible sensor specific to maleate and fumarate ions in an ensemble of common mono- and di-carboxylate ions in aqueous medium has eluded researchers to date.[3–8]

A new thiourea based receptor **A** was synthesized following a multistep procedure with reasonable yield and purity of this reagent was ensured.† Model reagents (**B** and **R**) were synthesized for unambiguous assignment of the spectral responses of **A** upon binding to fumarate or maleate ions (Scheme 1).

The electronic and emission spectra of compounds **A** and **B** were recorded in aq. HEPES buffer–CH_3CN (1 : 1, v/v; pH 7.4) medium at room temperature. The electronic spectrum of compound **A** showed three shoulders at ~353 nm, ~290 nm and ~244 nm, whereas in the case of compound **B** one distinct absorption band at 290 nm was observed.† The common shoulder observed at 290 nm could be ascribed to a charge transfer (CT) transition that is typical for the naphthalene moiety. A shoulder at ~353 nm for **A** was ascribed to a dansyl-based CT transition. UV-vis spectra of **A** and **B** remained unchanged in the presence of excess of all mono and di-carboxylate ions under identical experimental conditions.†

A solution of **A** in aq. HEPES buffer–CH_3CN (1 : 1, v/v; pH 6.0) medium showed a strong emission band at 542 nm (λ_{Ext} = 290 nm).† Emission spectra recorded for compound **B**, having only a comparable naphthalene moiety as the fluorescence active unit (λ_{Ext} of 290 nm), showed three emission bands at 313, 390 (broad) and 464 nm.† Bands at 313 and 390 nm were



Scheme 1 Molecular structures of **A**, **B** and **R**.

[a] *Organic Chemistry Division, CSIR-National Chemical Laboratory, Pune-411008, India. E-mail: a.das@ncl.res.in; Fax: +91 2025902629; Tel: +91 2025902385*

[b] *CSIR-Central Salt & Marine Chemicals Research Institute, Bhavnagar – 364002, India. E-mail: ganguly@csmcri.org; Fax: +91 278 2567562*

† Electronic supplementary information (ESI) available: Synthesis and characterization details of **A**, **B** and **R**. Energy optimized structures and analysis of fumaric acid in fruit juice by HPLC.See DOI: 10.1039/c3cc45051a

assigned as the vibronic bands of the naphthyl moiety, whereas a relatively less intense emission band at 464 nm was assigned to an emission process from an excited naphthyl dimer.[3h] Emission spectra of **R** showed a band at 528 nm for a λ_{Ext} of 355 nm, while a very weak emission band was observed when excited at 290 nm.† Naphthalene moiety is expected to absorb predominantly for λ_{Ext} of 290 nm. A comparison of the emission spectra of **A**, **B** and **R** clearly revealed the complete absence of the naphthalene-based emission and presence of strong dansyl-based emission in **A**.† All these tend to suggest that a Förster Resonance Energy Transfer (FRET) process was being operational for the probe molecule **A** at a λ_{Ext} of 290 nm with the naphthalene moiety as the donor and the dansyl moiety as the acceptor fragments. Such a possibility is further supported by the fact that two model compounds **R** (with predominant dansyl-based absorption) and **B** (with naphthalene-based emission) showed a significant spectral overlap.† Energy optimized structures of receptor **A** revealed that the distance between the dansyl moiety and two naphthalene units was 8 Å and 10 Å, respectively.† This further supported the feasibility of a FRET process. Efficiency of the FRET process for the energy transfer (ET) between naphthyl and dansyl moieties in **A** was evaluated to be 75% under the experimental conditions.†

Emission quantum yield (Φ_F of 0.0137) for the model compound **B** was evaluated in aq. HEPES buffer–CH_3CN (1 : 1, v/v; pH 6.0) medium (λ_{Ext} = 285 nm, λ_{Ems} = 394 nm) using naphthalene as the reference compound. It is known that the thiourea moiety could even act as a hydrogen bond donor for polar solvent molecules like DMSO.[9] Thus, more polar water molecules are expected to interact with **B** thereby favouring the non-radiative deactivation of the naphthalene based excited state. This could account for the low quantum yield value of FRET based ET (Φ_{ET} = 75%) despite a strong spectral overlap between **B** and **R**.†

