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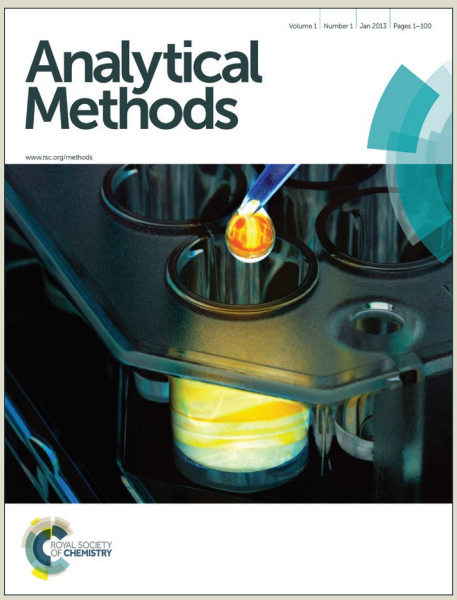

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Exploitation of a simple Schiff base as a ratiometric and colorimetric chemosensor for glutamic acid

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Abstract

A novel, exceptionally simple and rapid method has been developed for visual detection of L-glutamic acid (Glu) in aqueous solution. The chemosensor employed is easy to prepare and use with an added advantage of cost effectiveness. It exhibits an excellent selectivity and sensitivity towards Glu over other amino acids by both changes in absorption intensity and colorimetrically. The chemosensor provides a fast response time, with an LOD of about 7.96×10^{-7} M suggesting that the chemosensor may be useful as a valuable practical sensor for environmental analyses of glutamic acid.

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Introduction

Amino acids are the essential components of life processes. So sensing of amino acids is necessary in diverse fields such as nutritional analysis,[1] the diagnosis of Alzheimer disease[2] and pancreatitis.[3] Over other amino acids, glutamic acid is of great importance because it plays a vital role in a wide variety of brain functions, clinical applications and in food processing.[4] It is also well known as a flavour enhancer, commonly found in various foods. The excessive intake of this flavour enhancer can cause allergic effects such as headache and stomach pain.[5] L-glutamate is also an excitatory neuro transmitter in the central nervous system of vertebrates, and is a potent neuro-excitatory amino acid (EAA) associated with certain behaviour patterns such as aggressive behaviour, visual task learning, morphine-induced muscular rigidity and retrograde amnesia.[6] It plays a key role in brain function because the level of neuronal excitability depends on the relative balance of Asp A and Glu A. Any alterations in such a balance may cause several eurological or psychiatric disorders.[7] Furthermore, L-glutamate has importance in the diagnosis and treatment of myocardial and hepatic disease.[8] The measurement of liver enzymes- alanine amino transferase (ALT) and aspartate amino transferase (AST) in physiological fluids provide valuable information in the diagnosis of liver disease and both are widely used as a biomarker. Both ALT and AST measurements are based on glutamate detection.[9]

Massive efflux of Asp A and Glu A was observed in different neuro-pathological models of brain injury[10] which caused an uncontrolled excitotoxic stimulation of postsynaptic (mainly NMDA) receptors, membrane depolarization and energy depletion resulting neuronal cell death.[11] Hence, analysis of EAAs in biological samples was very relevant in biochemistry and clinical chemistry[12] to know the extent of neuronal damage due to the diseases like epilepsy, Parkinson's disease[13] and ischemic brain injuries.[14,15]

Recently, several researchers have focused to correlate the altered levels of EAAs in humans and some pathologies, including diabetes and cancer.[16] High level Glu A content in plasma will lead to acute ischemic stroke.[17] Glaucoma, one of the major causes of blindness, was characterized by the death of retinal ganglion neurons and optic nerve damage.[18,19] Pathological release of EAAs (particularly Glu and Asp) into the extracellular fluid was supposed to cause the deterioration of retinal ganglion cells after traumatic or ischemic damage to the CNS.[20] Therefore, the determination of EAAs in retina samples was important to know the pathological changes that occur in retinal ganglion cells in glaucoma.

