A Novel Design Strategy for Chitosan containing azo-based Schiff bases for Colorimetric Sensing of Anions

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ABSTRACT

The present investigation deals with the anion sensing studies of prepared chitosan/methyl red dye: chitosan/hydroxy nitro azobenzaldehyde (CHNAB) and chitosan/hydroxy methyl azobenzaldehyde (CHMAB) derivatives under mild conditions. These derivatives were synthesized by 79% and ≥ 90% deacetylated chitosan, in isopropyl alcohol/water mixture and dimethyl sulfoxide. The polymers were well characterized by thermal analysis (differential scanning calorimetry, DSC and thermogravimetric analysis,TGA), FTIR, ^{1}H-NMR, ESI-Mass spectra, birefringence and antibacterial activity and reported elsewhere. The optical property of the derivatives is an advantageous property prompted us to evaluate its anion sensing properties by UV-titrimetric method. UV–visible titration was carried out in DMSO to evaluate the binding affinities toward a set of anions (F^-, Cl^-, Br^-, I^-, CN^-, AcO^-, $HSO_4^-$and $H_2PO_4^-$). In the present study, it is observed that cyanide is mainly operating as a nucleophile. These cyanide anion sensing phenomena were analyzed by UV–vis titration in dimethyl sulfoxide. The plausible sensing mechanism of the chitosan derivative for CN-recognition was established. The study on the novel design strategy may be used for sensing anions.

KEYWORDS: *Chitosan, Schiff base, Anion sensing, Biological, Toxic cyanide.*

1. INTRODUCTION

There is a growing interest in sensing of anions due to their ubiquitous nature. Anions play significant role in a wide range of chemical and biological processes, in a various industries related to agricultural fertilizers, food additives and water, and in structures like amino acids, neurotransmitters, enzyme activity in organism, cofactors, nucleic acids etc.[1-3] Anions are present throughout biological systems and

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carry genetic information (DNA is a polyanion). The majority of the enzyme substrates and co-factors are anionic. A well known example is carboxypeptidase A,[4] an enzyme that coordinates to the C-terminal carboxylate group of polypeptides by the formation of an arginine-aspartate salt bridge, and catalyzes the hydrolysis of this residue. Anion also play essential role in medicine, catalysis and the environment,[5] like the function of fluoride ion use in dental care and treatment of Osteroporosis,[6,7] and a developing attention is the detection of fluoride ion due to its association with nerve gases, the analysis of drinking water,[8] and the refinement of uranium used in nuclear weapons manufacture.[9] Many industries like fishing, metallurgy, and mining as well as fabrication of polymer uses CN^- ion even though its toxicity is well recognized. CN^- ion inhibits the mitochondrial electron-transport chain leading to a decrease in the oxidative metabolism due to the non-use of oxygen because it is binding strongly to the active site of cytochromo-oxidase and so, it is extremely toxic and dangerous in very small amounts.[10-13] Phosphate ion and its derivatives also play important role in signal transduction and energy storage in biological systems.[14,15] Carboxylate ion also plays many biochemical roles in the enzymes, antibodies and in numerous metabolic processes.[16-19]

Recently, many optical chemosensors have been developed to perform selective anion detection visually and, in addition, to allow the quantification of such species.[20-23] The simplest strategy used for the development of anionic chemosensors involves the design of molecule that changes colour following an alteration in their molecular structure due to their binding with anions. Although a number of methods are available for concentration of anion analysis including voltammetry, potentiometry, electrochemical methods, and ion chromatography,[24] the major limitation of these methods is the use of time-consuming procedures that involve the use of sophisticated instrumentation. On the other hand, colorimetric (optical) anion sensors have been actively studied due to their operational simplicity, low cost, and rapid implementation.[25] Optical chemosensor (designed principally with covalently linked receptor and signalling subunits) with phenol as hydrogen-bond donor site for anion recognition has been relatively less studied.[26]

In this paper, colorimetric detection method for the cyanide with salicylaldehyde-based azo dyes is reported. These dyes are further incorporated into the chitosan biopolymer resulting into the formation of Schiff base. Hydrogen-bond donor site from phenol OH group, attached to azo dye, is utilized as the receptor unit. With this design strategy, any binding to the OH group would be indicated by a colour change due to change in conjugation of the dye. A second hydrogen-bond donor site was provided by an imine CH moiety in such a way that an elongated anion such as CN^- could coordinate simultaneously with both of the binding sites to generate a *bis*-chelate ring. In this case, the selectivity of the chemosensor towards an anion is related to the fact that anionic species have differentiated capabilities to interact with the receptor site in the chemosensor, for instance, through hydrogen-bonding. In this study, chitosan derivatives containing azobenzene chromophore were investigated for anion sensing by colorimetric method.

