



Selective Acetate Ion Sensing by Simple Isophthaloyl Based Fluorescence Receptor

N. B. Khadke¹, A. A. Patil², D. Y. Patil² and A. V. Borhade^{2,*}

¹Science Department, Government Residential women's Polytechnic College, Latur, 413512, India

²Department of Chemistry, Research Center, H.P.T. Arts and R.Y.K. Science College, Nashik 422 005, India
S. P. Pune University, Pune -07
INDIA

Abstract: In the present study Isophthaloyl-based symmetrical (12E,21E)-N1',N3'-bis((2-hydroxynaphthalen-1-yl)methylene)isophthalohydrazide, host (**H**) have been successfully synthesized and characterized. It shows high sensitivity towards acetate ion. The recognition behavior of Host (**H**) for various ions studied using fluorescence spectroscopy. The sensing ability of host (**H**) was investigated for various ions which were prepared in acetonitrile and water in 1:1 ratio (V/V = 1:1). The prepared host (**H**) shows change in fluorescence spectra with acetate ion. The binding constant (K_a) of host (**H**) with acetate ion was computed with the Benesi-Hildebrand plot, Scatchard and Connor's plot and the constant values evaluated are $9.45 \times 10^5 \text{ M}^{-1}$, $9.3784 \times 10^5 \text{ M}^{-1}$ and $9.3784 \times 10^5 \text{ M}^{-1}$ respectively. The 1:2 binding stoichiometry between (**H**) and acetate ion was established using Job's plot method.

Keywords: Anion chemo sensor, Acetate sensor, Acetate recognition, fluorescent sensing, Job's plot.

I. Introduction

The anions recognition and sensing possesses shows great attention in supramolecular chemistry because of its ubiquitous in chemical, biological processes and environmental process. Anions play a fundamental role in numerous applications such as transport of hormones, protein synthesis, DNA regulation and play a part in the activity of enzyme [1-4].

Acetate anion is biologically important among the various anionic analytes, due to its specific biochemical functions in the enzymes and antibodies. Acetate production and oxidation rate has been frequently used as an indicator of organic decomposition in marine sediments [5]. Acetate has unique chemical properties and can form the strongest hydrogen-bond interaction with hydrogen-bond donors because of the trigonal geometry and the high basicity [6].

Anion recognition can occur either through electrostatic interactions or hydrogen bonding [7]. While the sensing of biologically important anions should ideally be achieved using in aqueous organic media [8]. Numerous efforts have been devoted on synthetic receptors that operate in aqueous solution. However, anion recognition in water is quite tedious task because they are strongly hydrated in aqueous solvent. As compared with cations, anions are larger and they possess different geometries like spherical (F^- , Cl^- , Br^- , I^-), linear (N_3^- , CN^- , SCN^- , OH^-), trigonal (CH_3CO_2^- , CO_3^{2-} , NO_3^-), tetrahedral (PO_4^{3-} , HSO_4^-), octahedral ($[\text{Fe}(\text{CN})_6]^{4-}$, $[\text{Co}(\text{CN})_6]^{3-}$), etc. The synthetic anion receptors should be larger than cation receptors with complementary shapes [9-10].

Recently, a lot of researchers have focused on the fluorescent anion sensors having macro cyclic and acyclic amides as the hydrogen bond donors, because amide group forms a strong $\text{NH} \cdots \text{anion}$ hydrogen bond [11-12]. Many examples are available about the selective sensor molecules for acetate anion in the literatures [13-15]. Especially, fluorimetric technique is very attractive because of rapid, non-destructive and high sensitivity [16-18].

Herein, we report synthesis of Isophthaloyl-based fluorescent receptor and studied for its recognition behaviour toward various anions in semi aqueous solution. The binding constant and stoichiometry was computed with the Benesi-Hinderbrand plot and Job's plot respectively. The details of binding characteristics of receptor have been investigated by ^1H NMR, ^{13}C NMR and LC-MS technique.

II. Experimental

A. Chemicals and Instrumentation

All chemicals were purchased from sigma-Aldrich. All solvents and reagents used are of analytical grade and used directly without purification. The UV-Visible spectra were recorded on Shimadzu UV-Visible 2400 spectrophotometer at room temperature using a quartz cuvette of 1cm path length and 5 nm slit width. The infrared spectra were recorded on Shimadzu IR affinity spectrophotometer using KBr pellet. ^1H NMR and ^{13}C

NMR spectra were recorded on Bruker Avance II 400 MHz spectrometer in DMSO-D₆ using tetramethylsilane(TMS) as an internal standard (chemical shifts in ppm). Mass spectra were obtained on water Q-TOF micro mass (LC-MS).