In order to examine the response of the probe molecule **A** towards different carboxylic acids, we recorded the luminescence spectra of **A** in the absence and presence of various mono, di- and tri-carboxylic acids (Fig. 1). Luminescence spectra of **A** remained virtually unchanged when recorded in the presence of all other carboxylic acids, except Fum and Mal acids. For these two acids, a significant quenching of the FRET based luminescence at 542 nm was observed (Fig. 1), which was presumably due to the formation of a hydrogen bonded adduct between reagent **A** and the fumarate or maleate ion. The extent of changes was more pronounced for the fumarate ion. Luminescence responses of the model receptor **B** (λ_{Ext} = 290 nm) were also recorded in the presence of 200 mole equivalent excess of Fum and Mal acids, while a significant quenching of the naphthalene-based emission was observed.† This clearly suggested that the binding of the maleate/fumarate ion to either of two receptors (**A** and **B**) could cause an efficient quenching of the naphthalene-based luminescence. Such an observation is anticipated for the binding of the anionic analytes to a fluorophore without much change in the molecular rigidity.[9] Once the naphthalene based emission is effectively quenched, no FRET based ET and thus dansyl-based emission at 542 nm are anticipated for **A** upon excitation at 290 nm. Thus, the observed fluorescence quenching of receptor **A** at 542 nm (λ_{Ext} = 290 nm) could be attributed to the quenching of the luminescence of the naphthyl fragment and the subsequent interruption of the FRET process. The residual luminescence of receptor **A** in the presence of excess of maleate/fumarate ions could be accounted for by the weak dansyl-based emission at a λ_{Ext} of 290 nm. Low emission quantum yield measured for the model compound **R** at 528 nm (λ_{Ext} of 290 nm) further confirmed this.

Systematic luminescence titrations were carried out for **A** (2.0×10^{-5} M) with varying [Fum] (0 to 3.2×10^{-3} M) and [Mal] (0 to 2.8×10^{-3} M) in aq. HEPES buffer–CH_3CN (1 : 1, v/v; pH 6.0) medium at a λ_{Ext} of 290 nm and a λ_{Mon} of 542 nm (Fig. 1). A gradual decrease in emission intensity at 542 nm was observed upon addition of either of these two carboxylic acids (Fig. 1). Stoichiometry for the adduct formation between receptor **A** and the fumarate or maleate ion was evaluated to be 1 : 1 from the Benesi–Hildebrand (B–H) plot of the data obtained from the systematic fluorescence titration.† This was also confirmed from the data obtained from the ESI-MS analysis. Signals at *m*/*z* of 843.28 and *m*/*z* 843.66 were attributed to [**A** + fumarate + Na^+] and [**A** + maleate + Na^+], respectively. The respective binding affinity of Fum and Mal acids towards **A** was evaluated to be $(8.6 \pm 0.02) \times 10^2$ M^{-1} and $(2.1 \pm 0.04) \times 10^2$ M^{-1} (an average of four independent experimental data) from the subsequent B–H plot in aq. HEPES buffer-acetonitrile (1 : 1, v/v; pH 6.0) medium.†

The interactions between the TBA salt of fumaric acid (TBAF) and maleic acid (TBAM) with **A** were also investigated by ^{1}H NMR titration with varying [TBAF] (Fig. 2) or [TBAM].† The initial chemical shifts for signals for the four protons of two dissymmetric thiourea functionalities in **A** appeared as two sharp singlets at δ = 8.28 ppm (belonging to the N^1-atom) and δ = 6.52 ppm (belonging to the N^2-atom), respectively. Distinct downfield shifts of four thiourea-protons in **A** were observed upon subsequent increase in the [TBAF] or [TBAM]. These shifts were smaller for studies with TBAM, which