2. EXPERIMENTAL

2.1 Materials and reagents

The low molecular weight chitosan powder with degree of deacetylation (DD) >90% was a product of Sigma Aldrich, its average molecular weight was <5000g/mol and chitosan powder with DD of 90% was obtained as gift sample from Qingdao Yunzhou Biochemistry Co. Ltd China. Chitosan with a 79% degree of deacetylation was purchased from the Central Institute of Fisheries Technology (Cochin, India).

2-(4-Dimethylaminophenylazo) benzoic acid (methyl red) (BDH) was used as received. 4-nitro aniline, p-toluidine, salicyaldehyde, sodium nitrite (Fischer Scientific) were used as such. The chemicals isopropyl alcohol, chloroform (Central Drug House Pvt.Ltd New Delhi), N, N-dimethylacetamide (BDH), ethyl alcohol (made in China Changshu Yanguan Chemicals), methanol, glacial acetic acid, acetone, deionized water, hydrochloric acid and DMSO spectroscopy grade (Merck, Mumbai, India) were used directly without any further purification. The solutions of set of anions (CN, F^-,Cl^-, Br^-, I^-, $H_2PO_4^-$, HSO_4^- and AcO^-) were prepared from their terabutylammonium salts of analytical grade (Sigma Aldrich) and then subsequently diluted to prepare working solutions.

2.2 UV-Vis Titration method

UV–Vis spectra were measured on a Systronics double beam spectrophotometer 2203. It is the most common method to approach for titration method. One component (guest) is gradually added to the system (host) while monitoring a physical property such as specific absorption band (UV) of interest. To all the different methods available, titration by UV-Vis spectroscopy is particularly vulnerable to dilution and temperature effects and the presence of impurities in either host or guest solutions. If a low concentration of the host is required, special care needs to be taken in weighing out samples and solutions so that the concentration of the host can still be determined with good accuracy.

An aliquot (2 mL) of a freshly prepared solution of the azo dyes HNAB, HMAB and their respective schiff bases CHNAB and CHMAB in DMSO was transferred into a quartz cell (1 cm width). Small portions of a stock solution of the anions were added to the solution (TBA salt in acetonitrile) in an incremental fashion. The corresponding UV–vis spectra were recorded at room temperature. The change in absorbance at particular wavelength is plotted against anion concentration and fitted by non linear equation for 1:1 stoichiometry[19].

