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Design and Developed Novel and Ultrasensitive Chemosensor for Sequential Determination of Arginine and Glutathione Amino Acids

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Abstract
In this study, a novel mixed ligand (Dithizone-Co(II)-Alizarin red S: DTZ-Co-ALR-) ensemble is designed and developed ultra-sensitive, and highly selective to UV-Vis absorption and for naked-eye detection of Arginine (Arg), and Glutathione (GSH). The out coming high sensitivity and selectivity for new receptor (DTZ-Co(II)-ALRs-Arg) was attained by adding Glutathione. The indicator is released due to its displacement from the mixed ligand (DTZ-Co-ALRs-Arg) by Glutathione and the change in absorbance may be due to the further complexation of GSH with the additional coordination sites present in the Arg bonded with the mixed ligand (DTZ-Co-ALRs). The label-free DTZ-Co-ALRs receptor provided sensitive and selective detection of L-Arginine, and Glutathione with detection limits of 0.03, and 0.009µmol L-1, respectively. The protocol especially offers high selectivity for the determination of Arg, and GSH among amino acids found in real samples. Moreover, the investigation of the logic behavior of the proposed DTZ-Co-ALRs receptor indicated its capability of functioning as an INHIBIT-type colorimetric chemosensor with the chemical inputs and UV-Vis absorbance signal as the output. This mixed ligand receptor could also behave as a molecular "keypad lock" with the correct sequential addition of Arg and GSH inputs.

Keywords
Colorimetric chemosensor; Mixed ligand ensemble; L-Arginine; Glutathione; Keypad lock.

1.INTRODUCTION

Nowadays, the design of molecular sensors for selective recognition of various analytes is an area of growing interest due to their important role in a wide range of environmental, industrial, and biological applications 1, 2. One of these analytes is glutathione. Glutathione (GSH) (composed of three AAs- cysteine, glycine, and glutamic acid) is a detoxifier and antioxidant and can be found in virtually every cell of the human body [3]. Glutathione is the most abundant non-protein thiol in mammalian cells and plays an important role in maintaining redox homeostasis inside cells [4-6]. GSH plays some roles in the nervous system, including free radical scavenger, redox modulator of ionotropic receptor activity, and neurotransmitter [7]. Glutathione exists in reduced (GSH) and oxidized (GSSG); forms in cells and tissues, and the concentration of glutathione ranges from 0.5 to 10 mmol L^{-1} in animal cells. Its reduced form exists in healthy cells. A critical indicator of the health of the cell is the GSH/GSSG ratio. For healthy human, glutathione concentration usually ranges from 0.684 to 2.525 mmol L^{-1} in blood and from 2.22 to 11.36 µmol L^{-1} in plasma [8].

When oxidative stress occurs, GSH levels decrease and GSSG levels increase. GSH anti-oxidant effect cause its use as a potential therapeutic agent for several diseases [9, 10]. Glutathione is synthesized in both chloroplast and the cytosol and exists in both subcellular compartments at relatively high levels. An integral part of the reply of glutathione metabolism to oxidative stress becomes clear to contain an increase in the levels of the reduced form of the molecule [11]. Many kinds of detection assays have been used to quantify GSH such as high-performance liquid chromatography (HPLC) [12, 13], electrochemistry [14], colorimetry [15], and fluorescence [16]. Nonetheless, the selective detection of GSH over Cys and homocysteine (hCys) is difficult due to their alike thiol groups and structure, which can interfere with GSH detection. Lately, several assays have been suggested for the distinction of GSH from Cys/ hCys. Hu et al. designed a fluorescent hybrid probe with cyanine and [17] nitrobenzofurazan that replies to GSH and Cys/ hCys with separated emissions [16]. Notable endeavors have been also allocated to construct colorimetric and fluorescent probes for intracellular thiols [18-22]. These probes are usually designed based on various

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specific reactions between the probe and thiols, such as Michael addition [23], cleavage of GSH-sensitive bonds (e.g., sulfonamide and sulfonate ester [24], selenium-nitrogen bonds [25, 26], disulfide bonds [27, 28]), metal complex-displacement coordination [29], and metal complex oxidation-reduction [30]. However, these presented probes sometimes travail from the restrictions of high background fluorescence [31, 32], bad selective performance [33], or working in high pH aqueous solutions [34]. Thus, there is an enormous interest to develop a thiol probe with more desirable features.

The cooperative interaction of mixed organic functions with one metal ion has been widely explored in designing coordination networks with desirable applications [35–39]. However, a synergistic combination of simple receptors that are capable of detecting specific AAs with distinct selectivity and sensitivity has been rarely investigated. So far, our group has studied colorimetric and fluorescent sequential recognition of metal ions and anions or AAs [40-42] (in most colorimetric methods, one ligand alone or a ligand-cation complex is used to detect AAs).

Dithizone [3-hydroxy-4-(2-hydroxy-5-methyl phenyl-azo)-1-naphthalene- sulfonic acid] is a water-soluble dye and a chelating agent which is widely used as an indicator in complexometric and photometric methods. It reacts with transition metal ions and alkaline and alkaline earth metals to form stable complexes. The color of the Dithizone solution depends on the pH. It forms a red solution at pH below 7, blue solution at pH 9.1-11.4, and reddish-brown solution at pH above 13 [43]. Rhodamine is also an ideal platform for the development of colorimetric and fluorescent probes owing to its excellent photophysical properties [44, 45]. To date, a great deal of small molecule rhodamine derivatives has been synthesized to detect various metal ions due to their excellent spectroscopic properties [46-48] and vivid color-change development during the sensing procedure.

In this contribution, we introduce a colorimetric chemosensor based on two commercially available ligands: Dithizone (Diphenylthiocarbazone, 1,5-Diphenylthiocarbazone) (DTZ) and Alizarin Red S (3,4-Dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-sulfonic acid) (ALRs), for the sensitive and selective distinction of L-Arginine and Glutathione in 90% water media. Thus we applied a novel mixed ligand (DTZ-Co-ALRs) ensemble, without any further modification, for selective and sensitive determination of two analytes L-Arginine, and Glutathione. For this goal, the colors and UV-Vis spectra of the mixed ligand (DTZ-Co-ALRs) solution were being surveyed upon the addition of L-Arginine, and GSH. The interaction between the (DTZ-Co-ALRs)–Lys receptor and glutathione was via the indicator displacement assay approach. The color of the receptor solutions changed as a result of these interactions and the color-changes could be seen with naked eyes. This was while the (DTZ-Co-ALRs)–Arg receptor and glutathione were detectable with a naked eye. Furthermore, due to the great tendency of GSH toward oxidation in neutral form as well as basic pHs which necessitates GSH protection out of oxidation and also to achieve the most accurate and precise determination, all the procedures were performed in a pH as much physiologic as possible (pH = 7.0). Therefore, the proposed method is facile for no educated staff and it provides a cheap and rapid detection for quantification of L-Arginine, and Glutathione in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v).

2.EXPERIMENTAL

2.1.Materials and equipment

Solvents and reagents with analytical grades were obtained commercially and used for the preparation of solutions. : Dithizone (Diphenylthiocarbazone, 1,5-Diphenylthiocarbazone) (DTZ) and Alizarin Red S (3,4-Dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-sulfonic acid) (ALRs) was purchased from Merck and used as received, and amino acids including L-Arginine(Arg), L-Histidine (His), L-Lysine (Lys), L-Aspartic acid (Asp), L- Glutamic acid (Glu), L-Serine (Ser), L-Threonine (Thr), L-Glutamine (Gln), L-Cysteine (Cys), L-Glycine (Gly), L-Alanine (Ala), L-Valine (Val), L-Isoleucine (Ile), L-Leucine (Leu), L-Methionine (Met), L-Phenylalanine (Phe), L-Tyrosine (Tyr), L-Tryptophan (Trp), L-Proline (pro), Glutathione (GSH), Glutathione disulfide (GSSG) and dipeptides gamma-glutamylcysteine (gamma-Glu-Cys) and cysteinyl glycine (Cys-Gly) were purchased from Merck with analytical grade. Stock solutions (1.0×10^{-1} mol L^{-1}) of amino acids were prepared by direct dissolution of proper amounts of the relevant amino acid in deionized water. The standard working solutions were prepared daily by serial dilutions with doubly distilled deionized water from stock solutions. The UV-Vis spectroscopic measurement was carried out in the DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v). HPLC-grade reagents were purchased from Mallinckrodt Baker Inc. FDNB, iodoacetic acid (IA), N-ethylmaleimide (NEM), *tert*-butyl hydroperoxide (t-BOOH), and trichloroacetic acid (TCA) were obtained from Sigma-Aldrich Corp. Reversed-phase HPLC column Sephasil C_{18} was purchased from Pharmacia (Uppsala, Sweden).