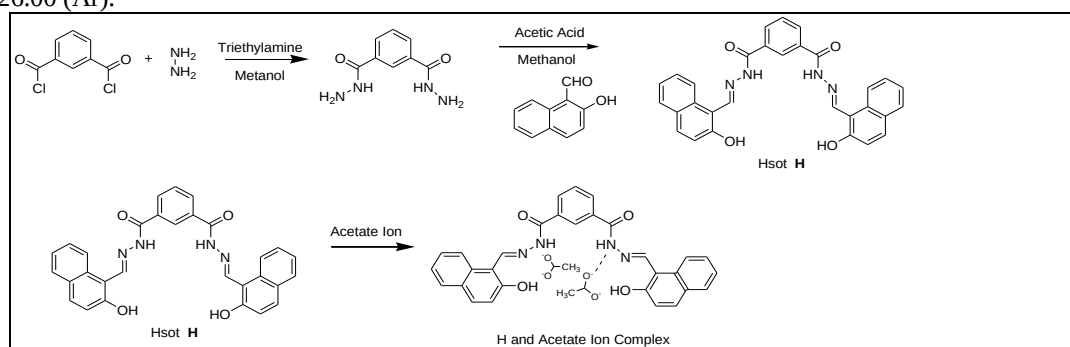
B. Sample preparation for photo physical measurement

The tetrabutylammonium salts of F⁻, Cl⁻, Br⁻, I⁻, CN⁻, NO₃⁻, CH₃COO⁻, H₂PO₄²⁻ and HSO₄⁻ were dissolved in water with concentration 1×10⁻³ M. Synthetic host (**H**) was dissolved in mixed solution of acetonitrile containing 50% water to give stock solution with concentration 1×10⁻² M. The stock solutions were used after appropriate dilution.

III. Synthesis of Host (H)

A. Synthesis of Isophthaloyldihydrazide

203 mg (1.0 mmol) Isophthaloyldichloride dissolved in 5 ml of dry methanol along with catalytic amount of triethylamine and 0.12 ml (2.2 mmol) hydrazine hydrate was added drop wise with continuous stirring at room temperature for 18 h gives (Scheme 1) Isophthaloyldihydrazide. Yield 84%, m.p. 222–50°C. ¹H NMR (DMSO-d₆, 400 MHz, δ in ppm) δ: 9.82s (2H, –NH–); 8.24 t (H, –CH– Ar); 7.91 m (H, –CH– Ar); 7.51 t (H, –CH– Ar); 4.53 s (4H, –NH₂); ¹³C NMR (DMSO-d₆, 100 MHz, in ppm): 165.41 (–CONH–); 133.49 (Ar); 129.26 (Ar); 128.40 (Ar); 126.00 (Ar).



Scheme 1 Synthetic procedure for host H

B. Synthesis of (12E,21E)-N1',N3'-bis((2-hydroxynaphthalen-1-yl)methylene)isophthalohydrazide:

To the solution of Isophthaloyldihydrazide (1 mmol) in dry methanol 2-hydroxynaphthalene-1-carbaldehyde (2 mmol) added drop wise with catalytic amount of acetic acid, stirred at room temperature for 8-10 h gives host **H**(Scheme 1). The precipitate was filtered, washed with methanol and dried under vacuum for 24 hours, (recrystallized from methanol column purified) Yield 83%, m.p. >300°C; IR (KBr) ν(cm⁻¹): 3563.64 (OH), 3421.87 (NH), 1663.68 (–NH–C=O), 1620.27 (C=O), 1560.48 (C=N), 1265.38 (N–N) cm⁻¹; ¹H NMR (DMSO-d₆, 400 MHz, δ in ppm): δ 7.24–8.64 (m, 16H, ArH), 9.54 (s, 2H, N=CH), 12.45 (s, 2H, NH), 12.73 (s, 2H, OH); ¹³C NMR (DMSO-d₆, 400 MHz, in ppm): 161.93 (–CONH–), 158.8 (–CH=N), 147.32 (–C–OH), 132.09 (C–C=O), 127.81 (C–C=N), 123.57 (Ar), 120.72 (Ar), 118.91 (Ar), 108.54 (Ar); LC-MS (ESI) *m/z* calc. for C₃₀H₂₂N₄O₄: 503.15848 [M+H]⁺, found: 503.1718 [M+H]⁺.