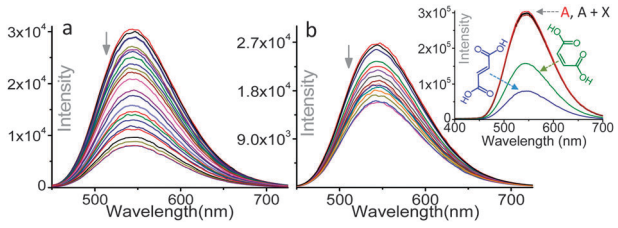


Fig. 1 Luminescence spectra **A** (2.0×10^{-5} M) in the presence of varying (a) [Fum] (0 to 3.2×10^{-3} M) and (b) [Mal] (0 to 2.8×10^{-3} M); inset: the emission response of **A** in the absence and presence of 200 mole equiv. of Fum & Mal and various carboxylic acids (X = acetic, oxalic, malonic, citric, succinic, adipic, glutaric, suberic, malic). All studies were performed in 10 mM aq. HEPES buffer–CH_3CN (1 : 1, v/v; pH 6.0) medium; λ_{Ext} = 290 nm, λ_{Mon} = 542 nm.

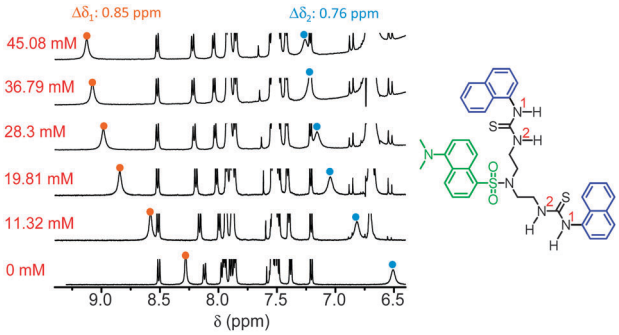


Fig. 2 Partial ^{1}H NMR spectra of **A**(2.83 mM) in the absence and in the presence of varying [TBAF] in a CD_3CN–DMSO(d_6) (99 : 1, v/v) medium.

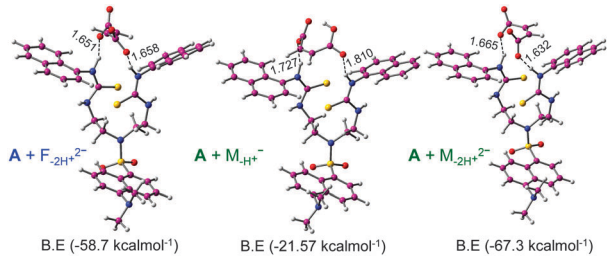


Fig. 3 B3LYP/6-31G*//RHF/PM3 calculated binding energies for **A** with $F_{-2H^+}{}^{2-}$ and **A** with $M_{-H^+}{}^{-}$ and $M_{-2H^+}{}^{2-}$. Distances are given in Å (atom colour code: red = O, blue = N, white = H, yellow = S, magenta = C).

further corroborate its weaker interaction with **A**.† These shifts were attributed to the net deshielding effect induced by the hydrogen-bonding interaction between the thiourea protons and the anion. Higher $\Delta\delta$ for the N^1-protons signified a stronger interaction with the fumarate or maleate ion than $\Delta\delta$ for N^2-protons. To understand this and to examine the relative binding affinities of **A** towards fumarate and maleate ions, detailed computational studies were performed.