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Perkin Elmer Lambda2 Spectrophotometer was used to record UV-Vis spectra in the wavelength range of 200 to 800 nm with a quartz cuvette (path length = 1 cm). Bruker Vector 22 Fourier Transmission Infrared spectrometer was applied for FT-IR spectra in the range of 400-4000 cm^{-1}. A Jenway 3510 pH-meter calibrated with two standard buffer solutions at pH 4.0 and 10.0 was used to measure the pH of the solutions. A Hamilton syringe (10 µL) was exploited to deliver small volumes of the reagent into the cell. HPLC separation was performed using a Bio-Tek Instruments KromaSystem 2000 apparatus (Bio-Tek Instruments, Milan, Italy). Detection was performed with a UV detector set (Bio-Tek Instruments) at 355 nm wavelength. Biosil NH_2 HPLC column was purchased from Bio-Rad Laboratories (Hercules, CA, USA).

2.2.UV-Vis titration

The 1.0×10^{-1} mol L^{-1} stock solution of Dithizone was diluted in several steps by the solvent solution, DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v), to make the final concentration of 5.0×10^{-5} mol L^{-1}. 2.5 mL of the prepared solution was transferred to the UV-Vis cuvette and titrated with 1.0×10^{-4} mol L^{-1} Alizarin Red S solution. The titration was continued by the addition of 1.0×10^{-2} mol L^{-1} of Arginine solution and Glutathione (GSH) solution respectively.

2.3. pH optimization

2.5 mL of 5.0×10^{-5} mol L^{-1} Dithizone in DMSO/10 mmol L^{-1} buffer solution, (1:9 v/v) was transferred to vials and 150 µL of auxiliary ligand, Alizarin Red S 1.0×10^{-4} mol L^{-1}, was added to each vial. The pH values were subsequently adjusted in a range from 2 to 12 by the addition of the buffers were: pH 3.0–4.0, CH_3COOH/NaOH; pH 4.5–7.0, MES/NaOH; pH 7.0–10.0, HEPES; pH 10.0–11.4, CABS. Upon achieving the solutions with desired pH values, 39.0 µL of 1.0×10^{-2} mol L^{-1} Arginine solution was transferred to each cell and after mixing for a few seconds, UV-Vis spectrum was recorded at room temperature. Thereafter, 5.0×10^{-5} mol $L^{-1}$of GSH solution was added to each cuvette and the UV-Vis spectra were taken.

2.4. Interference experiment

The following competition test was conducted separately for Arginine and Lysine solutions (1.0×10^{-2} mol L^{-1}) to investigate the effect of the presence of other amino acids on the UV-Vis spectra.

To a UV-Vis cell, 2.5 mL of 5.0×10^{-5} mol L^{-1} Dithizone in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v) and 150 µL of 1.0×10^{-4} mol L^{-1} Alizarin Red S were transferred and the spectrum was recorded. Afterward, 75.0 µL of 1.0×10^{-2} mol L^{-1}solutions of various amino acids including His, Arg, Pro, Cys, hCys, Val, His, Lys, Thr, Leu, Met, Ala, Ser, Glu, Ile, Leu, Orn, Asp, Tyr, Gly, Asn, Trp, Phe, Lys, and GSH were prepared and proper amount of each amino acid solution was added to the cell and the UV-Vis spectra were recorded. In the end, 1.0×10^{-4} mol L^{-1}, 5.0×10^{-5} mol L^{-1} of Arg was added to the cuvette respectively and the UV-Vis spectrum was taken.

2.5. Preparation of pharmaceutical samples

Two Arg dietary supplement products were purchased from a pharmacy. Product A was a capsule containing 500 mg L-arginine (acid free) as active ingredient. Product B was a supplement tablet containing 453 mg L-arginine hydrochloride plus glucosamine hydrochloride, vitamins C and B12, niacin, and boron. 2.0 g of capsule and tablet were ground in a mortar to achieve a smooth powder and then each of them was transferred to a separate 100 mL volumetric flask and dissolved in C_2H_5OH/H_2O media (7:3 v/v) to prepare stock solutions. The flasks were placed on the stirrer for a few minutes to dissolve the particles completely. Each solution was diluted to the desired and required volume, then shaken and used for analysis.

2.6. Reference method for arginine determination

After preparation, the sample solution was diluted 100-fold and 5.00 mL of it was transferred to a 100 mL Erlenmeyer flask and 15.0 mL of redistilled water was added. The solution titration was performed by perchloric acid ($HClO_4$, 11.1 mmol L^{-1}) which was calibrated with potassium hydrogen phthalate. The pH record was done for each 0.05 mL, and the graphing method was used for the determination of the titration endpoint [49].

2.7. Subjects of GSH determination study

This study includes two groups, i.e., diabetic (40-78 y) women, and nondiabetic (38-75 y) women. All study subjects were self-identified as Iranians. They were recruited from Namazi Hospital at Shiraz or from numerous health fairs affiliated. Immediately before the start of the study, a medical basal screening was performed by a physician. The 10 diabetic women participating in this study had suffered diabetes mellitus for 7-16 years and were receiving antidiabetic therapy. Patients whose fasting plasma glucose was higher than 130 mg/dL were excluded. The fasting plasma glucose level of the individuals in the nondiabetic young and old control groups was lower than 100 mg/dL. No participants were taking glutathione, which might affect cellular glutathione levels. Fully informed consent was obtained from all subjects. This study was approved by the Institutional Review Board of Shiraz Medical College.

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2.8. Preservation solution for blood sampling

Capillary blood was drawn by finger puncture with a sterile lancet and collected into 300µL capillary vials containing sodium heparin as an anticoagulant. Optimal sample processing procedures were utilized as determined previously for GSH and Cys [50- 52]. A solution of 100 mmol L^{-1} serine borate (pH 8.5) including 0.5 mg sodium heparin per mL, 1 mg bathophenanthroline disulfonate sodium salt (BPDS), and 2.0 mg iodoacetic acid is made using stock solutions of 100.0 mmol L^{-1} boric acid and 100 mmol L^{-1} sodium tetraborate. It is made by mixing 8 mL of a 100 mmol L^{-1} boric acid stock (0.62 g/100 mL), 2.0 mL of 100 mmol L^{-1} tetraborate stock (3.81 g/100 mL), 105 mg L-serine, 5 mg sodium heparin, 10 mg BPDS and 20 mg iodoacetic acid. This solution is used to prepare tubes, which are used for blood collection. These are clear 2.0 mL graduated microcentrifuge tubes to which 0.5 mL of the preservation solution has been added and 1.5 mL of the prepared blood sample has been added to these tubes, then the mixture is centrifuged to precipitate the proteins. These tubes can be stored at -80 °C for six months without adversely changing the assay [53].

2.5 mL solution of [DTZ-Co-ALs-Arg] was prepared in situ by simply mixing 5.0×10^{-5} mol L^{-1} Dithizone, 5.0×10^{-5} mol L^{-1} Co(II) in DMSO/10 mmol L^{-1} and 5.0×10^{-5} mol L^{-1} Alizarin Red S in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v), 5.0×10^{-5} mol L^{-1} of Arg, and the resulting solution of [DTZ-Co-ALRs-Arg] were transferred and the spectrum was recorded. Afterward, 25 µL solutions of blood samples were prepared above in the presence of 1.0×10^{-2} mol L^{-1} Cys-Gly and γ-Glu-Cys added to the cell, then this solution was delivered to volume of 2.5 mL by DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v), and the UV-Vis spectra were recorded.

2.9. GSH assay by HPLC method

2.9.1. Sample preparation

The required materials and solutions for HPLC determination assay of GSH as is reported by Tipple and coworkers [54] including buffers, stock solutions, solvents and HPLC mobile phases are summarized in Table 1. Firstly, Standards are made in concentrations from 10 to 40 nmol per assay for GSH dissolved in sample buffer. The thiols are unstable to freeze-thaw and should be made fresh each time. The standards are derivatized using the same methods as the experimental samples. Plasma samples are drawn into plasma buffer (Table 1, No.1), mixed thoroughly, and centrifuged to separate plasma from cells. Once separated, 200 mL of the plasma/buffer mixture is added to 200 mL of the sample buffer (Table 1, No.2) and mixed. After 5 min, the mixture is centrifuged to precipitate the proteins and the plasma supernatant may be frozen at -80°C for future analysis.

