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Novel Use of Congo Red and Alizarin Red S Dye Couple as Rapid and Selective Colorimetric Chemosensor for Naked Eye Detection and Determination of Carbonate in Aqueous Samples

Hossein Tavallali,*. Abolfath Parhami, Abbas Karimpour, Mohammad Ali Karimi

Department of Chemistry, Payame Noor University 19395-4697 Tehran, I.R.Iran

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Abstract

In this study, a new colorimetric sensor based on a couple of Congo Red (CR) and Alizarin Red S (ALRs) was ready for the detection of the carbonate in tracing levels in aqueous media. The linear range of carbonate derived from the results of this simple, selective, and rapid determination, was 1.21×10^{-6}–9.51×10^{-5} mol L^{-1} with the detection limit of 1.31×10^{-7} mol L^{-1}. In the examined cases, the concomitant cations, anions, and weak acids (I^-, Br^-, F^-, Cl^-, SCN^-, SO_3^{2-}, $S_2O_3^{2-}$, $S_2O_8^{2-}$, OAc^-, $H_2PO_4^-$, HPO_4^{2-}, NO_3^-, $C_2O_4^{2-}$, HCO_3^-, BrO_3^-, IO_3^-, Cit^{3-}), (K^+, Na^+, $<1.5 \times 10^{-4}$ mol L^{-1}) did not curb the analysis by creating the disturbance of chemical ions. The recoveries shown in the range of 98.6–102.5% indicate the high validity of the method in water samples. Typically, this compound presented was affordable and fast, with a trouble-free operation, which can consider as a good carbonate receiver in running water greenhouses and arable land. In addition, due to the sophisticated modifications made to filter paper strips, this chemical sensor has the potential capacity to be used as a carbonate solid colorimetric indicator.

Keywords
Colorimetric Chemosensor; Naked-Eye Detection; Alizarin-Red S; Congo-Red; Carbonate Recognition; Tap Water

1. INTRODUCTION

Most recently, anions have played an essential role in our daily life, being indispensable to physiological functions and various industrial processes [1]. The tracing of anions is a significant domain of investigation because several anions are necessary for the fundamental biochemical processes [2, 3]. Among anions, carbonate (CO_3^{2-}) plays a key role as buffering in seawater and human blood [4] or important roles in soil science [5], hydrology [6], and geology [7]. On the other hand, Potassium, sodium, magnesium, calcium, and ammonium carbonates are allowed to serve as food additives by the European Union [8].

Moreover, their determination is needed in environmental analysis [9] because they are a part of the carbon dioxide cycle in nature. Despite these Benefits facts, carbonate ions can have a negative effect. For example, carbonate, a common fraud found in milk, may interrupt the hormone signaling that adjusts growth and reproduction [10]. For these reasons, several analysis methods have arisen for the detection of CO_3^{2-}, including gas chromatography [11], the continuous flow method [5], the electrochemical method [12], selective ion electrodes [13], the acoustic method [14] and fourier transform infrared spectroscopy [15], pH ion-sensitive field-effect transistor [16]. However, these methods are invisible to the naked eye, time-consuming, costly, and at the same time complex and tedious [17].

Now considerable research endeavors are concentrated on finding inexpensive, reliable, and simple methods for detecting ions in solution, and new systems of selective ion receptors have been born as receptors with high sensitivity and selectivity in the last decade. [18-27]. A specific tendency in this field is related to the development of colorimetric anion sensors [28]. Such sensors detect anions with the naked eye without utilizing precision spectroscopy tools. [29].

Despite the report, the designed sensor is a combination of azo compounds for anions [30-35], and only a limited number of sensors for carbonate have been reported [36-38]. Perhaps this limitation further underscores the difficulty of designing a synthetic carbonate receiver in aqueous media due to its relatively powerful alkaline energy without much hydration [39].