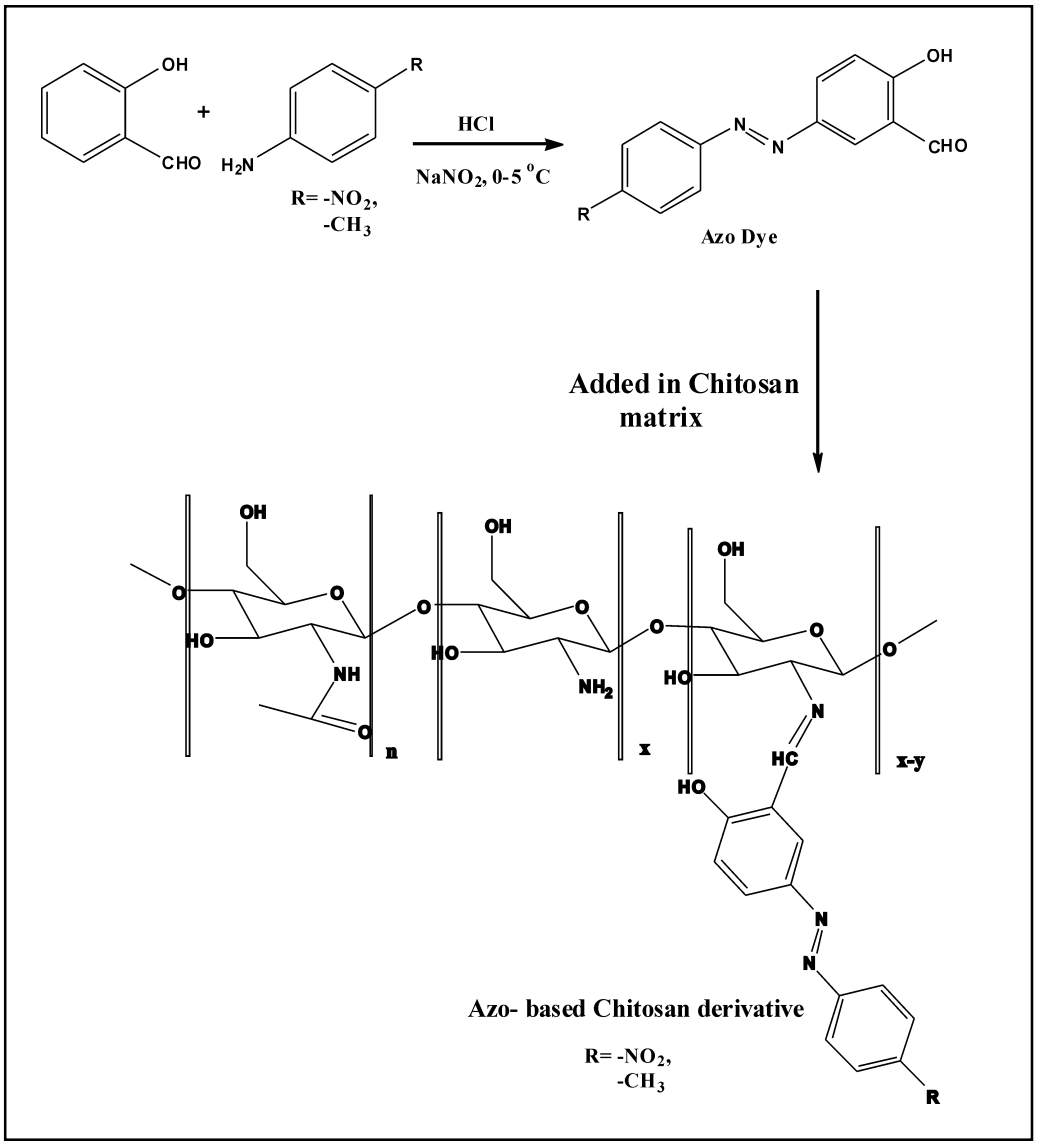
3. RESULTS AND DISCUSSION

The azo dyes hydroxy nitro azobenzaldehyde (HNAB) and hydroxy methylazobenzaldehyde (HMAB) were synthesized according to literature procedure.[2] The recrystallised azo dyes were finally added in chitosan matrix resulting in the formation of the corresponding Schiff bases (CHNAB and CHMAB). The prepared chitosan containing azo-based Schiff bases were used for anion sensing studies. The reaction scheme for preparation of derivatives is given as follows.

3.1 Characterization of the synthesized azo dyes

UV visible studies of dyes

UV-visible spectra of both the dyes are recorded in the dimethyl sulphoxide (DMSO) solvent. As seen in the Fig. 1 difference is due to the presence of functional group nitro ($-NO_2$) and methyl ($-CH_3$). Both are present at *para* position with respect to the Azo (-N=N-) group. It is acting as a bridge between the two aromatic rings as well as extending the conjugation and acting as a chromophore. This is one of the reasons why aryl azo compounds are called as dye, and extensively used in colour industries. Absorption peaks observed for dye containing nitro ($-NO_2$) group HNAB is bathocromic shifted as compared with the methyl ($-CH_3$) group containing dye HMAB. Observed Bathocromic shifted (red shift) is due to the extended conjugation present in the nitro



Scheme 1. Reaction Scheme

group. So, both the functional groups are acting as an auxochrome because these functional groups are responsible for the alteration of wavelength. Other anions of UV-titrations are shown in supplementary materials.

In case, of HNAB dye peaks are at 376 nm and 543 nm. Peak at 376 nm is due to the presence of azo(-N=N-) functional group and peak at 543 nm is due to the presence of -NO_2 group which is making the dye to show

response in visible range and hence, acting as an auxochrome. While in case of HMAB dye peak at 348 nm is due to the presence of azo (-N=N-) functional group and peak at 450 nm is due to methyl group.

