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ORIGINAL ARTICLE

Microwave Assisted Synthesis of N-Doped Carbon Dots: an Easy, Fast and Cheap Sensor for Determination of Aspartic Acid in Sport Supplements

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Abstract
In this work, determination of aspartic acid by N-doped carbon dots (N-CDs) was studied at optimum condition. Characterization and morphology of surface of N-CDs were carried out by FT-IR and HRTEM. N-doped carbon dots size was 10 nm. Quenching was very fast after addition of aspartic acid that is an important property of this sensor. Optimum conditions for pH and excitation wavelength were 8 and 360 nm, respectively. Linear dynamic range and limit of detection for aspartic acid were 0.5–50 μM and 90 nM, respectively. This method was used for aspartic acid determination in human serum and sport supplement powder as real samples. Performance of this sensor was also compared with other fluorescent sensors.

Keywords Carbon dots · Aspartic acid · Sensor · Fluorescence

Introduction

Amino acids are very important organic compounds because of their basic function in proteins, neurotransmitters and biosynthesis [1]. Aspartic acid, an aliphatic amino acid, contains α-amino group (as protonated form -NH_3^+) and α-carboxylic acid group (as deprotonated form –COO^-) under biological conditions. Aspartic acid can be synthesized in body from oxaloacetate [2]. Aspartic acid is found especially in sugar cane and sugar beets. It has an overall negative charge and plays an important role in the citric acid and urea cycles and in the synthesis of other amino acids such as asparagine, arginine, lysine, methionine and isoleucine [3]. Aspartic acid plays a key role in supplements for sportsman because lead to increasing muscle size and enhancing strength with testosterone and luteinising hormones increase [4].

Several methods were used for separation and determination of amino acids, such as ion exchange chromatography [5], capillary electrophoresis mass spectrometry [6], HPLC-MS, HPLC-UV [7] and GC-MS [8]. Most of common amino acids have not any chromophoric group and their derivatives with fluorescent dyes used in high performance liquid chromatography with laser-induced fluorescence. However, HPLC methods need to long analysis time, expensive instrumentation, sample pretreatment procedures, expensive material and complicated preparation process [9, 10]. Thus, it is essential to develop new methods to overcome these drawbacks.

Carbon dots have interesting properties including moderate reaction temperature, low toxicity, solubility in water, unique optical properties such as high quantum yield, photoluminescence, broad excitation spectrum, narrow emission bandwidth and size-dependent tunable [11]. Various synthesis techniques like physical and chemical vapor deposition, hydrothermal, electro templating and solvothermal methods can be used for CDs synthesis. These techniques need to long reaction times and post processing of the prepared samples [12]. A simple and easy technique is microwave-assisted method. Microwave-assisted method has many advantages such as environmental friendliness, energy saving, high yield and uniform size distribution of carbon dots [13, 14].

In this work, a selective and sensitive sensor based on N-doped CDs was used for cheap and easy determination of aspartic acid. N-doped CDs were synthesized by microwave-assisted method and used for aspartic acid determination in human serum and sport supplement powder as real samples.

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Experimental Section

Reagents

All chemicals with highest purity available were obtained from Merck. Stock solutions (10 mM) of aspartic acid and interferences were prepared from their salts and deionized water. Universal buffers (0.01 M) were used for pH adjustment.

Synthesis of Nitrogen-Doped Carbon Dots

A solution containing citric acid, 1 g, urea, 1 g and 10 mL deionized water was radiated at 450 W for 5 min in microwave oven. Obtained brown color solid was dissolved in 10 mL water, centrifuged and dried at room temperature. Dried solid was dissolved in water (final concentration was 20 mg/mL).

Characterization

UV–Vis (Varian Cary 300 Bio UV/Vis), FT-IR (Vertex 70 FT-IR) and fluorescence (Varian Cary) spectra were used for N-CDs characterization. HRTEM (MC30; Philips) was used for size and morphology determination of the N–CDs.