One of the problems these days is the presence of carbonate (CO_3^{2-}) in freshwater resources is one of the significant features of the geochemical environment. Thus, the environmental reasons such as sampling, transport, and storage that may

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*Corresponding Author: Tavallali@pnu.ac.ir

occur during the desalination can affect the concentration of carbonate contamin ants easily and convert the determination of the main content of carbonate ions in freshwater into a global challenge.

To solve this problem, in this study, we used a combination of complexes, namely 1,2-Dihydroxy-9,10-anthracenedione (Alizarin red S, ALRs) and 1- (1-hydroxy-4-methyl-2-phenyl azo) -2-naphthol-4-sulphonic acid(Congo red, CR) as a hybrid dye chemical sensor for selective detection.

ALRs known as allonym Mordant Red 3 with the IUPAC name 3,4-Dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-sulfonic acid, is an example of the most widely used anthraquinone dye [40], and is soluble in water [41]. ALRs is recalcitrant, durable, and possesses mutagenic and carcinogenic properties that are released especially by textile industries into aquatic ecosystems [40, 41]. According to some reports, ALRs is an anthraquinone dye that is difficult to break down naturally due to its aromatic structure. Hence, it causes irreparable damage to aquatic plants and animals [42]. Also, congo red is known with the IUPAC name disodium 4-amino-3-[4-[4-(1-amino-4-sulfonato-naphthalene-2-yl) diazenylphenyl] phenyl] diazenyl-naphthalene-1-sulfonate (CR) diazo is a monoazo compound with the usual structural unit of the azo chromophore linking two aromatic systems [43]. Congo red is a water-soluble secondary diazo dye, yielding a red colloidal solution and can be in the form of a pH indicator due to the color change from blue to red occurring at pH 3.0 to 5.2 [44], in biochemistry and histology to stain microscopic preparations [45], and as a chemosensor in color change studies to determine several anions and cations [37, 46, 47]. However, the use of this azo dye to detect anions has been less common in the literature. On the other hand, the content of carbonate (CO_3^{2-}) in the freshwater is counted as an important feature of the geochemical environment. However, it is clearly known that some environmental factors such as sampling, transportation, and storage which might come up in the course of the process, can easily exert influence on the concentration of the two ions, carbonate (CO_3^{2-}) and bicarbonate (HCO_3^{-}). That being the case, determining the real content of carbonate and bicarbonate ions in freshwater have turned into a worldwide outlook. To solve this problem, herein, we proclaim the use of a couple of Congo Red (CR) and Alizarin Red S (ALRs) as colorimetric chemosensor for the selective recognition of carbonate. At variance with the synthetic chemosensors which need time and energy for preparation, the proposed chemosensor is a readily accessible azo dye requiring no pretreatment.

The CO_3^{2-} due to the slow change caused by paper filter strips, we suggest it as a solid-state colorimetric indicator for carbonate. In contrast to synthetic chemicals, which require time and energy to prepare, the natural dyes used of monoazo and dihydroanthracene are readily available. To our knowledge, this is the first evidence of the use of a compound of two pigments with different chemical structures as sensor chemistry for the direct determination of carbonate ions.

2. EXPERIMENTAL

2.1.Materials and reagents

Analytical grade reagents and demineralized water were used in solution preparation. All reagents were obtained commercially and were used without further purification. ALRs and CR dyes were purchased from Merck and used as received without further purification. DMSO (Merck) was dried with CaH_2 and then distilled under reduced pressure. Solutions of CR with a concentration of 5.0×10^{-5} mol L^{-1} in H_2O media (HEPES buffer solution 10 mmol L^{-1}, pH=8.5) and Solutions of ALRs with a concentration of 50 µmol L^{-1} in DMSO/H_2O (25:75 v/v %, HEPES buffer solution10 mmol L^{-1}) were prepared. Later, the color solutions obtained from ALRs and CR are mixed in different percentages (ALRs/CR 20:80, 25:75, 40:60, 50:50, 60:40,75:25, 80:20 v/v%). Stock solutions (1.0×10^{-1} mol L^{-1}) of I^-, Br^-, F^-, Cl^-, SCN^-, CO_3^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, $S_2O_8^{2-}$, OAc^-, $H_2PO_4^-$, HPO_4^{2-}, NO_3^-, $C_2O_4^{2-}$, HCO_3^-, Cit^{3-}, Bromate and IO_3^- ions were prepared by direct dissolution of proper amounts of sodium salts of anions in deionized water. The standard working solutions were prepared daily by serial dilutions with doubly distilled deionized water from stock solutions. All spectroscopic experiments were carried out at room temperature.