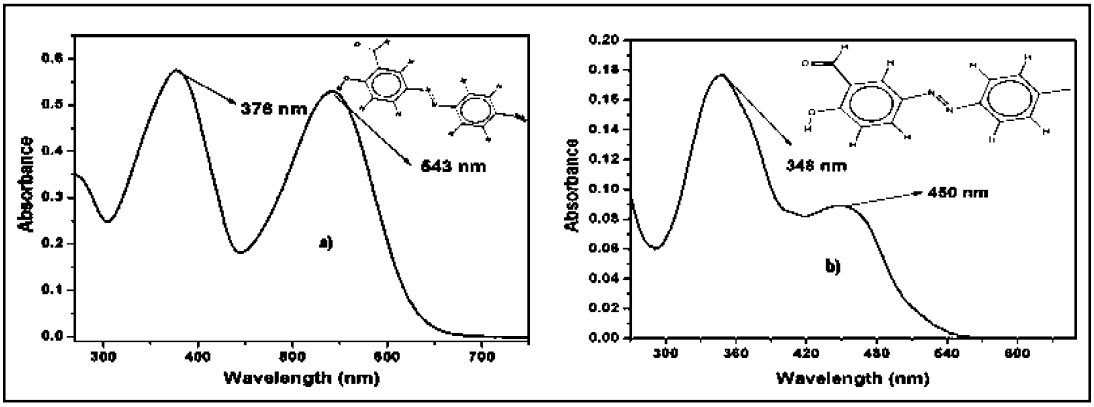


Fig .1. UV spectra of a) HNAB 50 μM in DMSO b) HMAB 10 μM in DMSO

UV visible studies of derivatives

Peak observed in CHNAB and CHMAB is at 350 nm, and 342 nm respectively (Fig. 2) which is mainly due to the presence of azo (-N=N-) functional group, because the chitosan itself is inactive in the UV-visible region. Here in this case, when the dye is incorporated in to the colourless chitosan polymer, properties and orientation of dye molecule is changed in the derivative which is the main reason for the hypsochromic shift as compared to the dyes.

3.2 Anion sensing studies

The recognition properties of the azo dyes and their respective Schiff bases toward different anions were studied by the several methods

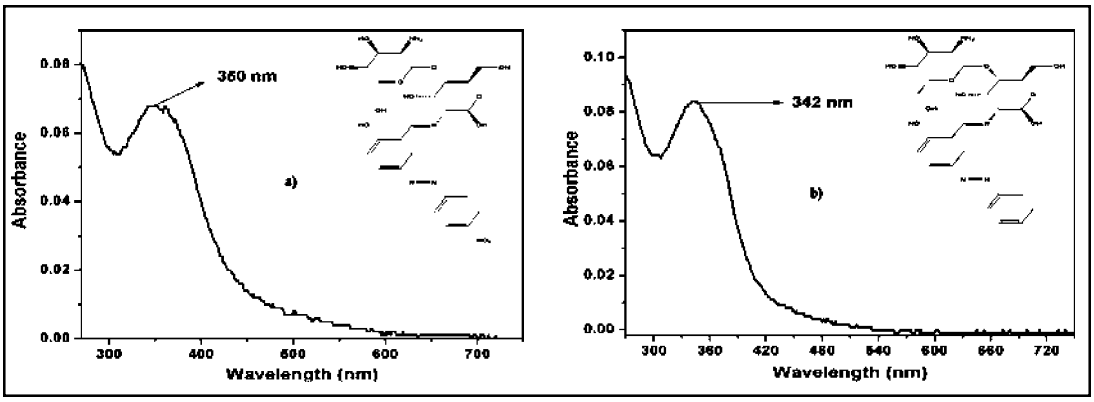


Fig. 2. UV spectra of a) CHNAB 2 μM in DMSOb) CHMAB 4 μM in DMSO.

such as the naked-eye experiment, the UV–vis titration. UV–vis titration was carried out in DMSO to evaluate the binding affinities toward a set of anions (F^-, Cl^-, Br^-, I^-, CN^-, AcO^-, HSO_4^- and $H_2PO_4^-$).

Anion sensing of dyes

Fig. 3 displays the changes in the UV-Visible spectra of the two dyes observed upon addition of CN^- in DMSO solution. Both the dyes are showing chromogenic response when titrated with the cyanide anion. In case of HNAB dye, the light yellow colour (λ_{max}=380nm) changes to the dark red colour (λ_{max}=540nm) upon addition of cyanide anion (Fig.3a).The peak at 380nm is bathochromic shifted on addition of CN^- anion. A clear isosbestic point is also observed at 414nm. This suggested that some new species is being formed on addition of cyanide anion. The hyperchromic shift at 540nm is observed due to the gradual addition of the CN^- anion to the dye solution. HNAB is showing a peculiar colour change in case of CN^-compared to other anions. Same observation is observed in case of HMAB but the isobestic point is observed at 380 nm (Fig. 3b) and increase in the absorbance resulting in a peak at 450nm.