IV. Results and Discussion

A. Sensitivity and Selectivity

The photo-physical properties such as anions recognition and sensing ability of host (**H**) (1×10⁻⁵ M) in acetonitrile/water (1:1 V/V) was investigated upon addition of various anions (**G** = F⁻, Cl⁻, Br⁻, I⁻, CN⁻, NO₃⁻, CH₃COO⁻, H₂PO₄²⁻ and HSO₄⁻) (1×10⁻⁴ M) by fluorescence spectroscopy at 350 nm as excitation wavelength shown in fig 1.

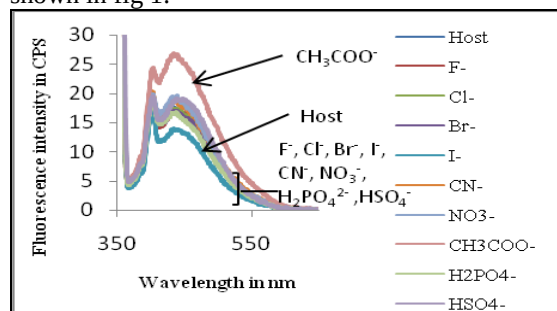


Fig 1 Change in fluorescence spectrum of host (**H**) (1×10⁻⁵ M, λ_{ex} = 350 nm) upon addition of various anions (**G**) (1×10⁻⁴ M) in acetonitrile/Water (1:1 V/V).

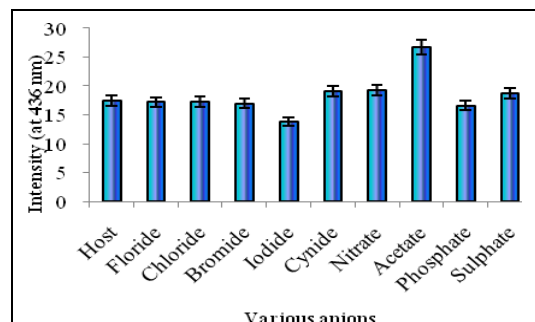


Fig 2 Fluorescence intensity of host (**H**) (1×10⁻⁵ M, λ_{ex} = 350 nm) upon addition of various anions (**G**) (1×10⁻⁴ M) at 436 nm.

Fluorescence response of host was recorded after addition of 3 equivalent of each anion. The acetate ion (CH_3COO^-) shows selective enhancement in fluorescence intensity at wavelength 436 nm and is shown in fig 2. In order to validate the selectivity of sensor the competitive experiments were also measured by addition of 1 equivalent of CH_3COO^- to the host (**H**) in presence of 1 equivalent of other anions such as of F^- , Cl^- , Br^- , I^- , CN^- , NO_3^- , CH_3COO^- , $\text{H}_2\text{PO}_4^{2-}$ and HSO_4^- as shown in fig 3. It is observed that all the coexisting anions hardly get affected with the detection of acetate (CH_3COO^-) ion. This leads to the conclusion that host has high selectivity towards acetate (CH_3COO^-) ion.

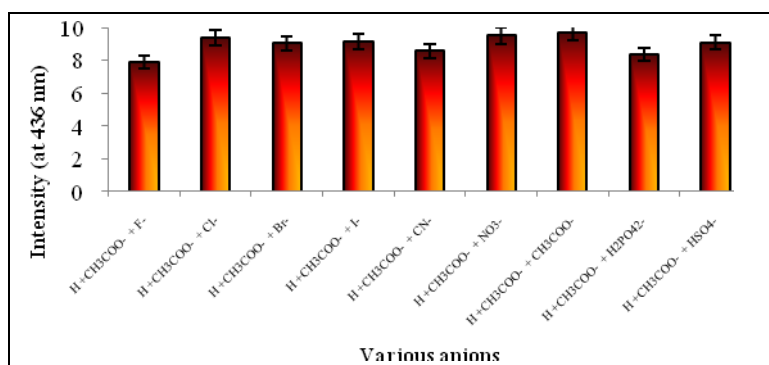


Fig 3 Interference study of various anions (1 equivalent) in presences of **acetate** ion (1 equivalent) at wavelength 436 nm.

B. Binding Stoichiometry and Binding Affinity

The binding stoichiometry was determined by Job's plot (continuous variation method) [19]. Fig 4 depicts the Job' plot between $[\text{HG}]$ and $[\text{H}]/([\text{H}]+[\text{G}])$ where the total concentration of host and guest was constant and molar fraction of host was continuously varied. The $[\text{HG}]$ is concentration of complex calculated as $[\text{HG}] = (\text{F}-\text{F}_0)/\text{F}_0 \times [\text{H}]$. The occurrences of maximum fluorescence at a 0.3 molar fraction indicated the formation of a 1:2 (host: guest) complex.