The binding of Fum and Mal acids with **A** could occur in the mono- or bis-deprotonated states of these diacids. The fractional distribution curve for respective acids reveals that at pH 6.0, mono ($M_{-H^+}{}^{-}$) and bis-deprotonated ($M_{-2H^+}{}^{2-}$) forms of the Mal acid exist in comparable concentrations, while the bis-deprotonated form ($F_{-2H^+}{}^{2-}$) exists almost exclusively for Fum acid.† Thus, binding energies were calculated for binding of **A** to $M_{-H^+}{}^{-}$, $M_{-2H^+}{}^{2-}$ and $F_{-2H^+}{}^{2-}$. The higher binding energy of the $F_{-2H^+}{}^{2-}$ with **A** was accounted for by the interaction of two $-N^1H$ groups adjacent to the naphthyl ring *via* strong H-bonding with the two O_{COO-} of $F_{-2H^+}{}^{2-}$ (Fig. 3). The binding mode of $M_{-2H^+}{}^{2-}$ was found to be similar to that of $F_{-2H^+}{}^{2-}$. The computed binding energy of **A** with $M_{-H^+}{}^{-}$ was reasonably lower when compared with $M_{-2H^+}{}^{2-}$. However, the presence of $M_{-H^+}{}^{-}$ in comparable concentration seems to mask the effective binding of the bis-deprotonated maleate ion at this pH.

To explore the possibility of evaluating unknown [Fum] in commercial fruit juice, a standard curve was generated from the plot of the $\Delta[I_0 - I]$ *vs.* known [Fum] (0 to 1.0×10^{-4} M) (Fig. 4).†

The linearity of the calibration curve for known [Fum] was ensured (Fig. 4) with the lowest detection limit of 10 ppm.

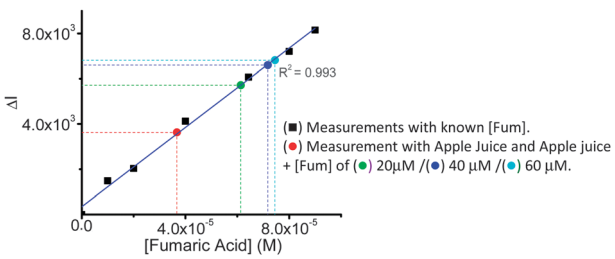


Fig. 4 Plot of $\Delta I = (I_0 - I)$ *vs.* [Fum], where I_0 and I are emission intensities of receptor **A** in the absence and presence of known [Fum] as well as apple juice and apple juice spiked with a known amount of fumaric acid.

These data support the suitability of the proposed method for its application to real samples. For analysis of the commercial sample of apple juice, 0.2 ml of this was used, which was eventually adjusted to the final volume of 5 ml after adding an appropriate amount of reagent **A** (1.0×10^{-4} M) and aq. HEPES buffer–acetonitrile (1 : 1, v/v; pH 7.4) as solvent. This solution along with solutions spiked with known [Fum] (20 μM, 40 μM and 60 μM) as an internal standard were used for emission measurements without further treatment. The fumaric acid concentration in the commercial apple juice was determined to be 3.8×10^{-5} M, which is within the allowed limit for fumaric acid content in good apple juice.[10] These results were compared and validated with the results of the HPLC studies.†

In brief, the new thiourea-based receptor (**A**) could be used for selective recognition of fumarate and maleate ions in an ensemble of several other mono- and di-carboxylic acids in an aqueous environment based on a binding induced modulated FRET response. This reagent could further be used for quantitative estimation of the fumarate ion in commercial fruit juice.

The authors thank the CSIR-Network project (M2D) for financial support. URG and RL thank UGC (India) and TB thanks CSIR (India) for their research fellowships.