Job's plot analysis indicates 1:1 stoichiometry (Fig. 4) between the dyes and CN^- ion. Absorption spectral change for upon addition of CN^- ion fits well in the following non linear equation which corresponds to 1:1 stoichiometry (Fig. 5).

$$\frac{\Delta A}{b} = \frac{Q_t K \Delta\varepsilon [L]}{1 + K[L]} \qquad \text{(Eq. 1)}$$

where, ΔA refers to the change in absorbance from initial value at the required wavelength, b

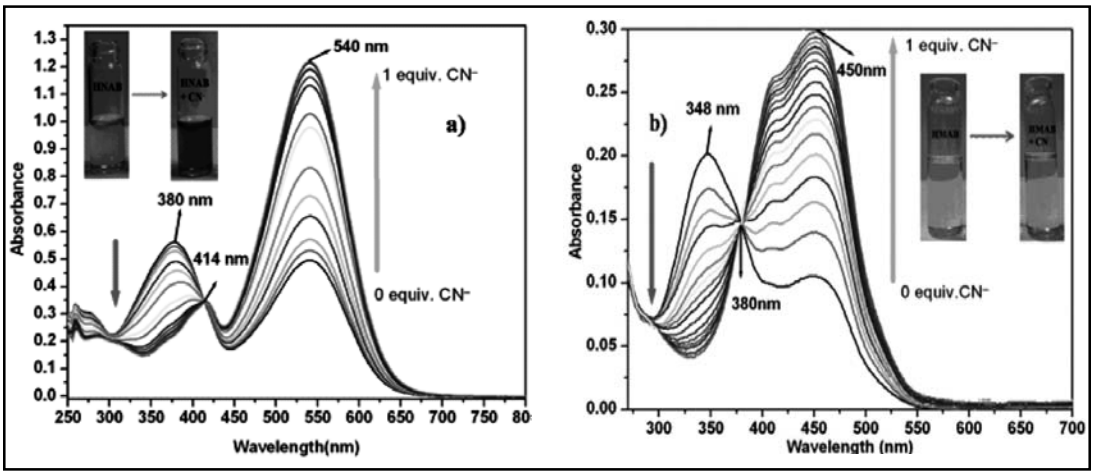


Fig. 3. UV-vis titration spectra of host a) HNAB and b) HMAB with cyanideat 298K, using [HNAB] = 50 μM in DMSO and [HMAB] = 10.7 μM in DMSO

is cuvette path length (in cm), Q_t is total concentration of sensors, K is the binding constant, $\Delta\varepsilon$ is the change in extinction coefficient between free and bound sensor and [L] is the concentration of titrated anion.[27,28] The binding constant data for both the dyes

are tabulated in the Table 1. The other anions UV-titrations results are shown in supplementary S1. Results observed with our chitosan derivatives (CHNAB and CHMAB) are similar to that reported by Rouzi et. al., where selective sensing of PO_4^{3-} and F^- were observed by a chitosan-urea receptor in DMSO-H_2O (1%) medium.[29]

Color change observed for HNAB and HMAB upon addition of different anions is shown in fig. 6. Color of DMSO solution of HNAB changed to violet upon addition of CN^-, F , AcO^- and $H_2PO_4^-$, while color change is observed with CN^- only for HMAB.

TABLE 1. Binding Constant (M^{-1}) of Azo dyes (HNAB and HMAB) and chitosan derivatives (CHNAB and CHMAB) with different anions, determined by UV-visible titration data at 25°C.

Anion	**HNAB**	**HMAB**	**CHNAB**	**CHMAB**
CN^-	2.3 x 10^4	5 x 10^4	1 x10^5	7.5 x 10^4
F^-	5.6 x 10^4	3 x 10^4	8.5 x 10^4	5 x 10^4
AcO^-	1.2 x 10^4	2.1 x 10^4	3 x 10^4	4 x 10^4
HSO_4^-	2.5 x 10^3	3.6 x 10^3	4.2 x 10^3	5.3 x 10^3
$H_2PO_4^-$	4 x 10^4	1.2 x 10^4	7.2 x 10^4	2 x 10^4