Table 1. Materials and solutions for HPLC assay [47]

Name	Composition/Preparation
Plasma buffer solution	8 mL 100 mmol L^{-1} H_3BO_3, 2 mL of 100 mmol L^{-1} $Na_2B_4O_7.10H_2O$, 105 mg L-serine, 5 mg sodium heparin, 10 mg BPDS and 20 mg IAA
Sample buffer solution	71 mL of 70% $HClO_4$, 6.2 g H_3BO_3 and 1.38 mg γ-glu-gly are dissolved in a total volume 500 mL of double distilled H_2O
Iodoacetic acid (IAA)	14.8 mg IAA in 2 mL distilled H_2O
KOH/tetraborate	Add 5.6 g KOH to 5 g $K_2B_4O_7{\cdot}4H_2O$ in 100 mL H_2O, let stand overnight and remove the supernatant solution.
Dansyl chloride	Pure lab. Grade dansyl chloride
Chloroform	Pure lab. Grade $CHCl_3$
Acetate stock	272 g of sodium acetate trihydrate, 122 mL H_2O, 378 mL glacial acetic acid
Mobile phase A	80% MeOH, 20% H_2O
Mobile phase B	640 mL MeOH, 200 mL acetate stock,125 mL glacial acetic acid, 50 mL H_2O

2.9.2. Derivatization of samples

Mix 300 mL of plasma, cell or homogenate supernatant with 60 mL of IAA (Table 1, No. 3). Adjust the pH to 9.0 ± 0.2 with KOH/tetraborate (~220 mL, Table 1, No. 4) and incubate the sample at room temperature for 20 min. Add 300 ml of dansyl chloride (Table 1, No.5), mix the solution, and place it in the dark for 16–24 h. Add 500 mL of $CHCl_3$ to the solution, mix, centrifuge briefly and remove the upper layer containing the derivatized sample. This process removes excess dansyl chloride from the sample. The sample can be stored at -80°C with the $CHCl_3$ layer for up to 12 months.

2.9.3. HPLC elution

Firstly Centrifuge samples for 2 min prior to loading into either autosampler vials or manually injecting into the HPLC. The typical injection volume is 25–35 µL of prepared sample. The column are first eluted with the mixture of 80% A and 20% B at 1 mL/min, hold these conditions for 10 min, then a linear gradient elution is performed upto 20% A, 80% B for 10–30 min, Hold at final

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conditions for 15 min, Return to initial conditions for column reequilibration, at least 15 min.

2.9.4. Detection and Quantification

Peaks are detected by fluorescence using an excitation wavelength of 328 nm and an emission wavelength of 541 nm. Sample concentrations are determined by experimentally derived standard curves [54].

3.RESULTS AND DISCUSSION

3.1.Chemosensor for arginine and glutathione

For this purpose, 1.0×10^{-4} mol L^{-1} solutions of various indicators such as Co(II), was prepared and added into the solution of 5.0×10^{-5} mol L^{-1} Dithizone in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v). After mixing for a short while, the subsequent titration experiments with 1.0×10^{-4} mol L^{-1} Co(II) solution and Job plot were performed and the spectra were recorded at 471 nm (Fig. 1 a and b) where the relationship asserted the 1:1 stoichiometry between the receptor (DTZ) and the analyte (Co(II)).

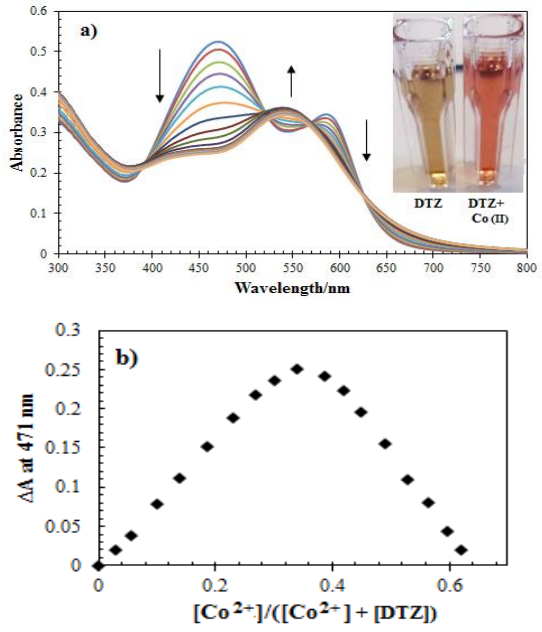

Fig. 1 a) UV–Vis spectra of 5.0×10^{-5} mol L^{-1} Dithizone in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v), to afford upon gradual addition of Co(II) (1.0×10^{-5}-2.0×10^{-3} mol L^{-1}), **b)** The stoichiometry analysis of Co(II) complex by Job plot analysis (λ_{max}=471 nm).

In order to investigate the selectivity in the presence of an auxiliary ligand, 5.0×10^{-5} mol L^{-1} Dithizone, 5.0×10^{-5} mol L^{-1} Co(II) in DMSO/10 mmol L^{-1} in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v) was titrated with 1.0×10^{-4} mol L^{-1} Alizarin Red S in two cuvettes, and the spectra were recorded. As can be seen in Fig 3, the characteristic band of Dithizone in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v) between 500-600 nm turned into a sharp peak at 556.5 nm in the presence of 5.0×10^{-5} mol L^{-1} Alizarin Red S (Fig. 2a).

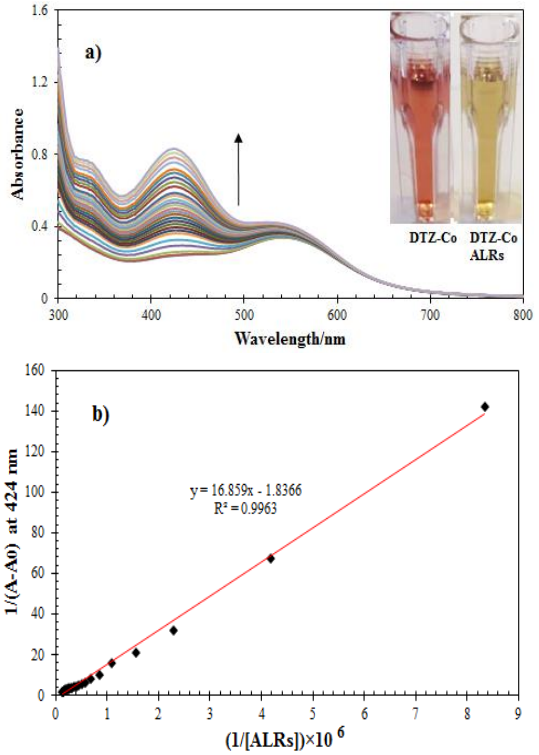

Fig. 2. a) UV–Vis spectra of 5.0×10^{-5} mol L^{-1} Dithizone and 5.0×10^{-5} mol L^{-1} Co(II) in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v), to afford upon gradual addition of ALRs (1.0×10^{-8}-1.8×10^{-3} mol L^{-1}), right inset: corresponding color change of DTZ-Co-ALRs upon addition of 39.0 µL of 1.0×10^{-2} mol L^{-1} ALRs (left to right) **b)** Benesi-Hildebrand plot of DTZ-Co solution(solution (2.5 mL, DTZ 5.0×10^{-5} mol L^{-1} and Co(II) 5.0×10^{-5} mol L^{-1} in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v) the variation of $1/(A–A_o)$ at 424 nm versus the function of 1/[ALRs] based on 1:1 binding stoichiometry with ALRs,

The calculations were carried out using the Benesi-Hildebrand equation [55] (eq. 1) where A_o and A indicate the absorbance of the receptor in the absence and in the presence of the analyte, respectively. A_{max} is the saturated absorbance of the receptor which means the absorbance intensity when an excess amount of analyte is added. Considering the wavelength of 424 nm as the λ_{max} of ALRs analysis, $1/(A-A_0)$ was plotted against 1/[ALRs] where the linear relationship asserted the 1:1 stoichiometry between the receptor and the analyte (Fig. 2b)

$$\frac{1}{(A-A_0)}=\frac{1}{(A_{max}-A_0)}\left[\frac{1}{K_{ass}[ALRs]^n}+1\right]$$

Eq. 1. The Benesi-Hildebrand equation

Dividing the intercept of each equation to its slope gave us the association constants, K_{ass}, of 1.09×10^5 mol^{-1} L for ALRs.