Amino acid analysis is usually performed by chromatographic,[21] UV or electrochemical methods.[22] These techniques are relatively expensive and require trained personnel. Chromatography methods suffered from drawbacks like lengthy clean-up and derivatization steps while electrochemical methods needed complex treatment of electrodes. Chemosensor is ideal for recognition of amino acids owing to their simplicity, high selectivity and sensitivity. [23] Still selective detection of a specific amino acid without interference from other amino acids is a challenging task. Recently, many optical sensors have been developed for cysteine (Cys) and homocysteine (Hcy).[24] Until now only a very few sensors are available for the detection of glutamic acid. [25]

Herein we present a simple Schiff base, **L** for trace level selective sensing of L-glutamic acid. It can perform rapid sensing by changing its colour from yellow to colourless which is easily discernible through naked eye. The chemosensor is easy to synthesize, eco-friendly, and cost effective also. The sensing process does not involve any complicated buffer-making procedure and use of nano particles. To the best of our knowledge, this is the first report of a Schiff base acting as a glutamic acid sensor in an aqueous medium.

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Experimental

General information

UV/Visible spectra were recorded on a Shimadzu UV 1800 spectrophotometer using a 10 mm path length quartz cuvette. ^{1}H NMR spectra were recorded on a Bruker Ultrashield 400 MHz spectrometer. High resolution mass (HRMS) spectra were recorded on Waters mass spectrometer using solvent HPLC methanol. All the chemicals and amino acids were purchased from Merck. Solutions of the receptor **L** (1×10^{-5} M) and amino acids (1×10^{-4} M) were prepared in CH_3OH–H_2O (2/1, v/v) and H_2O respectively.

Synthesis and characterisation of L

To a dehydrated methanolic solution of p-phenylenediamine (0.108 g, 1 mmol, in 50 mL methanol) pyridine-4-carboxaldehyde in methanol (0.214 g, 2 mmol, in 5 mL methanol) was added. The mixture was refluxed for 4h at 45^0C, maintaining dry condition. A yellow precipitate obtained was filtered and washed several times with *n*-hexane and then again recrystallized in methanol and dried in a vacuum to obtain the pure yellow solid. Yield: 82%. M.P- 205^0C. ^{1}H NMR: (DMSO-d_6, δ ppm, TMS): 8.77 (d, 4H); 8.75 (s, 2H); 7.87 (d, 4H) and 7.46 (s, 4H) (Fig. S1) ; ^{13}C NMR: (DMSO-d_6, δ ppm, TMS): 160.4, 152.1, 150, 148.6, 142.9,

122.6, 114 (Fig. S2). FT-IR: (KBr, cm^{-1}): 1597.08 (C=N). ESI-MS: m/z [M + H^+], 288.48 (100%) (Fig. S3). Anal. calcd for $C_{18}H_{14}N_4$: C, 75.50; H, 4.93; N, 19.57%. Found C,75.54; H,4.91; N,19.59%.

UV-Vis titrations

The chemosensor **L** (2.86 mg, 0.01 mmol) was dissolved in methanol-water solvent mixture (10 mL) and 30 μL of it was diluted to 3 mL with the solvent mixture to make a final concentration of 10μM. Glu (0.1 mmol) was dissolved in 10 mL of triple distilled water and 1.5–90 μL of the amino acid solution (10 mM) were transferred to the solution of **L** (10 μM) prepared above. After mixing them for a few seconds, UV-Vis spectra were obtained at room temperature.

Colorimetric test kit

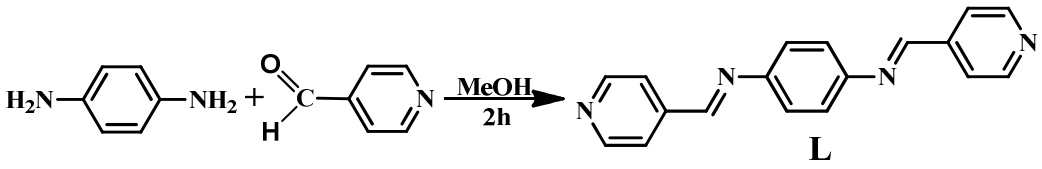
Chemosensor **L** (2.86 mg, 0.01 mmol) was dissolved in methanol (10 ml) to get 1mM solution. Test kits were prepared by immersing filter-papers into this solution (1 mM), and then dried in air to get rid of the solvent. Glutamic acid and other amino acids were dissolved in water (10 mL) to prepare 0.1 mM solution. The test kits prepared above were dipped into the aqueous solution of glutamic acid and other amino acids and then dried at room temperature.