2.2.Apparatus

UV-Vis spectra were recorded on a Perkin Elmer Lambda2 Spectrophotometer containing a thermal temperature-controlled cell holder at 298.2 ±0.1 K in the wavelength range of 300-700 nm with a quartz cuvette (path length = 1 cm). The measurements were taken and recorded in the wavelength range of 200–800 nm. A mixture of deionized water (HEPES10 mmol L^{-1}) and DMSO without any indicator was applied in the reference cell. Chemoffice for molecular drawing and energy evaluation. A HANNA HI2211 pH-meter calibrated with Merck standard buffer solutions was used for measuring the pH of the solutions. A Hamilton microliter syringe (50 µL) was used for titration experiments.

2.3.The pH dependence study on binding CO_3^{2-} to related chemosensor by UV– Vis spectroscopy

All pH measurements were carried out in DMSO/H_2O media (20:80 v/v, HEPES 10 mmol L^{-1}) in 298 k. 2.5mL solution of ALRs/CR(60:40 v/v, ALRs and CR solution 50 µmol L^{-1}) in DMSO/H_2O (25:75 v/v, HEPES buffer solution 10 mmol L^{-1}) was mixed with CO_3^{2-} in H_2O media to obtain [ALRs- CR]– CO_3^{2-}. The buffers (10 mmol L^{-1}) include pH 3.0–4.0, acetic acid/ sodium hydroxide; pH 5.0–6.0, MES/sodium hydroxide; pH 7.0–10.0, HEPES/sodium hydroxide, pH 10.0–11.4, CABS were utilized to study investigate the pH dependence of the proposed method.

2.4.General procedures of spectra detection

The suitable amount of the working required solutions (1.0×10^{-2} mol L^{-1}) of the anions (I^-, Br^-, F^-, Cl^-, SCN^-, CO_3^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, $S_2O_8^{2-}$, OAc^-, $H_2PO_4^-$, HPO_4^{2-}, NO_3^-, $C_2O_4^{2-}$, HCO_3^-, Cit^{3-}, Bromate and IO_3^- ions) was added to quartz cells including of ALRs/CR (60:40 v/v, 50 µmol L^{-1}) in DMSO/H_2O (25:75 v/v, HEPES buffer solution 10 mmol L^{-1}). Instrumental studies were performed with a short time delay to ensure the uniformity and homogeneity of the solutions as well as to stabilize the resulting color changes. By adding anions to the quartz cuvette, only the carbonate anion changed to purple, while the other anions showed no significant color effect (Fig. 1). Hence, the experiment was selectively performed for the detection and measurement of CO_3^{2-}. The colorimetric property of the current chemosensor towards CO_3^{2-} was subsequently investigated through UV-Vis titration. Therefore A combination of Alizarin red S and Congo red pigments with specifications (60:40 v/v, 50 µmol L^{-1}) in DMSO/H_2O (25:75 v/v, HEPES buffer solution 10 mmol L^{-1}, pH=8.5) was placed in a quartz cell (1cm width) and the UV–Vis absorption spectra were recorded after adding 1.99×10^{-7}–1.07×10^{-4} mol L^{-1} of carbonate ion.