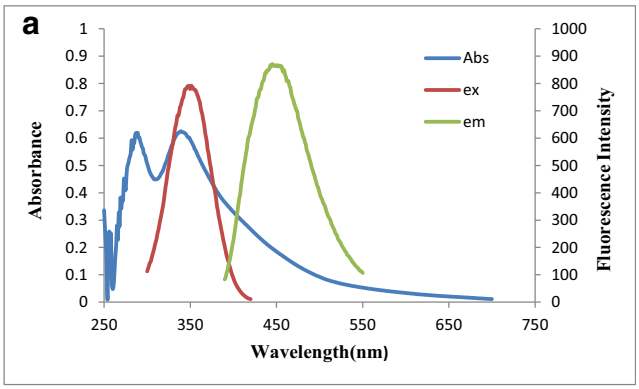

Fig. 1 **a** UV–vis absorption, excitation and emission spectra of CDs in aqueous solutions (0.05 mg mL^{-1}), $\lambda_{ex} = 360$ nm and $\lambda_{em} = 450$ nm. (**b**) FL spectra at different excitation wavelength

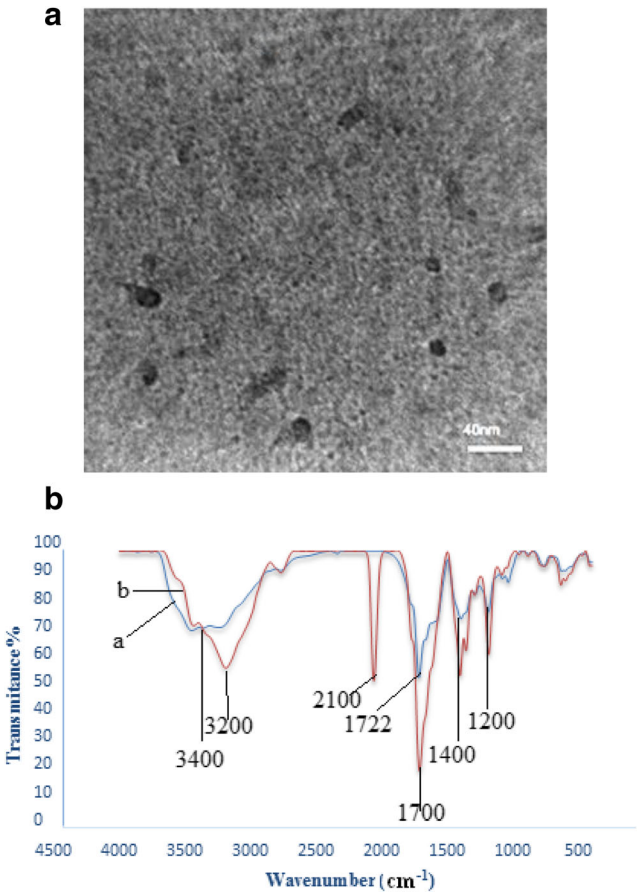

Fig. 2 **a** The typical HRTEM image (B) FT-IR spectra before (**a**) and after (**b**) addition of aspartic acid to N–CDs

Analytical Method

At initial N-CDs concentration, fluorescence intensity was very high, thus it was diluted 10 fold. A typical aspartic acid measurement was performed as follow. 75 μL of N-CDs were added to 3 mL of Britton–Robinson universal buffer (pH = 8, 0.01 M). Different concentration of aspartic acid was added to mixture and fluorescence intensity was measured (λ_{ex} = 360 nm, λ_{em} = 450 nm, slit widths 5 nm).

Real Samples

Real samples were human serum (central lab, Ilam, Iran) and sport supplement powder (Super-size, Apex Company, Isfahan, Iran). 1 mL aliquot of plasma was placed into a centrifuge tube and 60 μL of 0.2 M HCl was added. Then, 1 mL of acetonitrile was added in order to precipitate proteins. The solution was centrifuged for 20 min and the supernatant solution was transferred into a 25-mL volumetric flask and diluted to the mark with water [15].

Sport supplement dry powder (1 g) was dissolved in 10 mL of deionized water and sonicated up to 20 min at 300 W and 37 KHz (ultrasonic waves help to better dissolving and