Computational details

The GAUSSIAN-09 Revision C.01 program package was used for all calculations.[26] The gas phase geometries of the compound was fully optimized without any symmetry restrictions in singlet ground state with the gradient-corrected DFT level coupled with the hybrid exchange-correlation functional that uses Coulomb-attenuating method B3LYP.[27] Basis set 6-31++G was found to be suitable for the whole molecule. The electronic spectrum of the receptor **L** was calculated with the TD-DFT method and the solvent effect (in methanol) was simulated using the polarizing continuum model with the integral equation formalism (C-PCM).[28, 29]

Results and discussion

Synthesis and structure of L



Scheme 1 Synthetic procedure of the receptor **L**

Receptor **L** was obtained by the condensation reaction of p-phenylenediamine and pyridine-4-carboxaldehyde in methanol with 72% yield (Scheme 1) and characterized by ^{1}H NMR and ^{13}C NMR, I.R and ESI-mass spectrometry and elemental analysis.

DFT calculations were performed on the molecule **L**. The geometry optimizations staring from gauss view structure of **L** lead to a global minimum as stationary level. The optimized structure of the **L** shown in Fig. 1 and its geometry optimized selected bond length and angles are tabulated in Table S1. The absorption spectrum of **L** was simulated in presence of the solvent employing the TD-DFT methods with the same basis set and functional as used in geometry optimization. The findings were in good agreements with the experimental data (Fig. 2). Calculated spin-allowed electronic transitions with the experimentally observed data for **L** in methanol were summarized in Table S2. For **L,** the transitions having oscillator strengths greater than 0.001, were incorporated. Again, the transitions only with orbital contributions larger than 10% were taken into account for the molecules. A schematic representation of the contours of selected HOMO and LUMO orbitals and energy of MOs of **L** were presented in Figs. 3 and Fig S4 respectively. The HOMO to LUMO energy gap for **L** is 3.525 eV.

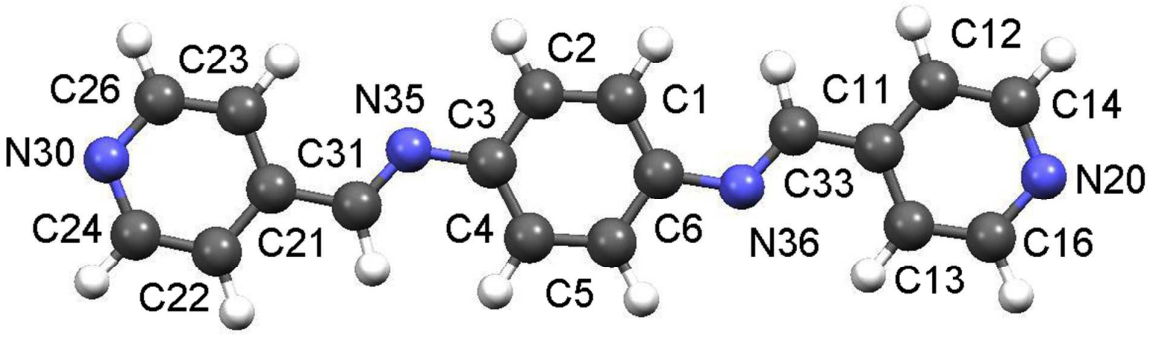


Fig. 1 Geometry optimized diagram of the molecule **L**.

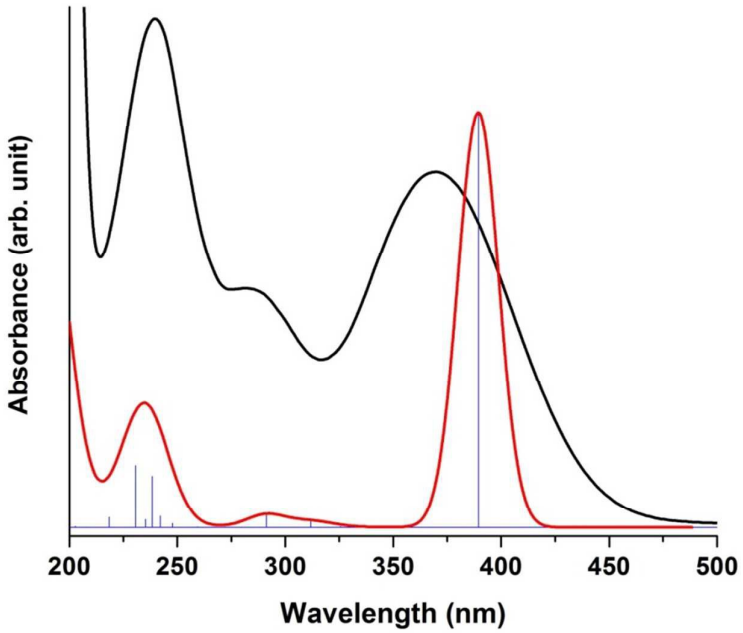


Fig. 2 The experimental (black), calculated (red) electronic absorption spectra and calculated electronic transition (blue) of **L**.