3. RESULT AND DISCUSSION

3.1.The UV-visible response of ALRs/CR to carbonate ions

The interaction of ALRs/CR (60:40 v/v, 50µmol L^{-1}) in DMSO/H_2O (25:75 v/v, HEPES buffer solution 10 mmol L^{-1}, pH=8.5), with carbonate, was studied through UV-Vis absorption method under optimized experimental conditions. As can be seen in Fig. 2, ALRs/CR (60:40 v/v) solution absorption band dominated at 503 nm. The molar absorptivity at λ = 503 nm was calculated to be 5.11×10^3 L mol^{-1} cm^{-1}.

The addition of carbonate ions induced the appearance of a new peak at 515 nm. Upon the successive addition of an incremental amount of CO_3^{2-} to ALRs/CR (60:40 v/v, 50 µmol L^{-1}) in DMSO/H_2O (25:75 v/v, HEPES buffer solution 10 mmol L^{-1}, pH=8.5), the maximum absorption at 503 nm started increasing, and instantaneously, a new red-shifted band was observed at 515 nm with the left shoulder fading at 430 nm and forming a very weak bulge at 597 nm (Fig. 2). This change was also discernible by naked eyes through the color change from orange to purple (Right inset of Fig. 2).

Two isosbestic points were detected at 372 nm and 456nm which confirm the binding of ALRs/CR and CO_3^{2-} ion and the existence of an equilibrium between ALRs/CR and its complexes. The binding affinity of ALRs/CR to an equal amount of different anions (I^-, Br^-, F^-, Cl^-, SCN^-, CO_3^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, $S_2O_8^{2-}$, OAc^-, $H_2PO_4^-$, HPO_4^{2-}, NO_3^-, $C_2O_4^{2-}$, HCO_3^-, Cit^{3-}, Bromate and IO_3^- ions) was also considered using the UV-Vis absorption changes and naked-eye detection. The addition of the above-mentioned anions did not cause any considerable changes even up to 25.0 fold excess amount (Fig. 1).

This is confirmed by the Benesi–Hildebrand analysis [48], of the absorption titration data. When assuming a 1: n stoichiometry for the association between ALRs/CR and CO_3^{2-}, the association constant, K_{ass}, is given by the following equation (Eq. (1):

$$\frac{1}{(A-A_0)} = \frac{1}{(A_{max}-A_0)}\left[\frac{1}{K_{ass}[CO_3^{2-}]} + 1\right] \quad (1)$$

Equation 1. The Benesi-Hildebrand equation: A_o is the absorbance of the receptor without carbonate; A is the absorbance of the obtained receptor with carbonate; A_{max} is the saturated absorbance of the receptor in the presence of an excess amount of the carbonate ion added at λ_{max} = 515 nm.

As shown in Fig. 3, the plot of 1/(A-A_o) against 1/[CO_3^{2-}] shows a linear relationship, indicating that ALRs/CR associates with CO_3^{2-} in a 1:2 stoichiometry (formation of ALRs/CR –CO_3^{2-} species). K_{ass} is determined from the intercept/slope to be $2.07(\pm0.25)\times10^8$ mol^{-2} L^2.

3.2.Sensing in the presence of competing anions

The selectivity of the system for carbonate was then examined by competition experiments. The UV–Vis absorption intensity of ALRs/CR complex changes upon addition of carbonate when 1.25×10^{-3} mol L^{-1} of different anions (I^-, Br^-, F^-, Cl^-, SCN^-, SO_3^{2-}, $S_2O_3^{2-}$, $S_2O_8^{2-}$, OAc^-, $H_2PO_4^-$, HPO_4^{2-}, NO_3^-, $C_2O_4^{2-}$, HCO_3^-, Cit^{3-}, Bromate and IO_3^- ions) and CO_3^{2-} and positive ions (K^+, Na^+) that are present simultaneously in the solution containing the receptor.