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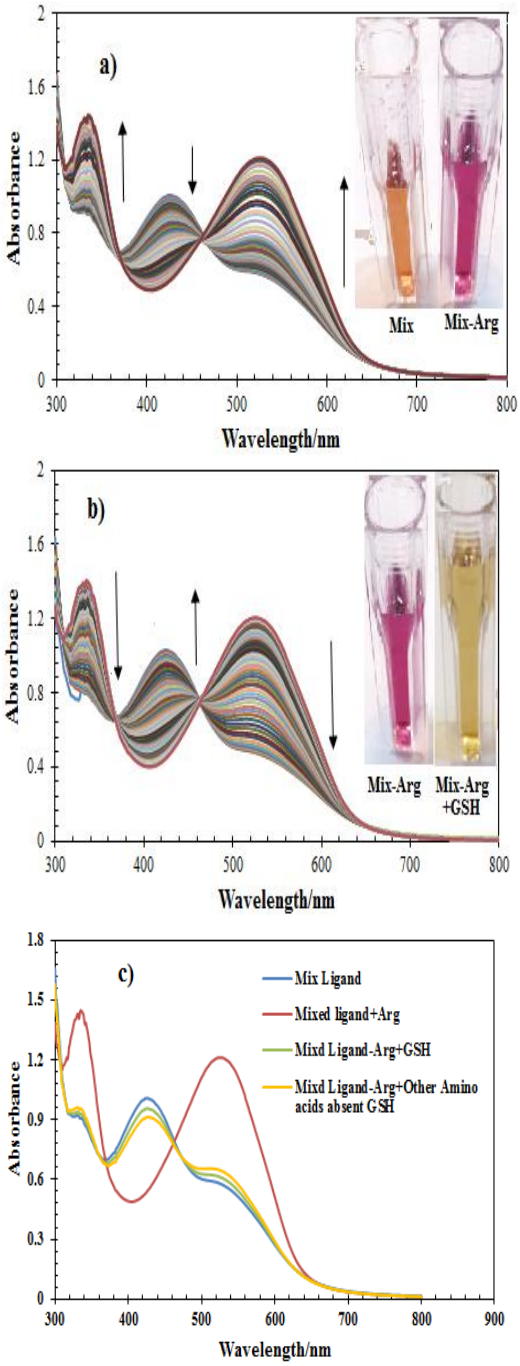

Fig. 3. a) UV–Vis spectra of 5.0×10^{-5} mol L^{-1} Dithizone, 5.0×10^{-5} mol L^{-1} Co(II) in DMSO/10 mmol L^{-1} and 5.0×10^{-5} mol L^{-1} Alizarin Red S in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v), to afford upon gradual addition of Arg (1.0×10^{-8}-2.3×10^{-3} mol L^{-1}, right inset: corresponding color change of DTZ-Co-ALRs upon addition of 5.0×10^{-5} mol L^{-1} Arg (left to right). **b)** UV–Vis spectra of 5.0×10^{-5} mol L^{-1} Dithizone, 5.0×10^{-5} mol L^{-1} Co(II) in DMSO/10 mmol L^{-1} and 5.0×10^{-5} mol L^{-1} Alizarin Red S in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v), and 5.0×10^{-5} mol L^{-1} Arg to afford upon gradual addition of GSH (1.0×10^{-9}-1.7×10^{-3} mol L^{-1}. **c)** Reversibility UV-Vis experiment and color changes of DTZ-Co-ALRs after the sequential addition of Arg (5.0×10^{-5} mol L^{-1}) and GSH (5.0×10^{-5} mol L^{-1}).

In order to investigate the selectivity in the presence of aminoacids, UV–Vis spectra of 5.0×10^{-5} mol L^{-1} Dithizone, 5.0×10^{-5} mol L^{-1} Co(II) in DMSO/10 mmol L^{-1} and 5.0×10^{-5} mol L^{-1} Alizarin Red S in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v), to afford upon gradual addition of Arg (1.0×10^{-8}-2.3×10^{-3} mol L^{-1}, was titrated with 1.0×10^{-3} mol L^{-1} aginine in two cuvettes, and the spectra were recorded. As can be seen in Fig 3, the characteristic band of DTZ-Co-ALRs (5.0×10^{-5} mol L^{-1} Dithizone, 5.0×10^{-5} mol L^{-1} Co(II) in DMSO/10 mmol L^{-1} and 5.0×10^{-5} mol L^{-1} Alizarin Red S in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v)) between 500-600 nm turned into a sharp peak at 527 nm in the presence of 1.0×10^{-4} mol L^{-1} Arg (Fig. 3a). UV–Vis spectra of DTZ_Co-ALRs-Arg (5.0×10^{-5} mol L^{-1} Dithizone, 5.0×10^{-5} mol L^{-1} Co(II) in DMSO/10 mmol L^{-1} and 5.0×10^{-5} mol L^{-1} Alizarin Red S in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v), and 5.0×10^{-5} mol L^{-1} Arg) to afford upon gradual addition of GSH (1.0×10^{-7}-1.7×10^{-3} mol L^{-1} (Fig. 3b and c).

The calculations were carried out using the Benesi-Hildebrand equation [55] (eq. 2) where A_o and A indicate the absorbance of the receptor in the absence and in the presence of the analyte, respectively. A_{max} is the saturated absorbance of the receptor which means the absorbance intensity when an excess amount of analyte is added. Considering the wavelength of 527 nm as the λ_{max} of Arg analysis, $1/(A-A_0)$ was plotted against 1/[Arg or GSH] where the linear relationship asserted the 1:1 stoichiometry between the receptor and the analyte (Fig. 4a and b)

$$\frac{1}{(A-A_0)} = \frac{1}{(A_{max}-A_0)}\left[\frac{1}{K_{ass}[Arg\ or\ GSH\ ]^n} + 1\right]$$

Eq. 2. The Benesi-Hildebrand equation

Dividing the intercept of each equation to its slope gave us the association constants, K_{ass}, of 4.04×10^4 and 1.67×10^5 mol^{-1} L for Arg and GSH, respectively.

3.2.*pH effect study*

Investigating the practical applicability led to the evaluation of pH effect on the two analyses. As it is indicated in Fig. 5, the addition of Arg, and GSH have the most obvious impact on the absorbance spectra 7.0 pH range. Hence, DTZ-Co-ALRs-Arg and DTZ-Co-ALRs-Arg-GSH is promising for biological applications due to the Arg, and GSH response range (pH= 7.0). As is clear from Fig 5, the optimum pH for GSH determination is 7.0 which is sufficiently acidic to protect GSH through inhibition of oxidation.

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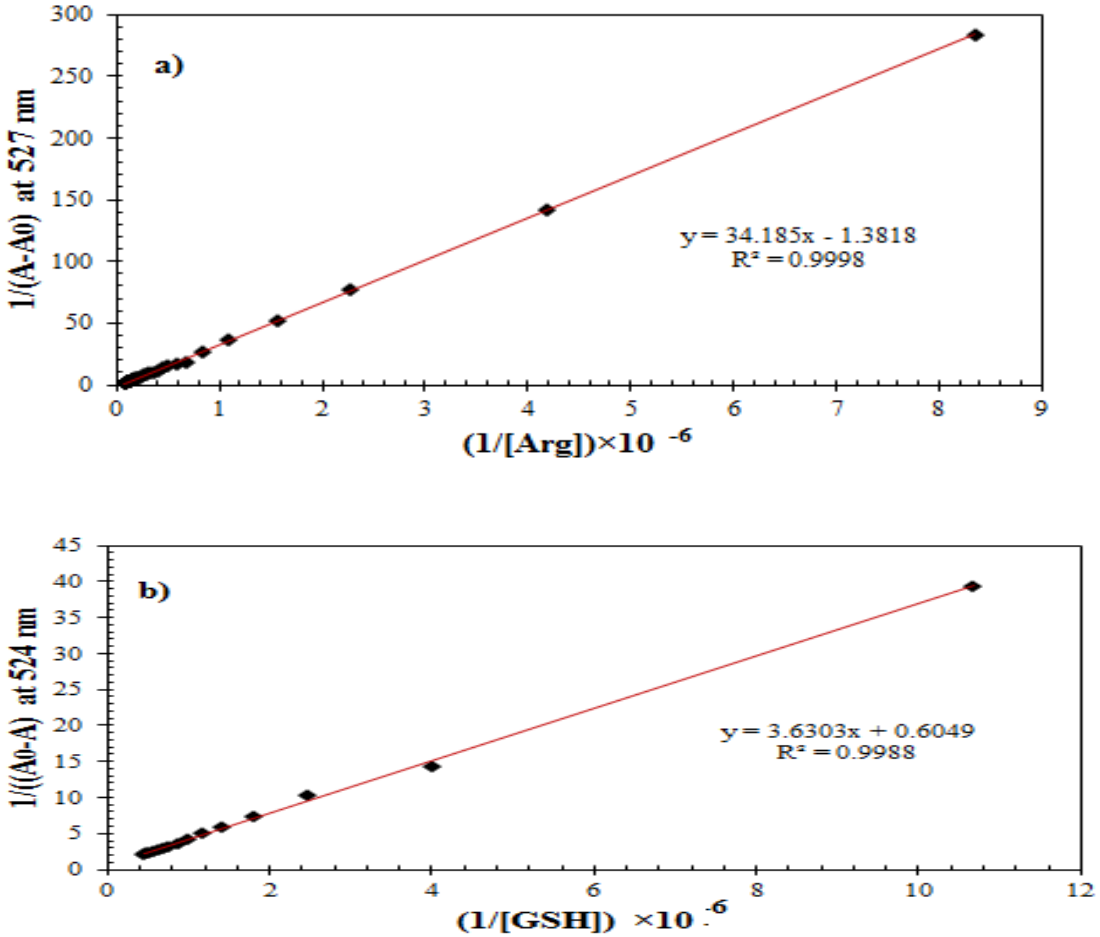