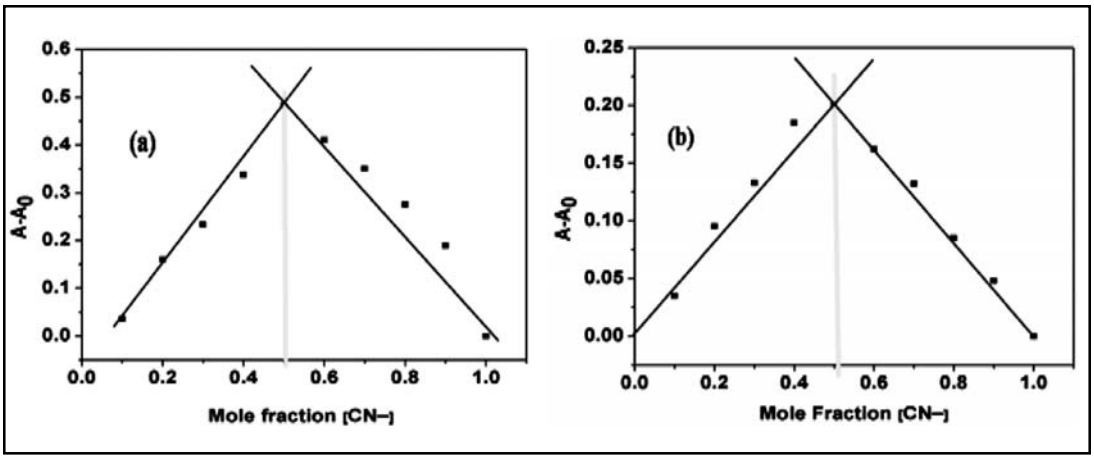


Fig. 4. Jobs plot of a) HNAB and b) HMAB with CN^-

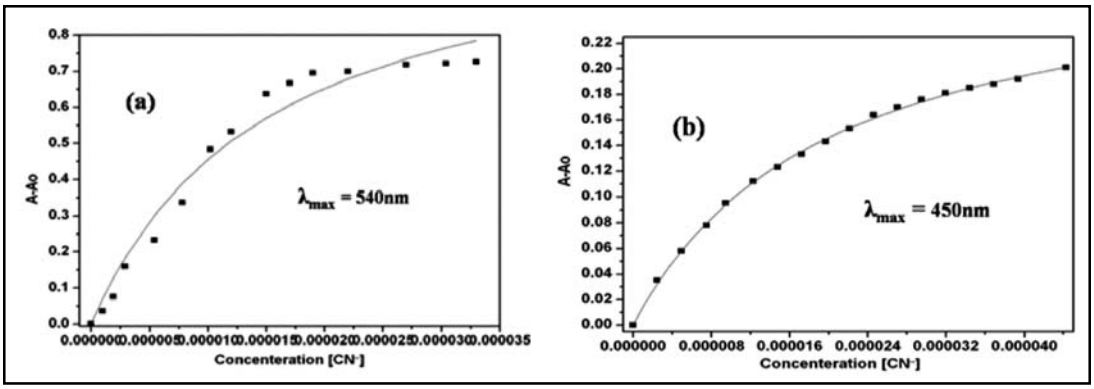


Fig. 5. Binding Constant determination of a) HNAB and b) HMAB

Anion sensing of chitosan derivatives

In both the derivatives i.e. CHNAB and CHMAB on addition of cyanide anion peaks at 347 nm and 343 nm is bathochromic shifted (Fig. 7) and the isobestic point is observed in both the cases at 384nm and 380nm respectively. Absorbance of peak at 450 nm and 439 nm is increased hyperchromic shift on subsequent