Notes and references

1 (*a*) I. M. Pérez-Díaz and R. F. McFeeters, *J. Food Sci.*, 2010, **75**, M204; (*b*) S. Rouse and D. J. van Sinderen, *J. Food Prot.*, 2008, **71**, 1724.
2 (*a*) D. M. Khiabani, A. Blank, T. Skripuletz, E. Miller, A. Kotsiari, V. Gudi and M. Stangel, *PLoS One*, 2010, **5**, e11769; (*b*) B. D. Trapp and K. A. Nave, *Annu. Rev. Neurosci.*, 2008, **31**, 247; (*c*) J. Wernerman and F. Hammarkvist, *Lancet*, 1990, **335**, 701; (*d*) R. H. Grubbs and W. Tumas, *Science*, 1989, **243**, 907.
3 (*a*) K. Ghosh and T. Sen, *J. Phys. Chem. B*, 2011, **115**, 8597; (*b*) E. Fan, S. A. V. Arman, S. Kincaid and A. D. Hamilton, *J. Am. Chem. Soc.*, 1993, **115**, 369; (*c*) T. R. Kelly and M. H. Kim, *J. Am. Chem. Soc.*, 1994, **116**, 7072; (*d*) J. M. Benito, M. G. Garćia, J. L. J. Blanco, C. O. Mellet and J. M. G. Ferńandez, *J. Org. Chem.*, 2001, **66**, 1366; (*e*) S.-Y. Liu, L. Fang, Y.-B. He, W.-H. Chan, K.-T. Yeung, Y.-K. Cheng and R.-H. Yang, *Org. Lett.*, 2005, **7**, 5825; (*f*) A. M. Costero, M. Colera, P. Gaviña and S. Gil, *Chem. Commun.*, 2006, 761; (*g*) T. Gunnlaugsson, A. P. Davis, J. E. O'Brien and M. Glynn, *Org. Biomol. Chem.*, 2005, **3**, 48; (*h*) Z. H. Lin, L. X. Xie, Y. G. Zhao, C. Y. Duan and J. P. Qu, *Org. Biomol. Chem.*, 2007, **5**, 3535; (*i*) S. Goswami, K. Ghosh and S. Dasgupta, *J. Org. Chem.*, 2000, **65**, 1907; (*j*) S. K. Kim, B.-G. Kang, H. S. Koh, Y. J. Yoon, S. J. Jung, B. Jeong, K.-D. Lee and J. Yoon, *Org. Lett.*, 2004, **6**, 4655; (*k*) P. Mateus, R. Delgado, P. Brandão and V. Félix, *J. Org. Chem.*, 2012, **77**, 4611.
4 (*a*) J. Raker and T. E. Glass, *J. Org. Chem.*, 2002, **67**, 6113; (*b*) A. Metzger and E. V. Anslyn, *Angew. Chem.*, 1998, **37**, 649; (*c*) J. J. Lavigne and E. V. Anslyn, *Angew. Chem., Int. Ed.*, 1999, **38**, 3666.
5 (*a*) D. A. Jose, I. Mon, H. F. Perez, E. C. E. Adan, J. B. Buchholz and A. V. Ferran, *Org. Lett.*, 2011, **13**, 3632; (*b*) M. Takeuchi, T. Imada and S. Shinkai, *Angew. Chem.*, 1998, **37**, 2096; (*c*) T. Ikeda, O. Hirata, M. Takeuchi and S. Shinkai, *J. Am. Chem. Soc.*, 2006, **128**, 16008.
6 Y. Wu, H. Guo, X. Zhang, T. D. James and J. Zhao, *Chem.–Eur. J.*, 2011, **17**, 7632.
7 (*a*) M. Hu and G. Feng, *Chem. Commun.*, 2012, **48**, 6951; (*b*) L. Tang, J. Park, H.-J. Kim, Y. Kim, S. J. Kim, J. Chin and K. M. Kim, *J. Am. Chem. Soc.*, 2008, **130**, 12606; (*c*) F. Li, S. Carvalho, R. Delgado, M. G. B. Drew and V. Félix, *Dalton Trans.*, 2010, **39**, 9579.
8 F. Sancenón, R. Martínez-Máñez, M. A. Miranda, M.-J. Seguí and J. Soto, *Angew. Chem.*, 2003, **115**, 671.
9 (*a*) A. Ghosh, B. Ganguly and A. Das, *Inorg. Chem.*, 2007, **46**, 9912; (*b*) A. Ghosh, S. Verma, B. Ganguly, H. N. Ghosh and A. Das, *Eur. J. Inorg. Chem.*, 2009, 2496.
10 V. Gokmen and J. Acar, *Food Addit. Contam.*, 2004, **21**, 626.