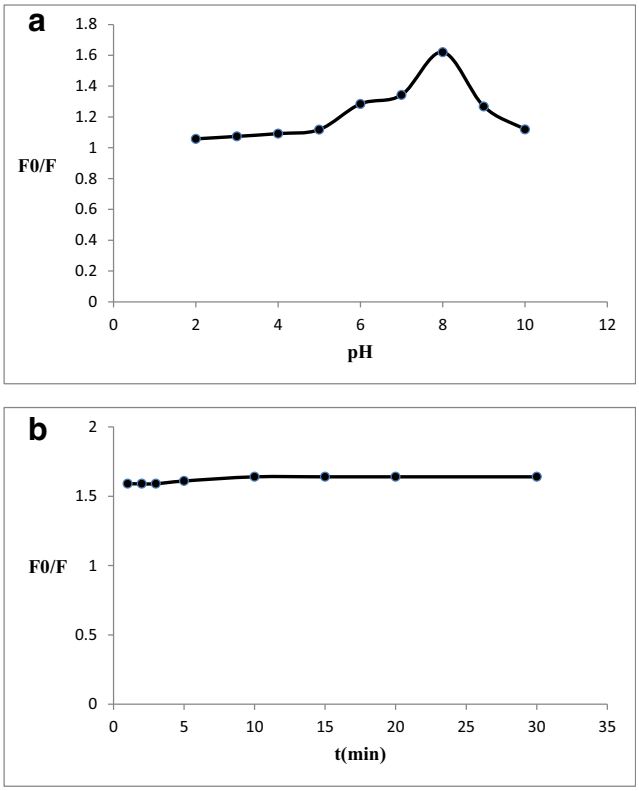

Fig. 3 Effect of pH (**a**) and time (**b**) on the relative florescence intensity in the presence of 25 μM aspartic acid

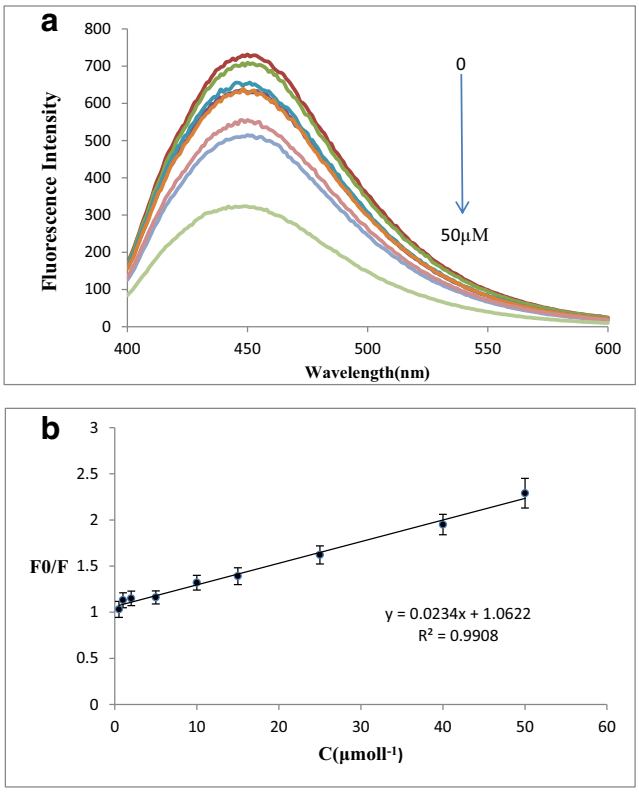

Fig. 4 **a** Fluorescence emission spectra of the N-CDs in aqueous solution after addition of various concentrations of aspartic acid (from top to bottom: 0, 0.5, 1, 2, 5, 10, 15, 25, 40 and 50 μM). **b** Stern-Volmer plot

dispersion of sample powder in water). Then, 20 mL of chloroform was added to sample solution. The suspension was centrifuged at 5000 rpm for 15 min and the liquid phase separated and analyzed [16, 17].

Result and Discussion

Characterization

As shown in Fig. 1a (UV–Vis absorption spectrum of the N-CDs), there are two absorption peaks at 250 and 350 nm. These peaks are related to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of aromatic system and non-bonding electron of nitrogen.

As shown in Fig. 1a, when N-CDs are excited at 360 nm, strong fluorescence emission peak is observed at 450 nm. As shown in Fig. 1b, fluorescence intensity of the N-CDs also changes with the change of excitation wavelength from 360 to 400 nm (with low shift at maximum peak from 445 to 460 nm). Thus, optimum wavelength for excitation is 360 nm.

High resolution transmission electron microscopy (HRTEM) image is used for morphological study and particles size determination of the synthesized N-CDs. As shown in Fig. 2a, the diameter of N-CDs is 10 nm.