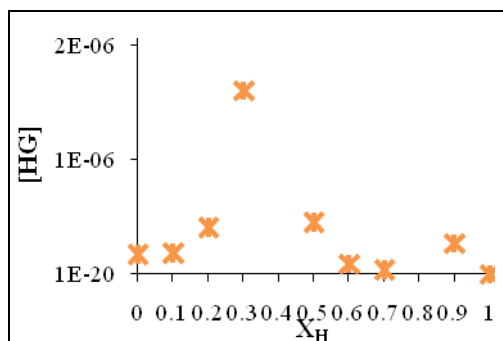


Fig 4 Fluorescence spectrum of **H** (10^{-5}M) and **acetate** ion (10^{-5}M) with varying molar fraction Inset job's plot for showing 1:2 stoichiometry of host and guest at wavelength 475 nm.

The changes in the fluorescence intensity at 436 nm were used to evaluate the binding affinity of **H** and acetate (CH_3COO^-) ion. The binding constant (K_a) was calculated by using Benesi-Hildebrand[20], Scatchard [21] and Connor's linear fitting equation[22].The K_a value obtained was found to be $(9.4142 \pm 0.0358) 10^5 \text{ M}^{-1}$ from fig 5-7.

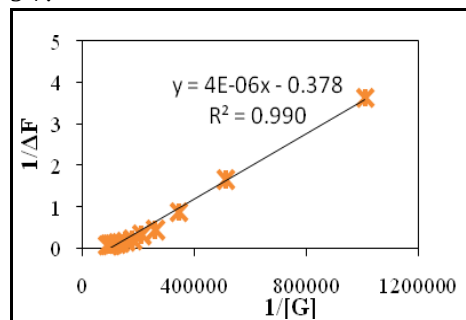


Fig 5 Benesi-Hildebrand plot of $1/\Delta\text{F}$ versus $1/[\text{G}]$, $K_a = 9.45 \times 10^4 \text{ M}^{-1}$

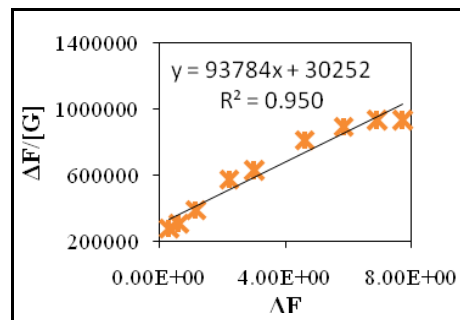


Fig 6 Scatchard plot of $\Delta\text{F}/[\text{G}]$ versus ΔF , $K_a = 9.3784 \times 10^4 \text{ M}^{-1}$

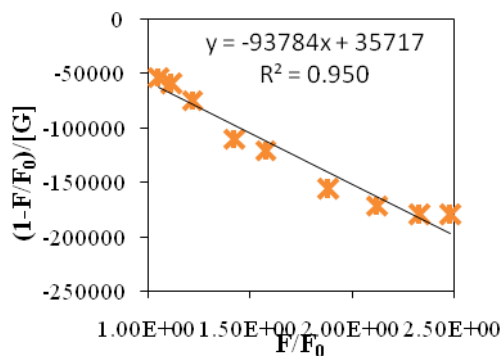


Fig 7 Connor's plot of $(1-F/F_0)/[G]$ versus F/F_0 , $K_a = 9.3784 \times 10^4 \text{ M}^{-1}$

C. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) of host (H) was investigated by spectral titration with the successive addition of acetate (CH_3COO^-) ion. Fluorescence titration of H (5ml $1 \times 10^{-5} \text{ M}$) with the successive addition of acetate (CH_3COO^-) ion ($1 \times 10^{-4} \text{ M}$, $0 \rightarrow 1 \text{ ml}$, $0 \rightarrow 5$ equivalents) was recorded as shown in fig 8. The fluorescence intensity of H at 436 nm was found to be increased with the incremental addition of acetate (CH_3COO^-) ion.

The limit of detection (LOD) and limit of quantification (LOQ) of host evaluated according to the IUPAC 3σ and 10σ method respectively [23]. $\text{LOD} = 3\text{Sb}/m$ and $\text{LOQ} = 10\text{Sb}/m$; Sb= standard deviation of blank, m = slope of regression line. The LOD and LOQ of host with acetate (CH_3COO^-) ion were $0.4377 \mu\text{M}$ and $1.459 \mu\text{M}$ from fig 9.