Fig. 4. Benesi-Hildebrand plot of DTZ-Co-ALRs solution (2.5 mL, 5.0×10^{-5} mol L^{-1} Dithizone, 5.0×10^{-5} mol L^{-1} Co(II) in DMSO/10 mmol L^{-1} and 5.0×10^{-5} mol L^{-1} Alizarin Red S in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v)) **a)** the variation of $1/(A-A_o)$ at 527 nm versus the function of 1/[Arg] based on 1:1 binding stoichiometry with Arg, **b)** DTZ-Co-ALRs-Arg solution complex the variation of $1/(A_o-A)$ at 524 nm versus the function of 1/[GSH] based on 1:1 binding stoichiometry with GSH

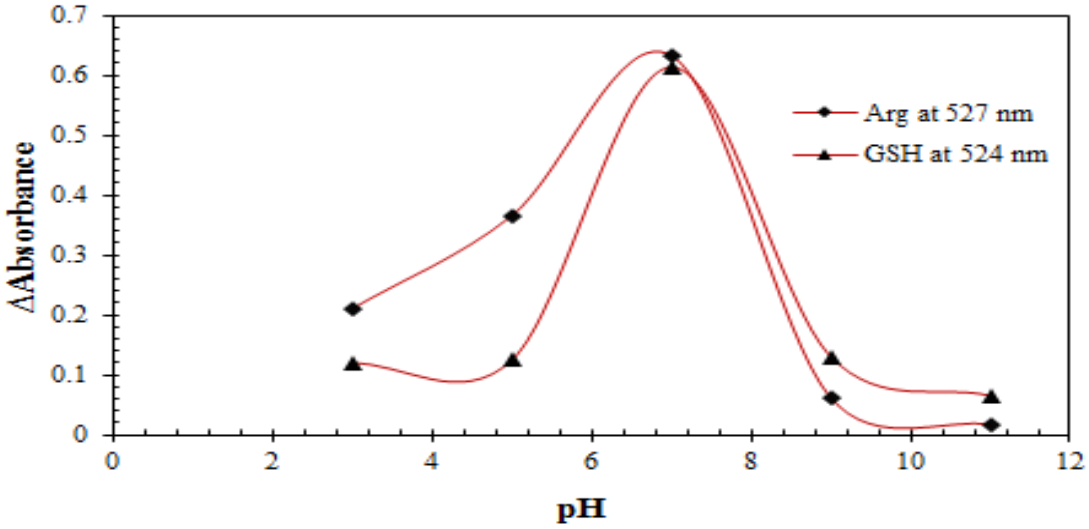

Fig. 5. Effect of the pH on the absorbance changes of DTZ-Co-ALRs solution (2.5 mL, 5.0×10^{-5} mol L^{-1} Dithizone, 5.0×10^{-5} mol L^{-1} Co(II) in DMSO/10 mmol L^{-1} and 5.0×10^{-5} mol L^{-1} Alizarin Red S in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v)) in the presence of Arg (39.0 µL of 1.0×10^{-2} mol L^{-1}) at 527 nm and DTZ-Co-ALRs-Arg solution complex in the presence of GSH (5.0×10^{-5} mol L^{-1}) at 524 nm.

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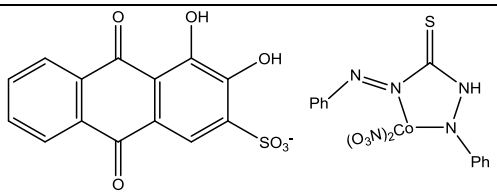
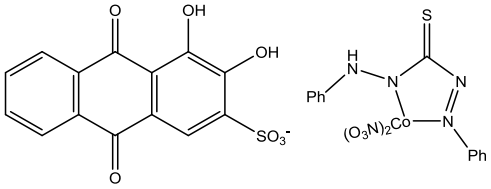
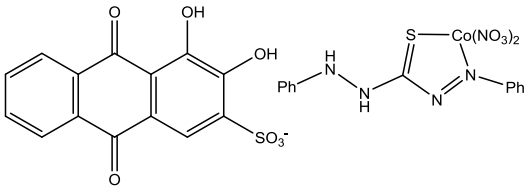
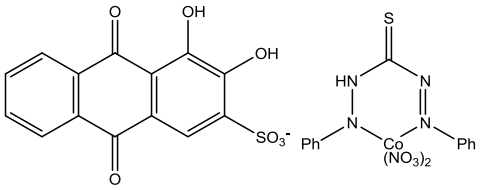
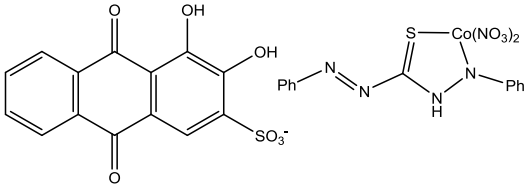
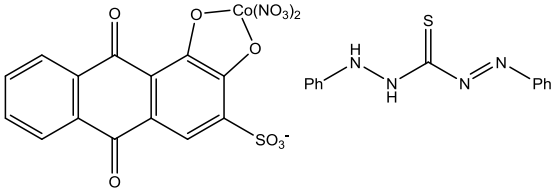
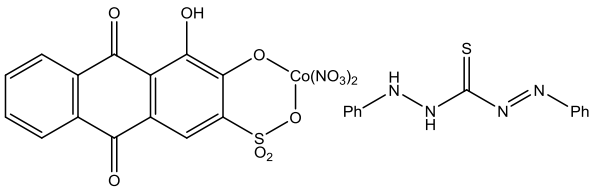
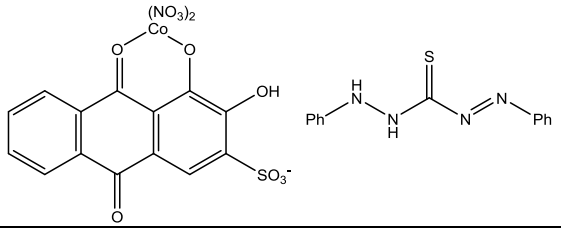
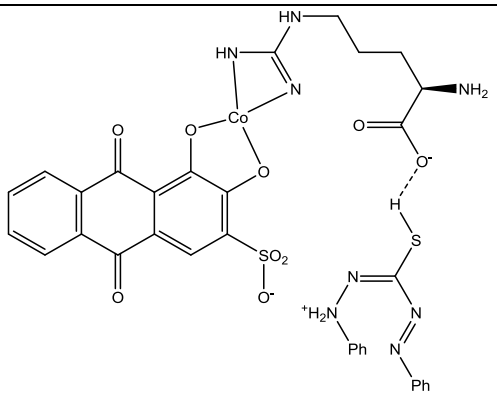
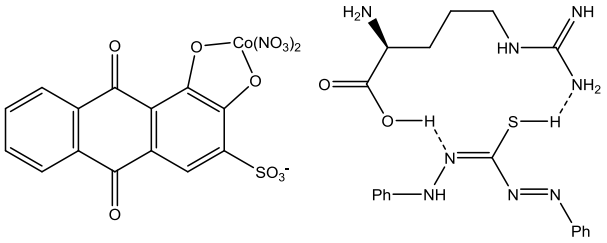
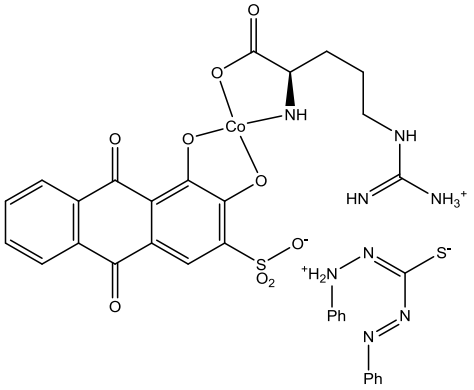
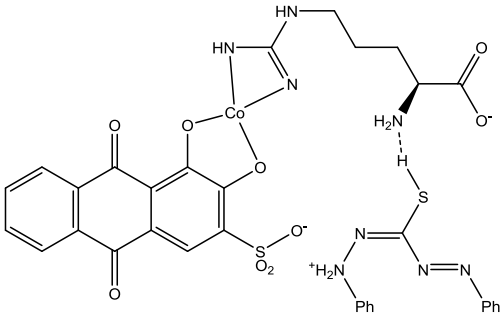
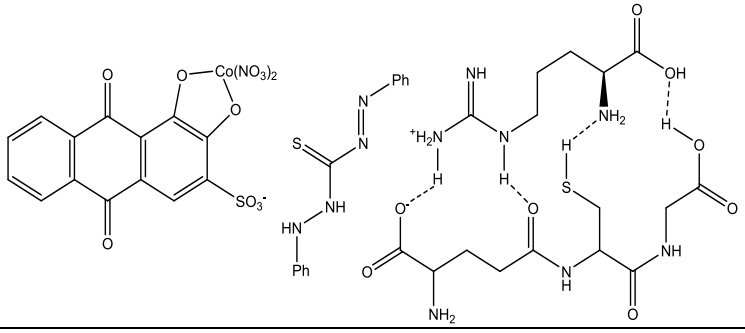
Table 2. Results of MM2 calculations for reactants and products of different steps of the mechanism.