Fourier transform infrared spectroscopy (FT-IR) can be used to investigate N-CDs functional groups. The FT-IR spectra of N-CDs before and after addition of aspartic acid are shown in Fig. 2b. As shown in Fig. 2b, there are clear band shifts and intensity changes in O–H and N–H vibrations bands at 3200–3400 cm^{-1}, while the sharp peak at 2100 cm^{-1} represents azide group. Vibrations at 1722 cm^{-1} were confirmed the existence of the carbonyl C=O groups. C-N stretching had medium absorption band at region 1201–1411 cm^{-1} [18].

Effect of pH and Time

One of the most important analytical parameters is pH. Effect of pH on the fluorescence quenching of N-CDs in the pH range of 2–10 in the presence of 25 μM of aspartic acid is shown in Fig. 3a. First, quenching increases and reaches to maximum value at pH 8 and then decreases at higher pHs. It could be emphasized that in acidic medium (pH = 2 to 5), there is little effect but in neutral and basic medium (6 to 9) fluorescence is highly affected. The effect of pH in this region is due to deprotonation of the functional groups (carboxyl and amine) on the surface of N-CDs and aspartic acid which leads to change in fluorescence intensity. Interactions reach to maximum value at pH = 8 and the highest quenching is observed.

Figure 3b shows effect of response times in the range of 1 to 30 min on the fluorescence intensity of N-CDs at room temperature and pH 8. Quenching is very fast after addition of aspartic acid. It is an important property of this sensor.

Table 1 Comparison of different fluorescence sensors for aspartic acid

Sensor	Detection limit (μM)	Linear range (μM)	Ref.
Dansyl Hydrazine Dextran Conjugate	–	100–2500	[18]
Supramolecular binary ensemble based on an imidazolium-modified cationic dansyl derivative/ anionic surfactant (SDS)	0.6	40–200	[19]
2-(2-Pyridyl) benzimidazole based Co(II) complex	10	10–300	[20]
Polythiophene–gold nanoparticles composite	0.032	0.075–6	[21]
Glucosamine modified near-infrared cyanine fluorescent probe	–	0.1–10	[22]
Co^{2+} complex of triazole linked imino-phenol based calix[4]arene conjugate	2.5	–	[23]
N-CDs	0.09	0.5–50	This study

Calibration

At optimized conditions, the fluorescence intensity of the N-CDs is gradually decreased due to fluorescence quenching by aspartic acid in the range of 0.5–50 μM (Fig. 4a). As shown in Fig. 4b, Stern-Volmer plot (F_0/F versus aspartic acid concentration) is $F_0/F = 0.0234\ C_{Asp}$ (μM) + 1.0622, $R^2 = 0.99$. The limit of detection (LOD) for aspartic acid is 90 nM using $LOD = 3\sigma/s$. Comparison of detection limit and linear range of various fluorescence methods for aspartic acid is presented in Table 1. Most of the reported sensors [18–23] have complex structure, complicated preparation process and need to expensive material, while our synthesis method is green, easy, fast and cheap. Advantage of the new sensor is very fast response time with respect to previous sensors. 2-(2-Pyridyl) benzimidazole based Co(II) complex [20] has 2 h reaction time. Glucosamine modified near-infrared cyanine fluorescent probe [22] need to 20/80 ethanol/water solvent. Good water solubility, low detection limit and acceptable linear dynamic range are another advantages of this sensor.

Table 2 Tolerance of diverse ions on the fluorescence intensity of 25 μM aspartic acid

Materials	Tolerance*
Arginine, Sucrose, Glucose, Proline, Urea, L-Phenyl Alanine, Glycine, Melamine, Cysteine, Leucine, Methionine, Lysine, Serine	500
Mg^{2+},Na^{+}, K^{+}, Ca^{2+}, I^{-}	100
Ag^{+}, Fe^{3+}	50
Fe^{2+}, Tyrosine	10
Glutamic acid	5

*The tolerance limits were determined based on maximum error of 5% in fluorescence intensity

Selectivity

Fluorescence turn off synthesized N-CDs after addition of various cations, anions and molecules is shown in Fig. 5. As shown, F/F_0 ratio decreases in present of aspartic acid, but other ions and molecules have no remarkable effects. Electron transitions of nonbonding pair electron in the carbon dots structure cause fluorescence emission. Aspartic acid, as an acidic amino acid (with $pK_{a1} = 1.92$ and $pK_{a2} = 3.87$) acts as an electron withdrawing group, can attract free electrons, and lead to fluorescence quenching of carbon dots.