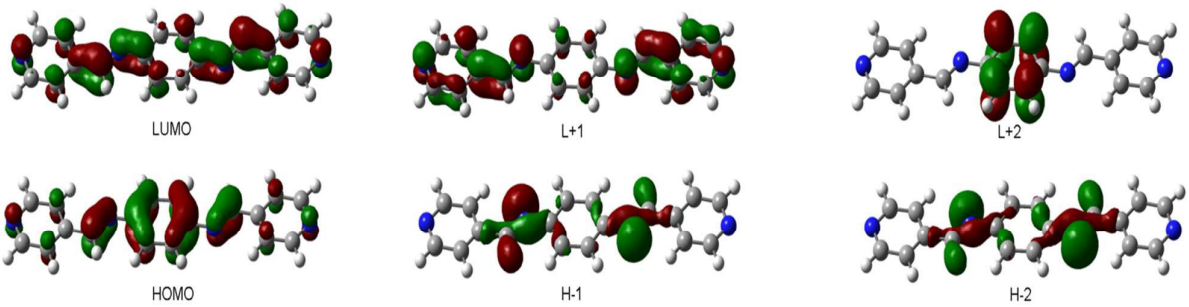
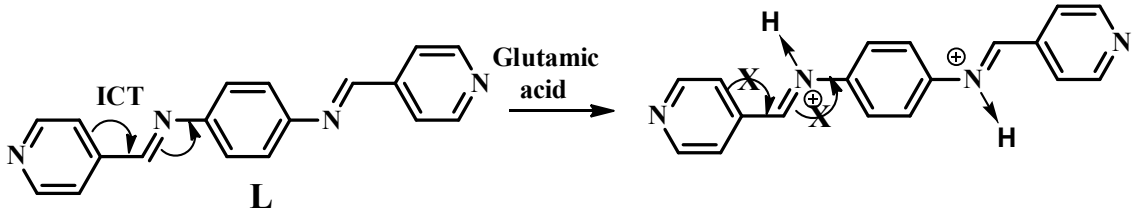


Fig. 3 The contours diagram of selected HOMO and LUMO orbitals of **L**. Positive values of the orbital contour are represented in purple (0.04 au) and negative values in green (-0.03 au).



Scheme 2 Sensing mechanism of chemosensor **L**

Absorption studies of L toward different Amino acids

The colorimetric selective sensing abilities of chemosensor **L** with various amino acids in water were investigated by UV-Vis absorption spectrometry (Fig. 4a). Only the addition of glutamic acid induced distinct spectral changes while other amino acids did not induce any spectral change. In Fig. 4b, bar graph showing the relative absorption intensity of **L** upon treatment with various amino acids has been given.

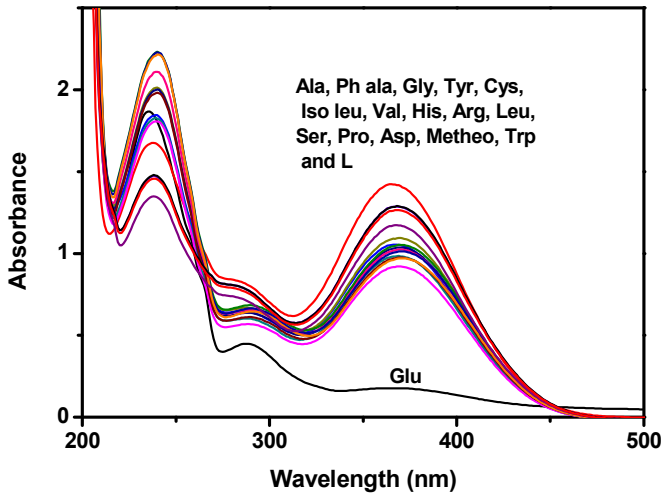


Fig. 4a Absorption spectra of **L** (10 μM) changes in presence of 10 equiv. of different amino acids.