It is noticeable that the absorption intensity was almost identical to that obtained in the absence of anions. The miscellaneous competitive anions did not lead to any significant spectral and color changes (Fig. 4).

Fig. 1. Naked-eye visible color changes of UV–Vis spectra of ALRS/CR (60:40 v/v, ALRs and CR solution. 50 µmol L^{-1}) in DMSO/H_2O (25:75 v/v, HEPES buffer solution 10 mmol L^{-1}, pH=8.5) in the presence of 1.25×10^{-3} mol L^{-1} of different anions (I^-, Br^-, F^-, Cl^-, SCN^-, SO_3^{2-}, $S_2O_3^{2-}$, $S_2O_8^{2-}$, OAc^-, HCO_3^-, $H_2PO_4^-$, HPO_4^{2-}, NO_3^-, $C_2O_4^{2-}$, Cit^{3-}, Bromate and IO_3^- ions) and 1.07×10^{-4} mol L^{-1} carbonate ions.

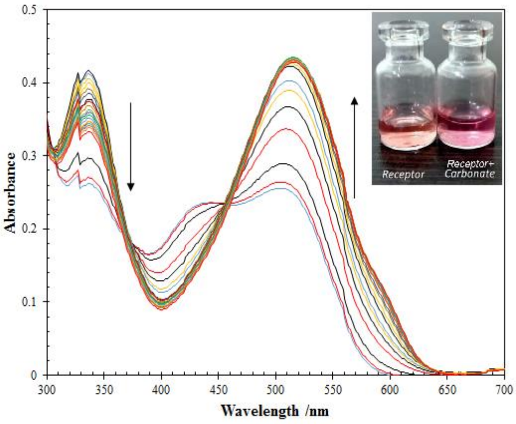

Fig. 2. UV–Vis spectra of ALRs/CR (60:40 v/v, 50 µmol L^{-1}) in DMSO/H_2O (25:75 v/v, HEPES buffer solution 10 mmol L^{-1}, pH=8.5) upon gradual addition of carbonate ion (1.99×10^{-7} –1.07×10^{-4} mol L^{-1}), Right inset: corresponding color change of ALRs/CR and ALRs/CR upon addition of 1.07×10^{-4} mol L^{-1} carbonate ions in H_2O media.

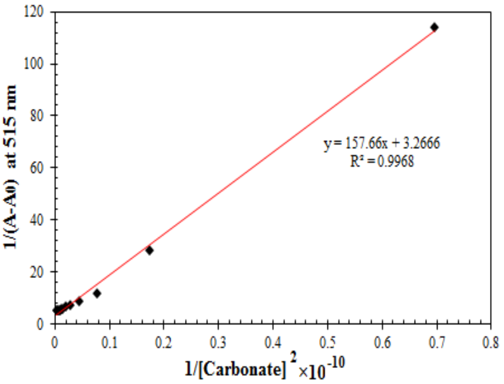

Fig. 3. Benesi-Hildebrand plot (absorbance at 515 nm) of ALRs/CR (the variation of 1/(A_A$_o$) at 515 nm versus the function of 1/[carbonate]^2) based on 1:2 binding stoichiometry with carbonate ions (relative standard deviation of 3 individual measurements = ± 0.25).

These substances are generally present in a quantity of less than 1.25×10^{-3} mol L^{-1}, so their influence could be ignored. In the case of suspended solids, colloids microorganisms and other such interferences, we can adjust the receptor to remove them by the appropriate filtration. The results confirmed the supreme selectivity of the chemical sensor, ALRs/CR, for CO_3^{2-} determination. The above results clearly show that the designed ALRs/CR protocol for carbonate has preferable selectivity work for detecting carbonate in biological liquids. More importantly, this system is very suitable for qualitative and quantitative detection.