Entry	Structure	Total energy (Kcal/mol)
1		226.7171
2		239.3803
3		298.5559
4		227.4437
5		413.2438
6		203.7614
7		296.0684
8		1166.5005

9		114.2304
10		270.7858
11		303.545
12		527.8247
13		108.6934

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3.3.Interference studies

The addition of 1.56×10^{-4} mol L^{-1} solutions of Arg to the mixture of 5.0×10^{-5} mol L^{-1} Dithizone, 5.0×10^{-5} mol L^{-1} Co(II) in DMSO/10 mmol L^{-1} and 5.0×10^{-5} mol L^{-1} Alizarin Red S in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v), and upon the addition 5.0×10^{-5} mol L^{-1} of Arg, the absorption peak demonstrated a remarkable change and the color of the solution changed from yellow to purple which could be easily observed by the naked eye (Fig. 3).

To examine the selectivity of the mixed ligand receptor in the presence of other amino acids, 1.0×10^{-4} mol L^{-1} solutions of His, Arg, Pro, Cys, hCys, Val, His, Lys, Thr, Leu, Met, Ala, Ser, Glu, Ile, Leu, Orn, Asp, Tyr, Gly, Asn, Trp, Phe,dipeptides Cys-Gly γ-Glu-Cys, and GSSG, and biological species, including K^+, Mg^{2+}, Fe^{3+}, Ca^{2+}, Zn^{2+}, Na^+, glucose, urea, uric acid, and ascorbic acid were prepared and added to the mixture of 5.0×10^{-5} mol L^{-1} Dithizone, 5.0×10^{-5} mol L^{-1} Co(II) in DMSO/10 mmol L^{-1} and 5.0×10^{-5} mol L^{-1} Alizarin Red S in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v) and 5.0×10^{-5} mol L^{-1} Arg and the spectra were recorded after each addition (Fig .3c). The transference of the mentioned amino acid solutions to the ligands mixture had no impact on the color and spectra, except for Glutathione which caused an insignificant spectral variation and a negligible color change to yellow (Fig. 3b).

3.4.Probable structure for receptor and the proposed mechanism of interactions

Following our studies in the development of the colorimetric sensor for the sequential detection of arginine (Arg) and glutathione (GSH), we decided to propose a reasonable mechanism for the process using the MM2 molecular mechanics calculation method (ChemBio3D software package: Perkin-Elmer Company) to determine the role of each component in the process. In the first step, in the preparation of the sensor, we add an equivalent amount of alizarin red s (ALRs) to the dithizone cobalt complex (DTZ-Co). As a result, the color changes to something very similar to DTZ. This event can be a visual evidence of substitution of ALRs instead of DTZ, formation of ALRs-Co and therefore release of DTZ. In order to provide computational evidence for this step and also for every other step, we calculated the energy of all possible structures for the reactants (DTZ-Co+ALRs, Table 2: entries 1-5) and products (ALRs-Co+DTZ, Table 2: entries 6-8), the results of which are displayed in Table 2. Among all these species, the lowest energies are related to entries 1 and 5 equal to 226.7171 and 203.7614 kcal/mol, respectively (Table 2). The data in Table 1 shows that the substitution step of ALRs instead of DTZ is an exothermic and spontaneous thermodynamically favorable reaction (Fig. 6). The second and third steps of the mechanism, respectively, include the sequential increase of Arg and then GSH to the ALRs-Co+DTZ mixture. After adding Arg, the color change is completely different from the previous steps, which indicates the formation of a completely different species. The interaction of Arg can be with DTZ (270.7858 kCal/mol, entry 10) or ALRs-Co (114.2304 kCal/mol, entry 9), which the latter is more favorable due to its lower energy. Finally, in the last step, GSH is added to the mixture and the color change is such that the color of the first step is reproduced again, which means that there are species in the mixture that are completely similar to the first step (ALRs-Co+DTZ, Table 2: entry 6). Since GSH and Arg are completely colorless and therefore do not absorb at all in the visible region, we come to the conclusion that the main interaction is preferably between GSH and Arg, which leads to elimination of Arg from the ALRs-Co-Arg complex and ALRs-Co is again reproduced in the mixture and that's why the color becomes completely similar to the that of the second stage.

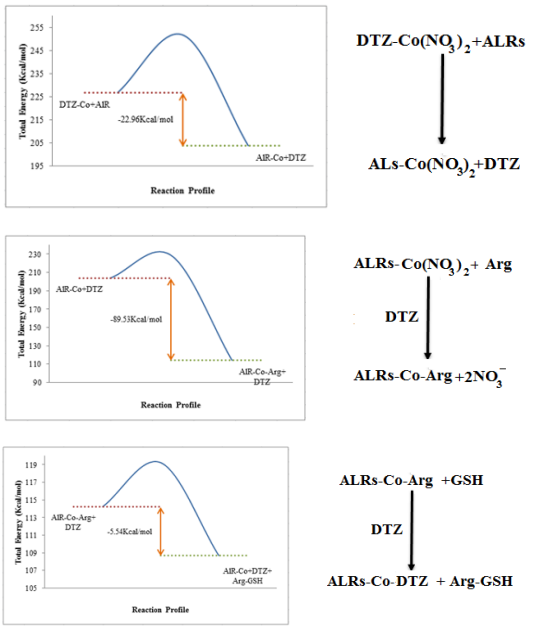

Fig. 6. Proposed mechanism of interactions based on colorimetric evidences and MM2 calculations

It is noteworthy that both the second and third steps are exothermic, spontaneous and so thermodynamically favorable (Fig. 6). Therefore, considering all the evidences, including the color changes and the calculated energies (Table 2), the following general mechanism (Fig. 6) is suggested for the process under study. It should be noted that the role of DTZ, despite the fact that it exists freely in most steps and does not react with other species,

is very essential to the extent that none of the interactions will occur in its absence, and its role is mostly catalytic, or in other words, reduces the activation energy of the process. *Reversibility of the chemosensor*

As known, the reversibility and regeneration of chemosensors are very important factors for sensing the analytes in practical applications. To examine the reversibility of the designed DTZ-Co-ALRs receptor, we used alternate addition of constant concentration of Arg and GSH to the DTZ-Co-ALRs receptor. As shown in Fig. 7 intermittent increases of Arg and GSH into the DTZ-Co-ALRs receptor solution results in the disappearance and appearance of the absorption band at 524 nm.

3.5.Logic gate construction of the proposed colorimetric sensor

Recently, various Boolean logic operations with applications for biosensors and bioimaging have been developed [56]. Here, the proposed probe inspires us to fabricate INHIBIT molecular logic gates so as to prove its practical application in the future. If the significant characteristics are considered on the optical signal response of this DTZ-Co-ALRs receptor, Arg and GSH as two inputs for INHIBIT gate can be employed. The absence and presence of the inputs are respectively defined as "0" and "1".

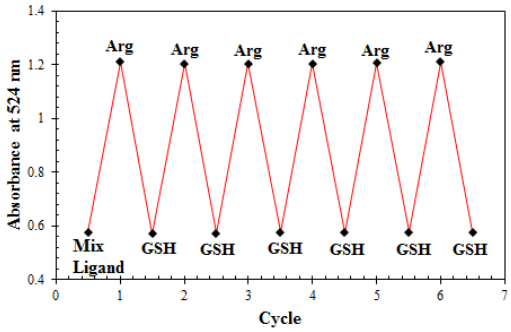

Fig. 7. The reversible and reproducible absorption signal at 524 nm imposed by alternate addition of Arg (5.0×10^{-5} mol L^{-1}) and GSH (5.0×10^{-5} mol L^{-1}) into the DTZ-Co-ALRs solution (2.5 mL, 5.0×10^{-5} mol L^{-1} Dithizone, 5.0×10^{-5} mol L^{-1} Co(II) in DMSO/10 mmol L^{-1} and 5.0×10^{-5} mol L^{-1} Alizarin Red S in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v)) mediums induces reappearance and disappearance of the absorbance band centered at 524 nm.