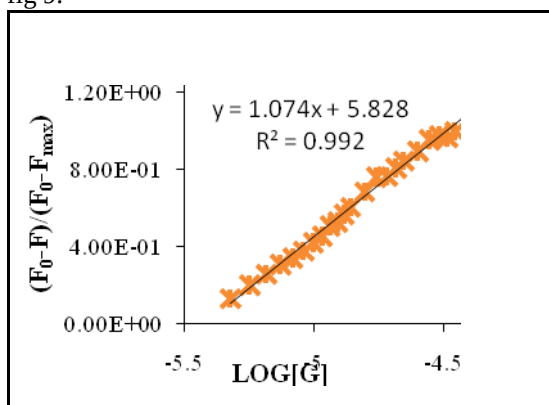


Fig 8 Normalized response of fluorescence signal to changing **acetate** ion concentrations in acetonitrile/water (1:1 V/V) at 436 nm.

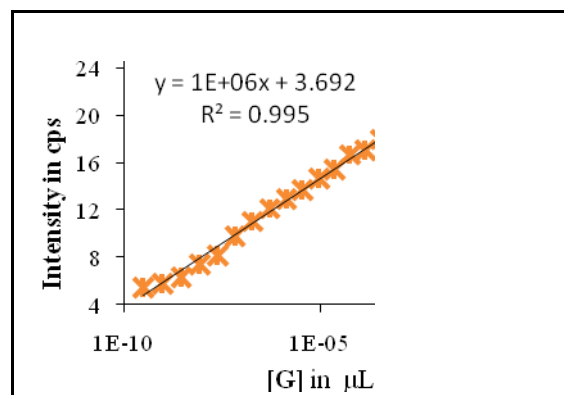


Fig 9 Fluorescence intensity **H** (2 ml 10^{-5} M) versus of μL of **acetate** ion solution added

D. The Stern–Volmer Quenching Constant

The quenching can be mathematically expressed by the Stern–Volmer Eq. (1), which allows for calculating quenching constants [24].

$$F_0/F = 1 + K_{SV} [Q]^2 \dots\dots\dots(1)$$

Where, F is Fluorescence of host during spectral titration, F_0 is fluorescence of host H , $[Q]$ is the concentration of the quencher and K_{SV} is the Stern–Volmer quenching constant.

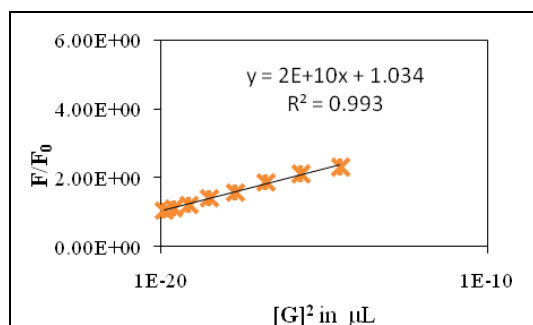


Fig 10 Stern- Volmer plots for titration of host **H** (2 ml 10^{-5} M) versus of μL of **acetate** ion solution added

In the presence of a quencher (guest) the fluorescence intensity is reduced from F_0 to F . The ratio (F/F_0) is directly proportional to the quencher concentration $[Q]^2$. According to Eq. (1), a plot of F/F_0 versus $[Q]^2$ shows a linear graph an intercept of 1.0344 and a slope of K_{sv} with good regression. A typical plot of F/F_0 versus Acetate ion concentration is depicted in fig 10.

V. Conclusions

In conclusion, the synthesized chemosensor i.e. (12E,21E)-N1',N3'-bis((2-hydroxynaphthalen-1-yl)methylene)isophthalohydrazide, host (**H**) was selective and sensitive towards the detection of acetate (CH_3COO^-) ion in acetonitrile/water (1:1, v/v) medium. Furthermore, the sensor was not affected by the common interference of other anions like F^- , Cl^- , Br^- , I^- , CN^- , NO_3^- , $\text{H}_2\text{PO}_4^{2-}$ and HSO_4^- . The 1:2 stoichiometry of the host guest complex was established from the Job's plot and the binding constant value was obtained from Benesi-Hildebrand, Scatchard and Connor's plot were found to be $9.45 \times 10^5 \text{ M}^{-1}$, $9.3784 \times 10^5 \text{ M}^{-1}$ and $9.3784 \times 10^5 \text{ M}^{-1}$ respectively. The limit of detection (LOD) and limit of quantification (LOQ) of host with acetate (CH_3COO^-) ions were 0.4377 μM and 1.459 μM respectively.

VI. References

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