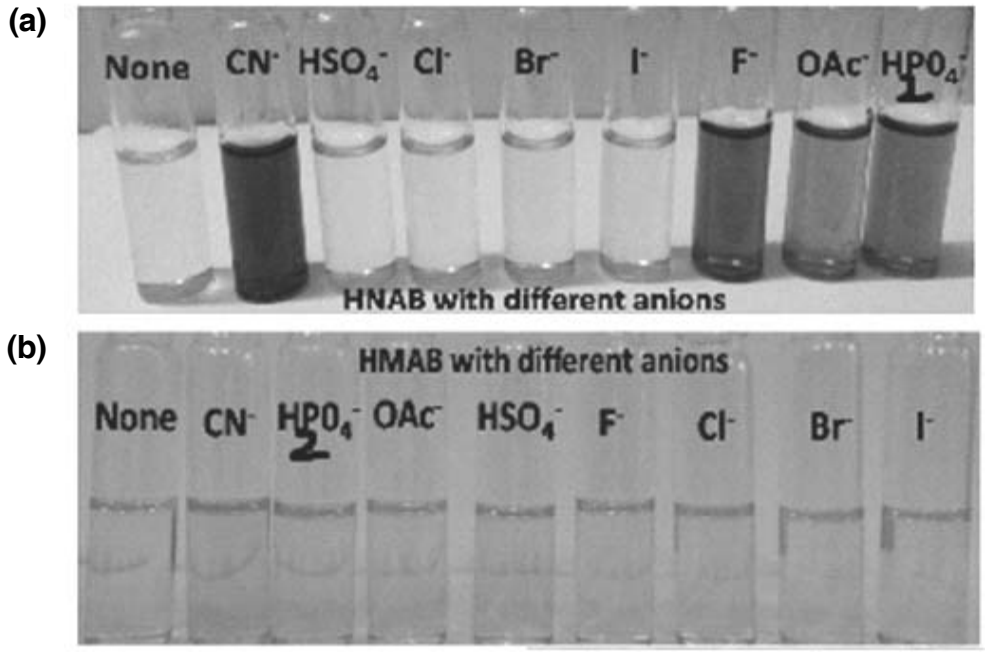


Fig. 6. Colour changes observed for a) 50 μM HNAB b)10.7μM HMAB with different anions

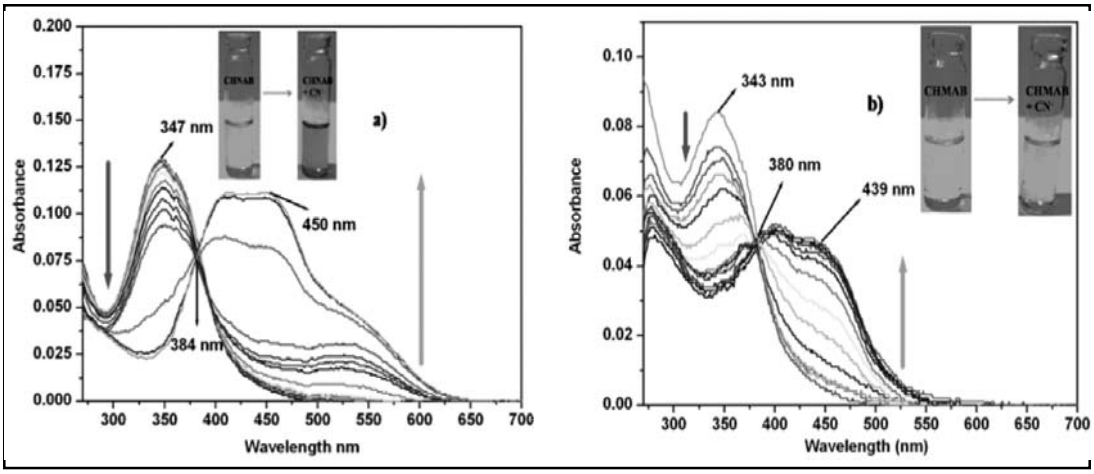


Fig. 7. UV-vis titration spectra of host a) CHNAB and b) CHMAB with cyanide at 298K, using [CHNAB] = 2 μM in DMSO and [CHMAB] = 3.8 μM in DMSO.

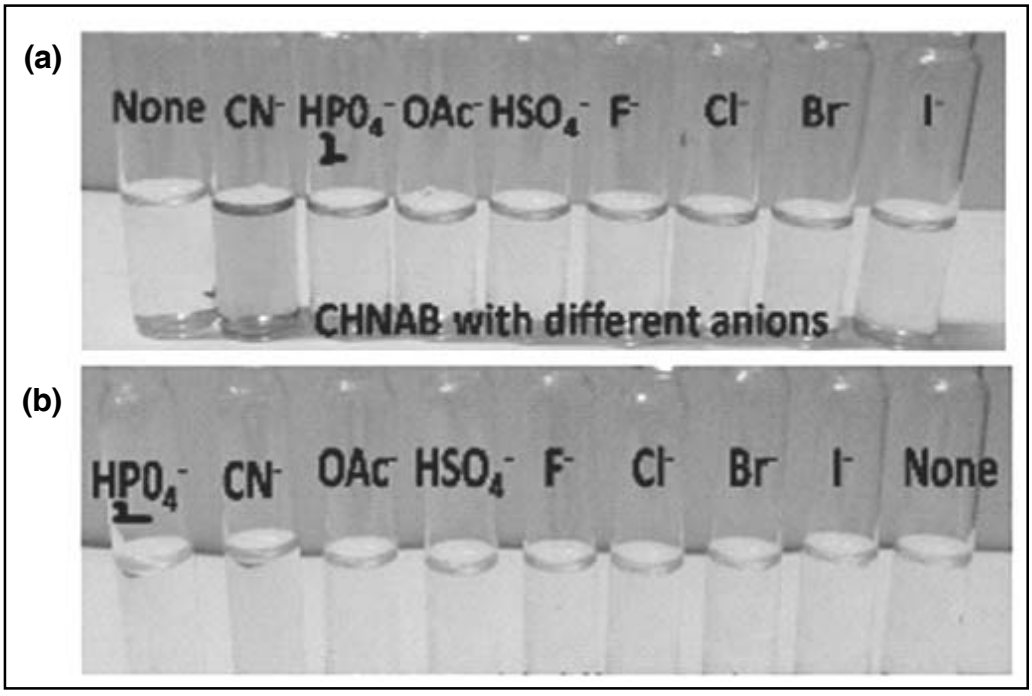


Fig. 8. Colour changes observed for a) 2 μM CHNAB b) 3.8 μM CHMAB titrated with different anions