We focused on the absorption signal output at 524 nm after the addition of Arg and GSH inputs to the DTZ-Co-ALRs receptor. First, the operation of the sensor by Arg and GSH as INHIBIT logic function was expressed. The output was the specific value of A_{524}. The value of 0.01 represented the threshold. Values higher than 0.6 were considered as "1" and lower values as "0". Only the presence of Lys input (1/0) resulted in a higher specific value of A_{524} (>0.6), due to the effective binding between the DTZ-Co-ALRs system and Lys, suggesting the detection of Lys (Fig. 2c). When both inputs were absent (0, 0) or in the presence of either input (1/1, 0/1), the colorimetric system provided output 0. These studies clearly demonstrated an INHIBIT logic gate for these ions 524 nm (Fig. 8).

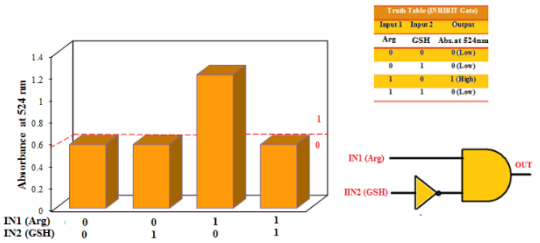

Fig. 8. Illustration of "INHIBIT" logic gate: The histogram of different inputs values based on UV-Vis absorption at 524 nm, and truth table corresponding to the "INHIBIT" logic gate. The complementary INHIBIT logic gate and its truth table INP1 and INP2 represent Input Arg and Input GSH, respectively.

3.6.The system as a keypad lock

Based upon the response of the mixed ligand (DTZ-Co-ALRs) receptor toward Arg and GSH, we further examined the system as a keypad lock and observed the absorption behavior of the mixed ligand (DTZ-Co-ALRs) during the sequential addition of these two inputs. The first input sequence, Arg as input A and excess of GSH (50.0 µL of 1.0×10^{-2} mol L^{-1}) as input B, resulted in the absorbance decreases from 1.21 to approximately 0.6 at 524 nm (Fig. 2c). On reversal of chemical inputs, as GSH input A and Arg as input B, the absorbance value at 524 nm increases from ~0.6 to 1.21(Fig. 3b).

Therefore, the sequence of the addition of Arg and GSH to the solution of DTZ-Co-ALRs results in changes (increase/decrease) in the absorbance value at 524 nm. While the reversal orders of inputs were not produced repeating output (ON). From the above results of absorbance outputs, it is clear that we can construct our system as a security keypad lock. To generate the input sequence as a password and receptor DTZ-Co-ALRs as a keypad lock, inputs Arg and GSH were applied as "A" and "G" respectively (Fig. 9). For the first input sequence, "A" followed by "G" results in absorption "OFF" at 524 nm and created a secret code "AGF" where "F" defines the "OFF" state. On reversal of input sequence, i.e. first input "G" followed by "A", the absorbance was in the "ON" state at 524 nm. So this sequence i.e. "GAT" where "T" defines the "ON" state, is a wrong entry and unable to open the keypad lock. This concludes that a security code "GAT" is developed, which

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one can use to open the absorption lock of DTZ-Co-ALRs at 524 nm.

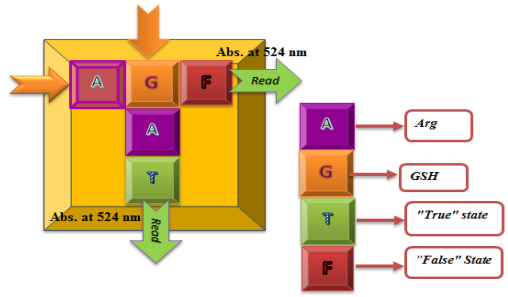

Fig. 9. Absorbance keypad locks with the inputs of Arg and GSH to access a secret code at 524 nm.

3.7.*nalytical characteristics*

The linear range of the quantitative detection for the analytes involved in the assay was determined by plotting the relevant calibration graphs. As demonstrated in Fig. 10a, the absorbance intensity at λ_{max}=527 nm was graphed against the concentration of Arg. With the curve equation of A = 0.048C_{Arg} + 0.5405 and the Coefficient of Determination (r^2) of 0.9902, the linear range for Arg detection was ascertained to be 0.1-13.6 µmol L^{-1}. GSH was one of the key components to increase the selectivity of the experiment. Plotting its calibration graph at the λ_{max} of 524 nm gave us the equation of A = -0.1106C_{GSH} +1.1957 (r^2 = 0.9942) and the linear range of 0.09-5.270 µmol L^{-1} for this tripeptide (Fig. 10b).

The aforementioned data were also used to determine the Limit of Detection and the Limit of Quantification of the sensor toward Arg, and GSH, using the equation LOD = 3S/m for the Limit of Detection and equation LOQ = 10S/m for the Limit of Quantification, where S represented the standard deviation of the blank solution and m was the slope of the calibration curve. The LOD of the sensor toward Arg, and GSH was 0.03, and 0.009 respectively. The LOQ of the sensor was calculated to be 0.10, and 0.03 for Arg, and GSH respectively.

55.0×10^{-5} mol L^{-1} Dithizone, 5.0×10^{-5} mol L^{-1} Co(II) in DMSO/10 mmol L^{-1} and 5.0×10^{-5} mol L^{-1} Alizarin Red S in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v)) at 527 nm and the concentration of Arg b**)** DTZ-Co-ALRs-Lys (solution (2.5 mL, 5.0×10^{-5} mol L^{-1} Dithizone, 5.0×10^{-5} mol L^{-1} Co(II) in DMSO/10 mmol L^{-1} and 5.0×10^{-5} mol L^{-1} Alizarin Red S in DMSO/10 mmol L^{-1} HEPES buffer solution, pH 7.0 (1:9 v/v), and 5.0×10^{-5} mol L^{-1} Arg) at 524 nm and the concentration of GSH.

3.8.*Analysis of real samples*

3.8.1.*Determination of Arg in real samples*

The practical feasibility of the sensor was assessed by using DTZ-Co-ALRs receptor for the determination of Arg in two dietary supplements. The analysis results which are summarized in Table 3 appeared to be comparable with the results from the reference method (there were not any statistically significant differences by *t*-test [57]). Therefore, the use of this sensor in the application of real samples was accredited with high sensitivity.

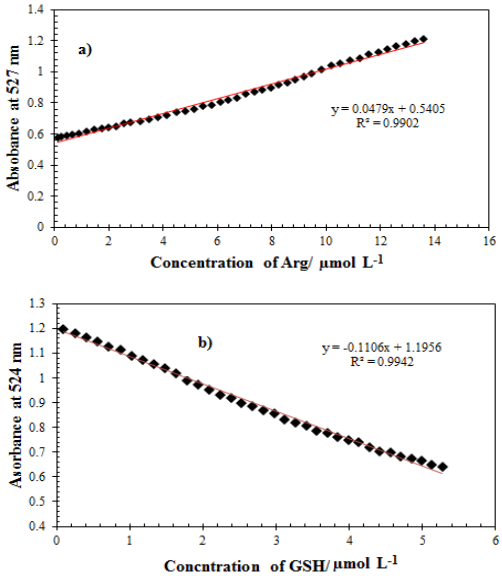

Fig. 10. The linearly proportional relationship between the absorbance of. a**)** DTZ-Co-ALRs (solution (2.5 mL,

3.8.2.*Analysis of GSH in real samples*

The GSH concentrations were measured in plasma samples of 10 healthy and 10 diabetic subjects (diabetic (36–72 y), and non-diabetic (34–77 y) women) together with anthropometric and clinical characteristics (Table 4). Data from this report revealed that plasma glutathione (GSH) is significantly higher in diabetic patients than in age-matched nondiabetic controls. The SD of GSH determination in the samples was between 0.13 and 0.45, which is acceptable for quantitative detection attained in complex real samples. On the other hand, there were no statistically major differences between the results developed from the proposed method and those obtained using the high-performance liquid chromatography (HPLC) method. The application of the *t*-test to both sets of results manifested that there is no substantial difference at the 95% confidence level. The student *t*-test [57] was applied to both analytical data including the developed and HPLC methods. No substantial difference using the Student t-test was observed between the methods. The tabulated t value at 95% confidence limit was 2.31 and the calculated t value by employing Student's t-test to the results of real samples were found less than 2.31 for five measurements. The results show that this method is as appropriate for the determination of GSH concentrations in such samples as the known representative HPLC method. On the other hand, the present method is much cheaper in comparison to the HPLC method.

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Table 3 Analytical results for determination of arginine (mg mL^{-1}) in dietary supplement by using the proposed and reference method (Mean Value[1] ± Standard Deviation).