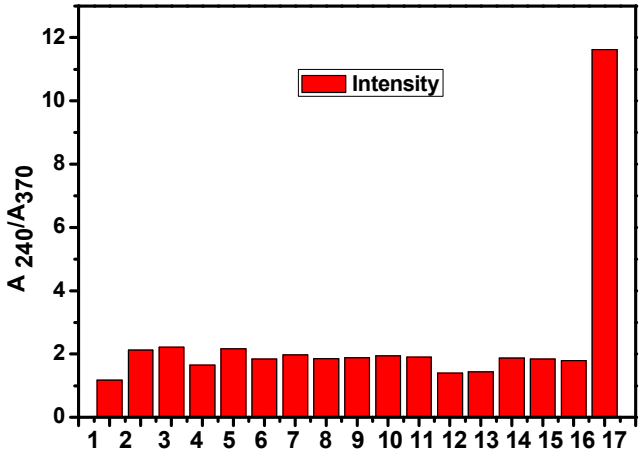


Fig. 4b Bar graph shows the relative absorption intensity of **L** upon treatment with various amino acids (1.L 2.Trp 3.Arg 4.Ala 5.Val 6.His 7.Leu 8.Ph ala 9.pro 10.Isoleu 11.Ser 12.Asp 13.Cys 14.Metheo 15.Tyr 16.Gly 17.Glu).

The chemosensor **L** initially exhibited three absorption bands at 240, 282 and 370 nm. Among them two strong bands at 240 nm and 370 nm were assigned to the phenyl π- π* and n-π* electron transition respectively. But addition of glutamic acid to a solution of **L** led to an abrupt decrease in absorption intensity along with a colour change from yellow to colourless, which allows the detection of glutamic acid with the naked eye (Fig. S5). The exclusive selectivity of chemosensor **L** towards glutamic acid was further studied ratiometrically (Fig. 4b). However absorption study carried out with common metal cations and anions showed no significant change, indicating their noninteractive nature with **L** (Fig. S6). This indicates that under signalling conditions, the possible interference by them is not a practical problem in glutamic acid sensing by **L**.

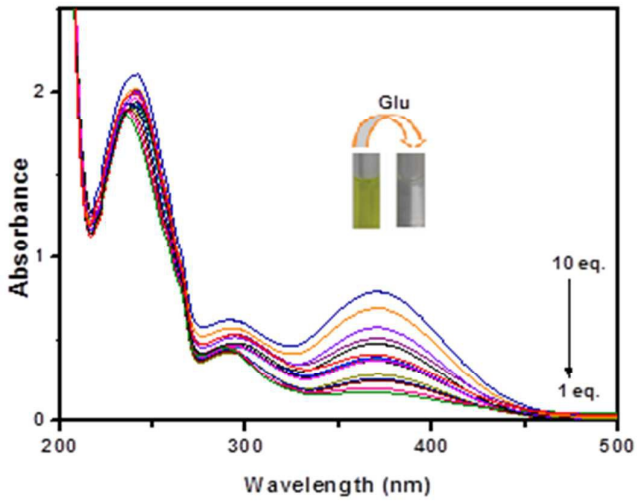


Fig. 5 Absorption spectra of **L** changes after addition of Glu up to 10 equiv.

The binding properties of **L** with glutamic acid were further studied by UV-Visible titration experiment. As shown in Fig. 5, upon increasing the concentration of glutamic acid, the absorbance of **L** at 370 nm almost vanished with slight decrease in the absorption bands at 240 nm and 282 nm. The π conjugate system of the chemosensor **L** undergoes intramolecular charge transfer (ICT) from the donor to the acceptor upon excitation by light. On addition of glutamic acid, the imino nitrogen atoms of chemosensor **L** get protonated and thereby reduced the electron-donating ability of imino nitrogen atoms (Scheme 2). Thus the efficiency of intramolecular charge transfer (ICT) process is affected resulting in decrease in intensity at 370 nm. To identify the ICT property of **L**, we have checked the change of its absorption spectra in several solvents such as dimethylsulfoxide, methanol, ethanol and acetonitrile because it has been reported that the solvent dipole can relax the ICT excited by polar solvents.[30] As shown in Fig. S7 and summarized in Table 1, the absorption spectra of **L** featured a marginal red-shift

of absorption maxima ($\Delta\lambda_{abs}$ = 13 nm), indicating an apparent solvent dependence of the absorption band.