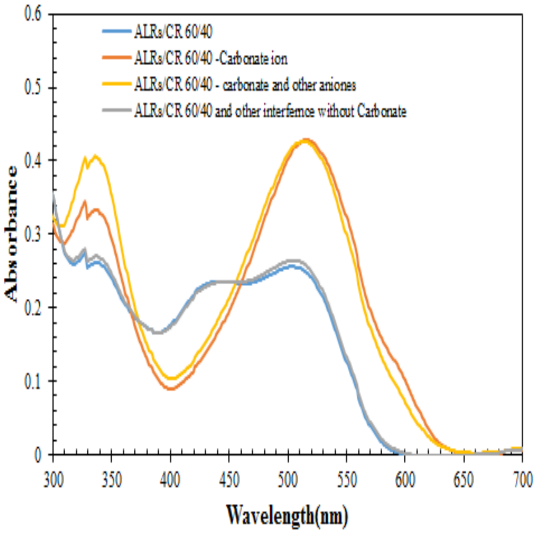

Fig. 4. The UV-Vis absorption changes of ALRS/CR (60:40 v/v, ALRs and CR solution. 50 µmol L^{-1}) in DMSO/H_2O (25:75 v/v, HEPES buffer solution 10 mmol L^{-1}, pH=8.5) media toward carbonate ion in the presence of 1.25×10^{-3} mol L^{-1} of different anions (I^-, Br^-, F^-, Cl^-, SCN^-, SO_3^{2-}, $S_2O_3^{2-}$, $S_2O_8^{2-}$, OAc^-, HCO_3^-, $H_2PO_4^-$, HPO_4^{2-}, NO_3^-, $C_2O_4^{2-}$, Cit^{3-}, Bromate and IO_3^- ions) and positive ions (K^+, Na^+) and CO_3^{2-}(1.84×10^{-4} mol L^{-1}).

3.3.Optimization analysis conditions Effect of pH

To access the appropriate pH range, the effect of pH on the absorbance of ALRs/CR in the presence of carbonate ions was studied by UV-Vis spectroscopy. The pH range of 3.0 to 11.0 was selected for taking the measurements. The spectra were recorded before and after the insertion of CO_3^{2-} (1.84×10^{-4} mol L^{-1}). As demonstrated in Fig. 5, the addition of CO_3^{2-} ion has the highest impact on the absorbance intensity in the pH close to 8.5. Hence, further studies of UV-Vis spectroscopy ere carried out at a pH equal to 8.5.

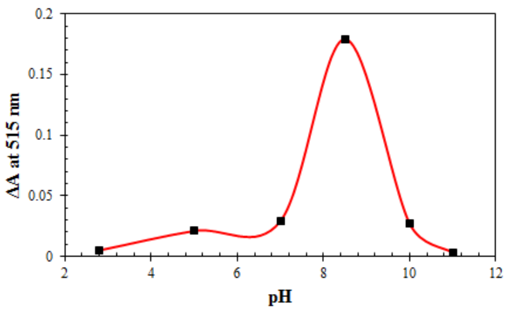

Fig. 5. Effect of the pH on the absorbance changes of ALRs/CR (60:40 v/v, 50µmol L^{-1}) in the presence of carbonate ion (1.07×10^{-4} mol L^{-1}) at 515 nm in DMSO/H_2O (25:75 v/v, HEPES buffer solution 10 mmol L^{-1}, pH=8.5) media.

3.4. Temperature optimization

Discovering the desired temperature is very important because of the effect it has on sensitivity. For this reason, the effect of temperature on the ALRs/CR spectrum was investigated by performing a series of experiments. The spectrum of UV-Vis receptors and their combination with carbonate were studied systematically at different temperatures. Significant changes in adsorption were observed at 25 °C (Fig. 6). It is noteworthy that we performed all the experiments at around 25 °C.