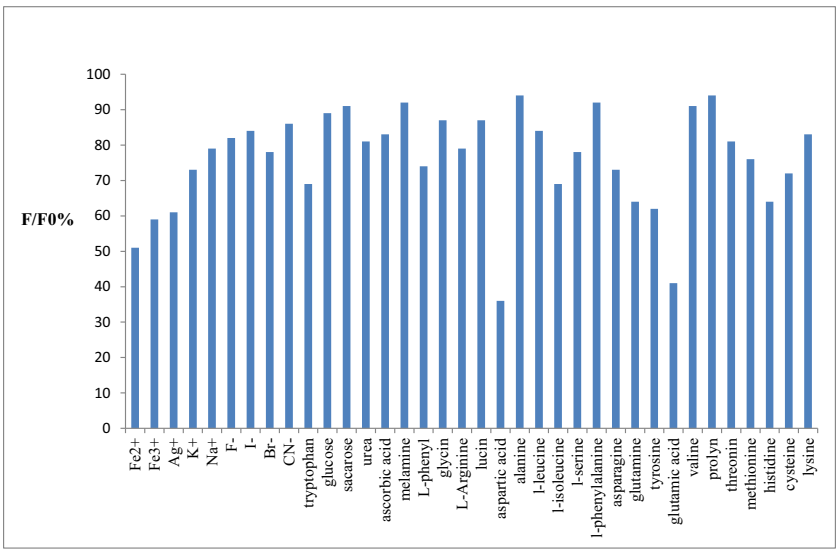

Fig. 5 Fluorescence responses of N-CDs system to different ions and molecules (concentration was 25 μM)

Table 3 Determination of aspartic acid by the suggested fluorescence sensor in real samples

Samples	Added (μM)	Found (μM)	Recovery (%)	RSD
Human serum	0	8.0	–	–
	10	18.5 ± 0.13	105.0	1.2
	25	35.0 ± 0.43	108.0	1.6
	50	59.5 ± 0.98	103.0	1.9
Sport supplement powder	0	18.0	–	–
	10	28.9 ± 0.22	109.0	2.1
	25	46.5 ± 0.75	114.0	2.6
	50	74.5 ± 1.70	113.0	3.0

Interference Effect

The interference effect of other ions on aspartic acid determination (25 μM) is presented in Table 2. No remarkable interferences from ions including: Sn^{2+}, Sn^{4+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Sb^{2+}, Ti^{2+}, Ba^{2+}, Li^{+}, W^{6+}, Na^{+}, K^{+}, Cu^{2+}, Zn^{2+}, Ca^{2+}, Li^{+}, Al^{3+}, Fe^{3+}, Fe^{2+}, Pb^{2+}, Bi^{3+}, Mo^{6+}, La^{3+}, Ba^{2+}, Ag^{+} are observed. Only Glutamic acid has significant interferences, because it has acidic properties ($pK_a = 4.1$) similar to aspartic acid and can attract free electrons and lead to fluorescence quenching.

Real Samples

Real sample analysis is carried out in order to investigate efficiency of new methods in complicated matrix. Recovery of aspartic acid in human serum and supplement powder is presented in Table 3. As shown, this sensor has suitable recoveries, thus has good ability for analysis of aspartic acid in real samples.

Conclusions

Application of N-CDs as amino acid sensor is cheap, fast, easy and environmental friendly. N-CDs can be synthesized by microwave method and characterized by FT-IR and HR-TEM. Aspartic acid can be determined in human serum and sport supplement powder as real samples by turn-off fluorescence sensor at optimum conditions (pH 8 and excitation wavelength of 360 nm).