Table 1 Absorption properties of **L** in various solvents

Solvent	λ_{abs} [nm] (log ε)
Ethanol	368 (6.92)
Methanol	370 (6.95)
Acetonitrile	378 (6.87)
DMSO	381 (7.09)

Therefore, this solvatochromic behaviour demonstrates the occurrence of the ICT transition in receptor **L**.[30, 31] From the titration profile, the detection limit calculated is 7.96×10^{-7} M (Fig. S8).

In order to get better insight into the sensing mechanism, ^{1}H NMR and mass spectral studies of **L-Glutamic acid** have been carried out. In ^{1}H NMR spectra (Fig. S9), the generation of a new peak at 7.22 ppm with little shifts of other peak positions to lower δ values with respect to **L** establishes the fact that the two imino nitrogen atoms of **L** get protonated in presence of glutamic acid (as shown in Scheme 2). Moreover, no significant change is found in HRMS spectral analysis of **L-Glutamic acid** (Fig. S10), which further supports the protonation mechanism.

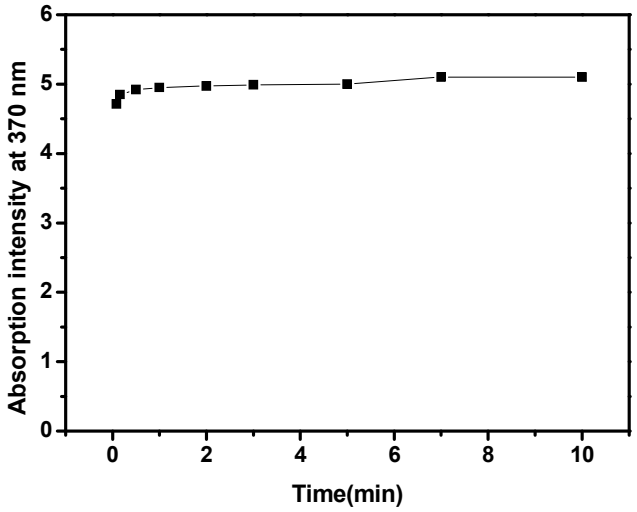


Fig. 6 Time evolution for glutamic acid

To get more details on the spectral changes of probe **L** towards glutamic acid, scanning kinetics were then performed. As shown in Fig. 6, the absorption intensity decreased suddenly

within few seconds and remained almost constant up to 10 min on addition of 2 equiv. of glutamic acid.

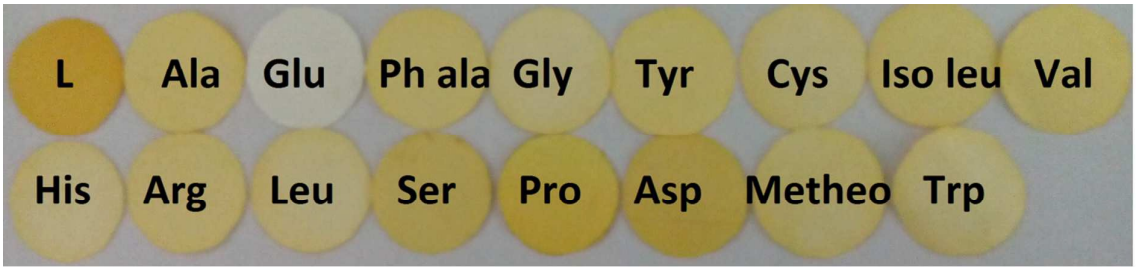


Fig. 7 Photographs of the test kits with **L** (0.5 mM) for detecting glutamic acid among other amino acids.

To check the practical application, the test kits were utilized to sense glutamic acid among different amino acids. As shown in Fig. 7, when the test kits coated with **L** were added to different amino acid solutions, the obvious colour change was observed only with glutamic acid in aqueous solution. Therefore, the test kits coated with the chemosensor **L** solution would be convenient for detecting glutamic acid. These results showed that chemosensor **L** could be a valuable practical sensor for environmental analyses of glutamic acid.

Conclusion

In summary, we have developed a novel, exceptionally simple and rapid method for visual detection of glutamic acid in aqueous solution. The chemosensor **L** employed is easy to synthesize, eco-friendly, and cost effective. It exhibits an excellent selectivity and sensitivity towards glutamic acid by both changes in absorption intensity and colorimetrically. The detection limit of **L** for glutamic acid is sufficiently below in comparison to the most of the reported chemosensors. On the basis of these results, we believe that the chemosensor **L** may be useful as a valuable practical sensor for environmental analyses of glutamic acid.

Acknowledgements

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Notes and references

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