Sample	Arg (mg mL^{-1}) Found with mixed ligand (DTZ-Co-ALRs)	Recovery (%)	Arg (mg mL^{-1}) Reference method[b]	t_{exp}[c]
Product A (Capsules)	(4.82±0.21)[a]	104.4	(4.99±0.39)	0.86
Product B (Tablet)	(3.49±0.42)	98.6	(3.33±0.19)	1.93

[a] Mean±standard deviation (n = 5)
[b] Reference method [49]
[c] Note: $t_{0.05,\,8}$=2.31 [57]

Table 4. Application of the proposed method for determination of GSH in plasma samples using (A) Mixed ligand (DTZ-Co-ALRs)and (B) HPLC method for diabetic (36–72 y), and nondiabetic (34–77 y) women (mean±standard deviation (SD), n =5).

Sample[a]	Found (A)± SD (µmol L^{-1})	Found (B)± SD (µmol L^{-1})	*t*-Test[b]
N.W.[c] (34 y[d])	2.41 ± 0.19 [e]	2.52 ± 0.48	1.29
A.W[f]. (36 y)	5.29 ± 0.17	5.16± 0.37	2.09
N.W. (40y)	3.66± 0.26	3.58 ± 0.71	0.89
A.W. (37y)	6.25 ± 0.18	6.31 ± 0.56	1.08
N.W. (42 y)	4.07 ± 0.33	4.09 ± 0.58	1.24
A.W. (45 y)	6.12 ± 0.17	6.21 ± 0.53	1.01
N.W. (47 y)	3.43 ± 0.13	3.40 ± 0.54	2.11
A.W. (49 y)	5.28 ± 0.29	5.21 ± 0.66	1.64
N.W. (50 y)	6.25 ± 0.26	6.30 ± 0.59	1.15
A.W. (51 y)	7.78 ± 0.14	7.66 ± 0.45	2.09
N.W. (55 y)	5.97 ± 0.25	5.85 ± 0.43	0.97
A.W. (54 y)	6.89 ± 0.15	6.67 ± 0.55	1.05
N.W. (64 y)	4.89 ± 0.19	4.98 ± 0.73	1.72
A.W. (63 y)	6.25 ± 0.17	6.57 ± 0.68	1.07
N.W. (66y)	6.77± 0.19	6.84 ± 0.64	1.92
A.W. (65 y)	7.94 ± 0.23	7.78 ± 0.49	1.21
N.W. (68 y)	5.11 ± 0.45	5.02 ± 0.64	1.67
A.W. (68 y)	7.66 ± 0.27	7.49 ± 0.57	0.86
N.W. (72 y)	5.12 ± 0.22	5.29 ± 0.44	1.75
A.W. (77y)	8.42 ± 0.31	8.51 ± 0.38	0.86

[a] In the presence of 50.0 µL of 1.0×10^{-2} mol L^{-1} Cys-Gly and gamma-Glu-Cys
[b]Tabulated value at 95% confidence limit was 2.31
Nondiabetic Woman
[d] Years old
[e] Values determined after10^2 times dilution
[f] Diabetic Woman

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Table 5 Performance comparison for the proposed method with other detection methods of GSH

Methods	Detection Limit (μmol L^{-1})	Linear Range (μmol L^{-1})	Ref.
Calorimetric flow-injection method	-	500 - 10000	[33]
Enzymatic electrochemical sensor	4	40 - 1	[34]
Voltammetric method by a modified carbon nanotube paste electrode	0.07	700 - 25	[58]
Colorimetric method based on the reduction of oxidized 3,3′,5,5′-tetramethylbenzidine (TMB)	0.1	0.26–26	[59]
Fluorimetric method based on target-induced agglomeration of silvernanoclusters	0.38	0.5–6.0	[60]
Colorimetric method based on Ag [I] ion–3,3′,5,5′-tetramethylbenzidine (TMB)	0.05	0.05–8.0	[61]
Fluorimetric method based on a rhodamine B and N-[4-(carbonyl) phenyl]maleimide conjugate	0.219	2.0 – 26	[62]
Fluorimetric method polymer dots-manganese dioxide	0.1	0.5–200	[63]
Enzymatic method, MnO_2 nanosheets as an artificial enzyme to mimic oxidase	0.3	1.0–25	[64]
Colorimetric method based on carbon quantum dots	0.016	0.05–20	[65]
Colorimetric method based on multiple ligands	0.0009	0.007–0.163	[15]
Colorimetric method based on multiple ligands (DTZ-Co-ALRs-Arg)	0.009	0.09–5.27	This work

Table 6 Evaluation of analytical features of the reported colorimetric chemosensor in comparison with the recently reported methods for Arg determination

No	Sensing probe	Spectroscopic method	LOD (μmol L^{-1})	Linear range (μmol L^{-1})	Ref
1	Cu^{2+} complex of rhodamine derivative	Fluorescence and Naked-eye	2.20	0-60.0	[66]
2	8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt	Fluorescence and colorimetric	1.94	0-45.0	[67]
3	Rhodamine complex	Naked-eye and fluoresence	2.30	0-16.0	[68]
4	Polydiacetylene-Mg^{2+} system	Fluorescence and colorimetric	4.27	0-150.0	[69]
5	Cu^{2+} complex of Reactive Blue 4	Colorimetric	1.06	1.75−92.38	[70]
6	Multiple ligands **(**DTZ-Co-ALRs-Arg)	Colorimetric	0.03	0.1–13.6	This work

4.CONCLUSION

Our proposed novel designed and developed mixed ligand (DTZ-Co-ALRs) is of particular importance in understanding the capabilities for the detection of α-amino acids. In consequence, a new type of label-free, rapid, highly sensitive, and selective colorimetric assay for the detection of arginine, and glutathione using a mixed ligand ensemble is demonstrated. The probe is simple in design and fast in operation and is more convenient and promising than previous methods. This new method eliminated the need for separation processes, chemical modifications, enzymatic reactions, and sophisticated instrumentations. The limits of detection of the proposed procedure are lower or at least comparable to the reported techniques for GSH [33, 34, 58-65], and Arg [66-70] (Table 5 and 6). And in between the first and foremost, the method suggests high selectivity for the determination of Arg, and GSH amino acids found in biological samples. Nevertheless, the selective determination of a specific amino acid is a complicated matrix. So, the evaluation showed great potential for practical utilization as a disease-interdependent biomarker and would be needed to amuse the great demand for amino acid determination in fields such as food analysis, clinical investigations, biochemistry, and pharmaceuticals. Therefore, as many biomolecules could form stable mixed ligands selectively, these results suggest a potential approach to the determination of multiple analytes.

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طراحی و توسعه حسگر شیمیایی جدید و فوق حساس برای تعیین متوالی اسیدهای آمینه آرژنین و گلوتاتیون

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چکیده

در این مطالعه، یک گروه لیگاند مخلوط جدید، دایتیزون- کبالت(II)-آلیزارین رد اس (Dithizone-Co(II)-Alizarin red S: DTZ-Co-ALR) طراحی و توسعه داده شد که بسیار حساس و انتخابی با روش جذب UV-Vis و برای تشخیص با چشم غیر مسلح آرژنین (Arg) و گلوتاتیون (GSH) کاربرد دارد. حساسیت و انتخاب پذیری بالا بعد از افزایش آرژنین به گیرنده اولیه، گیرنده جدیدی(DTZ-Co-ALRs-Arg) را برای شناسایی و اندازه گیری ایجاد گلوتاتیون نمود. این اندیکاتور به دلیل جابجایی آرژنین از لیگاند مخلوط (DTZ-Co-ALRs-Arg) توسط گلوتاتیون آزاد می شود و تغییر در جذب ممکن است به دلیل کمپلکس شدن بیشتر GSH با مکان های هماهنگی اضافی موجود در آرژنین باشد که با لیگاند مخلوط پیوند خورده است. DTZ-Co-ALRs). گیرنده DTZ-Co-ALRs بدون برچسب، تشخیص حساس و انتخابی ال-آرژنین وگلوتاتیون را با حد تشخیص های به ترتیب ۰/۰۳و۰/۰۰۹میکرومول بر لیتر ارائه می کند. این روش به ویژه گزینش پذیری بالایی را برای تعیین Arg و GSH در میان اسیدهای آمینه موجود در نمونه های واقعی ارائه می دهد. علاوه بر این، بررسی رفتار منطقی گیرنده پیشنهادی DTZ-Co-ALRs توانایی آن را برای عملکرد به عنوان یک حسگر شیمیایی رنگ سنجی نوع INHIBIT با ورودی های شیمیایی و سیگنال جذب UV-Vis به عنوان خروجی نشان داد. این گیرنده لیگاند مخلوط همچنین می تواند به عنوان یک "قفل صفحه کلید" مولکولی با افزودن متوالی صحیح ورودی های Arg و GSH رفتار کند.

کلید واژه ها

حسگر شیمیایی رنگ سنجی؛ گروه لیگاند مخلوط؛ ال-آرژنین؛ گلوتاتیون؛ قفل صفحه کلید.

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