References

1. Vukosav P, Mlakar M (2014) Speciation of biochemically important iron complexes with amino acids: L-aspartic acid and L-aspartic acid-glycine mixture. Electrochim Acta 139:29–35
2. Hai H, Yurika M, Reiko K, Masashi M (2015) Changes in d-aspartic acid and d-glutamic acid levels in the tissues and physiological fluids of mice with various d-aspartate oxidase activities. J Pharm Biomed Anal 116:47–52
3. Prasad BB, Srivastava A, Tiwari MP (2013) Molecularly imprinted polymer-matrix nanocomposite for enantioselective electrochemical sensing of d-and l-aspartic acid. Mater Sci Eng C 33:4071–4080
4. Burke L, Deakin V (2015) Clinical sports nutrition, 5th edn. McGraw-Hill
5. Martens DA, Frankenberger WT (1992) Pulsed amperometric detection of amino acids separated by anion exchange chromatography. J Liq Chromatogr 15:423–439
6. Simionato AVC, Moraes EP, Carrilho E, Tavares MFM, Kenndler E (2008) Determination of amino acids by capillary electrophoresis-electrospray ionization-mass spectrometry: An evaluation of different protein hydrolysis procedures. Electrophoresis 29:2051–2058
7. Gu L, Jones AD, Last RL (2007) LC− MS/MS assay for protein amino acids and metabolically related compounds for large-scale screening of metabolic phenotypes. Anal Chem 79:8067–8075
8. Zhang X, Yang L, Mester Z (2012) Determination of amino acids in selenium-enriched yeast by gas chromatography–mass spectrometry after microwave assisted hydrolysis. Anal Chim Acta 744:54–59
9. Lee SH, Jeon CW, Wabaidur SM (2008) Flow-injection chemiluminescence determination of aspartic acid in tea leaves using tris (2, 2′-bipyridyl) ruthenium (II)–Ce (IV) system. J Fluoresc 18:655–660
10. Zhang C, Wan LY, Wu S, Wu D, Qin X, Ko F (2015) A reversible colorimetric chemosensor for naked-eye detection of copper ions using poly (aspartic acid) nanofibrous hydrogel. Dyes Pigments 123:380–385
11. Ma R, Zhou PJ, Zhan HJ, Chen C, He YN (2013) Optimization of microwave-assisted synthesis of high-quality ZnSe/ZnS core/shell quantum dots using response surface methodology. Opt Commun 291:476–481
12. Danish R, Ahmed F, Koo BH (2014) Rapid synthesis of high surface area anatase titanium oxide quantum dots. Ceram Int 40: 12675–12680
13. Chen D, Huang F, Cheng YB, Caruso RA (2009) Mesoporous anatase TiO_2 beads with high surface areas and controllable pore sizes: a superior candidate for high-performance dye-sensitized solar cells. Adv Mater 21:2206–2210
14. Xuan T, Wang X, Zhu G, Li H, Pan L, Sun Z (2013) One-step microwave-assisted synthesis of water soluble CdSe quantum dots for white light-emitting diodes with excellent color rendering. J Alloys Compd 558:105–108
15. Chen S, Tian J, Jiang Y, Zhao Y, Zhang J, Zhao S (2013) A one-step selective fluorescence turn-on detection of cysteine and homocysteine based on a facile CdTe/CdS quantum dots–phenanthroline system. Anal Chim Acta 787:181–188
16. Brabcová I, Hlaváčková M, Šatínský D, Solich P (2013) A rapid HPLC column switching method for sample preparation and determination of β-carotene in food supplements. Food Chem 141: 1433–1437
17. López-García M, García MSD, Vilariño JML, Rodríguez MVG (2016) MALDI-TOF to compare polysaccharide profiles from commercial health supplements of different mushroom species. Food Chem 199:597–604
18. Nasomphan W, Tangboriboonrat P, Tanapongpipat S, Smanmoo S (2017) Selective fluorescent detection of aspartic acid and glutamic acid employing dansyl hydrazine dextran conjugate. J Fluoresc 24: 7–11
19. Zhang Y, Cao J, Ding L (2017) Fluorescent ensemble based on dansyl derivative/SDS assemblies as selective sensor for Asp and Glu in aqueous solution. J Photoch Photobio A 333:56–62

20. Das S, Guha S, Banerjee A, Lohar S, Sahana A, Das D (2011) 2-(2-Pyridyl) benzimidazole based co(ii) complex as an efficient fluorescent probe for trace level determination of aspartic and glutamic acid in aqueous solution: a displacement approach. Org Biomol Chem 9:7097–7104
21. Guan H, Zhou P, Zhou X, He Z (2008) Sensitive and selective detection of aspartic acid and glutamic acid based on polythiophene–gold nanoparticles composite. Talanta 77:319–324
22. Zhao X, Wei R, Chen L, Jin D, Yan X (2014) Glucosamine modified near-infrared cyanine as a sensitive colorimetric fluorescent chemosensor for aspartic and glutamic acid and its applications. New J Chem 38:4791–4798
23. Pathak RK, Dessingou J, Rao CP (2012) Multiple sensor array of Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}complexes of a triazole linked imino-phenol based calix[4] arene conjugate for the selective recognition of Asp, Glu, Cys, and His. Anal Chem 84:8294–8300