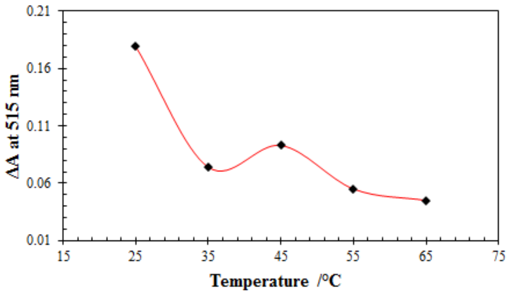

Fig. 6. Effect of the temperature on the colorimetric detection of ALRs/CR (60:40 v/v, 50 µmol L^{-1}) in the presence of carbonate ion (1.07×10^{-4} mol L^{-1}) in DMSO/H_2O (25:75 v/v, HEPES buffer solution 10 mmol L^{-1}, pH=8.5) media. [In panel A, $A_{ALRs/CR}$ is the absorbance value of ALRs/CR in the absence of carbonate and A_i is the absorbance value after addition of carbonate ion] at 515 nm. The Signal change in the temperature range of 25 to 65 °C was not almost ineffective.

3.5. Response time

Since long response time is a common problem in many chemical sensors [49,50], this factor was investigated in the present study. To the ALRs/CR solution (60:40 v/v, 50µmol L^{-1}), the appropriate amount of CO_3^{2-} (1.84×10^{-4} mol L^{-1}) was added. Visible color change and change in absorption intensity occurred in the first few seconds, which remained relatively constant for the next 60 minutes (Fig. 7). The results showed the ability of the anion sensor to respond to the anion in a short time.

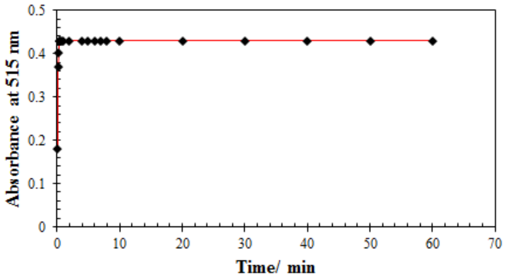

Fig. 7. Response time for the colorimetric detection of carbonate ion (1.07×10^{-4} mol L^{-1}) by ALRs/CR (60:40 v/v, 50 µmol L^{-1}, DMSO/H_2O (25:75 v/v, HEPES buffer solution 10 mmol L^{-1}, pH=8.5) receptor, at 511 nm during 60 min.

3.6. FT-IR study on possible binding interaction of ALRs/CR and carbonate ion

The FT-IR spectra of the carbonate salt (analyte), ALRs/CR (receptor) and their binary mixture are compared in Fig. 8 to investigate the mode of interaction as well as possible binding sites on the receptor and analyte. Normal vibrational peaks of free carbonate ion (trigonal planar D3h geometry) as indicated in Fig. 8 include ν1 (CO_3^{2-} symmetric stretching, 1084 cm^{-1}), ν2 (out of plane bending, 884 cm^{-1}), ν3 (CO_3^{2-} asymmetric stretching, 1450 cm^{-1}), ν4 (in-plane bending, 707 cm^{-1}) and two overtone combined peaks assigned as ν1+ ν4 and ν1+ ν3 (at 1783 and 2504 cm^{-1} respectively). Also, the absorption peaks of ALRs/CR azo dye are assigned in Fig. 8 in which the main vibrations comprise azo bond (N = N), C-N and C-O stretching vibrations identifiable at 1518, 1298 and 1059 cm^{-1} respectively.