addition of cyanide anion. A noticeable colour change is observed more in case of the nitro derivative than the methyl derivative. These derivatives can possibly be used as a kit for the detection of lethal cyanide anion.

In the colorimetric experiment, the derivative gave colour change from light yellow to light orange in the presence of CN^- (Fig.8). However, addition of a large amount of Cl^-, Br^-, I^-, F^-, AcO^-, HSO_4^-, and $H_2PO_4^-$ could not result in any colour changes of the receptors.

As shown in Fig. 9, the electron-withdrawing nitro or ester group at the end increases not only the degree of conjugation but also the hydrogen bond donor ability of the phenolic OH. The strong hydrogen bonding to, or deprotonation /protonation of the phenolic

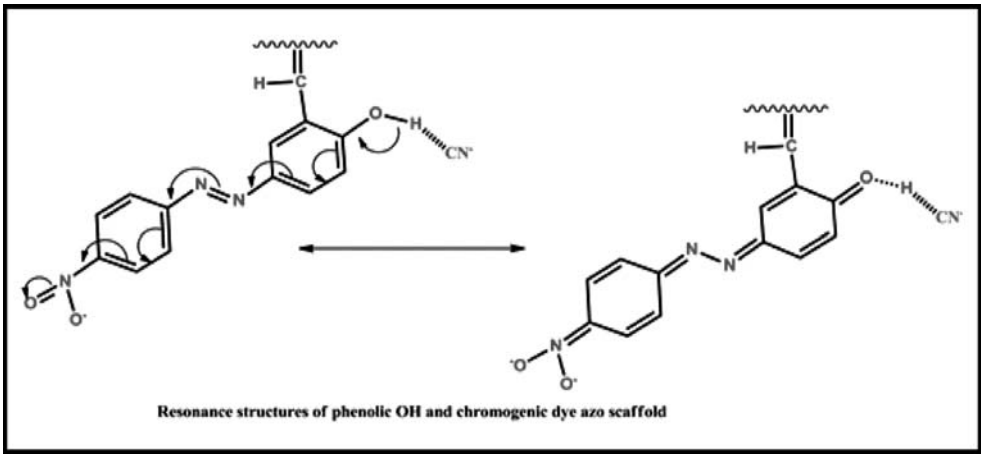


Fig. 9. Cyanide recognition by chitosan derivatives

moiety might modulate the electronic properties of the chromophore and give rise to the colour change. In case of derivative CHNAB cyanide anion concentration is varied from 0.5 μm to 180 μm whereas derivative CHMAB cyanide anion concentration is varied from 0.5 μm to 110 μm. A small amount of derivative CHNAB is sufficient for the detection of cyanide anion; only 2 μm is consumed for showing the colour change. It suggests that the amount of azo dye binded with chitosan is more i.e. why the consumption of cyanide anion is so high in both the cases. Similar binding mechanism is applicable for the azo dyes HNAB and HMAB with the anions also.

It is proposed that the CN– recognition occurs by the initial hydrogen bonding of the anion to the receptor, followed by deprotonation which brings electron density onto the π conjugated framework through bond propagation.

4. CONCLUSIONS

An azo compound based on salicylaldehyde displays a reddish colour upon binding to the cyanide anion with micromolar sensitivity; same response is obtained in the derivative with

much more sensitivity. The high affinity is caused by an intramolecular hydrogen bond of phenol hydrogen to carbonyl oxygen, which stabilizes the anionic character of the intermediate during the nucleophilic attack of a cyanide anion. In case of derivative also affinity is due to the intramolecular hydrogen bond of phenol hydrogen to imine bond nitrogen, which stabilizes the anionic character of the intermediate during the nucleophilic attack of a cyanide anion. This nucleophilic character of the cyanide anion can be utilized in the detection of biological toxic cyanide in various systems. The studies indicate that the materials can be used as optical sensors for detection of cyanide ions even in very small concentration.

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