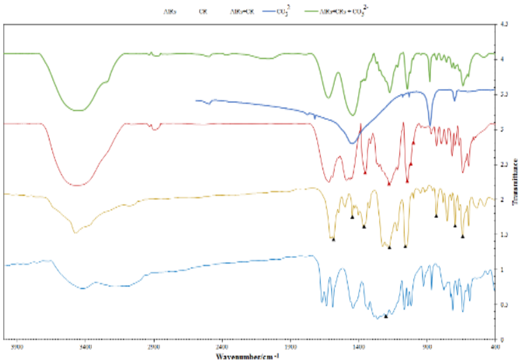

Fig. 8. FT-IR spectra of ALRs,CR, ALRs/CR, Na_2CO_3 and ALRs/CR + Na_2CO_3

To investigate possible binding interactions, the FT-IR spectrum of the binary mixture (ALRs/CR + CO_3^{2-}) was compared with the spectra of both receptor and analyte to recognize frequency shifts as well as changes in peak intensity. Significant changes in the frequency and intensity of carbonate stretching (ν2 from 884 to 921 and ν4 from 707 to 691 cm^{-1}), as well as bending peaks (ν3 from 1450 to 1481 cm^{-1}), suggest a strong interaction between the receptor molecule and the analyte. It is noteworthy that the combined overtone peak of ν1+ ν3 has completely disappeared in the binary mixture spectrum, indicating strong binding of carbonate to ALRs/CR and thus, the limitation of freedom degrees in carbonate. Similarly, significant changes in the range of 32–34 cm^{-1} towards higher frequencies are observed in the C-O and C-N stretching peaks associated with ALRs/CR in the binary mixture. The augmentation in the vibrational frequencies of C-O and C-N bonds indicates increased bond order and confirms the binding of carbonate through hydrogen bonding into acidic protons of phenolic hydroxyl groups. The hydrogen bonding of basic carbonate into OH protons injects O-H bond electron pair to the aromatic ring and subsequently towards the N = N bond, which consequently increases the bond order for C-N and C-O while decreasing the bond order for the N = N.

3.7.Mechanism of interactions through MM2 molecular mechanics calculations

In order to investigate and propose a rational mechanism for the interaction between the dye-couple receptor (ALRs-CR) and the analyte (CO_3^{2-}), we used the semi-empirical molecular mechanics MM2 calculations in the ChemBioOffice software package. Considering ALRs-CR as a bimolecular adduct, therefore, initially, different possibilities for the interaction of Congo Red (CR) and alizarin (ALRs) from the energy viewpoint include two single hydrogen bonded a) $Al(O_1)$-$CR(N_1)$ and b) $Al(O_2)$-$CR(N_1)$ as well as two double hydrogen bonded c) $Al(O_1/N_{az})=CR(N_1/O_2)$ and d) $Al(O_2/N_{az})=CR(N_1/O_1)$ () were studied through MM2 calculation. The results revealed that the single hydrogen bonded adducts are more stable than double hydrogen bonded species among them $Al(O_1)$-$CR(N_1)$ is the most stable (575.9683 kcal/mol) and therefore was considered as the receptor of choice.

Being aware of the most stable structure for dye-couple as the probable receptor, we then focused on the possible interaction states through which one or two carbonate ions interact with this receptor. (Fig. 10) It's noteworthy mention that docking the carbonate ions on the receptors is not satisfactory without the assistance of water molecules. This cleALRs the role of water molecules in the aqueous media. Docking one or two carbonate ions on both single hydrogen bonded receptors (Recep1 and Recep2) approves that the more stable receptor provides more stable interactions. The proposed interaction state according to the experimental stoichiometric ratio (1:2) is assigned on Fig. 9.

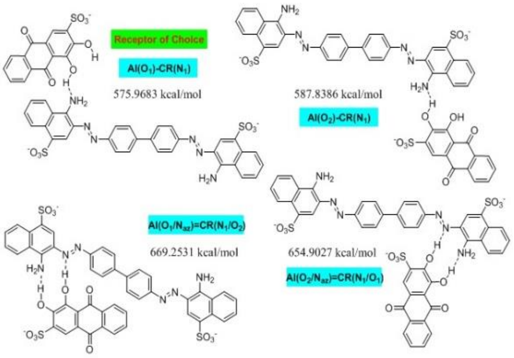

Fig. 9. Various interaction states for sensing carbonate ions.

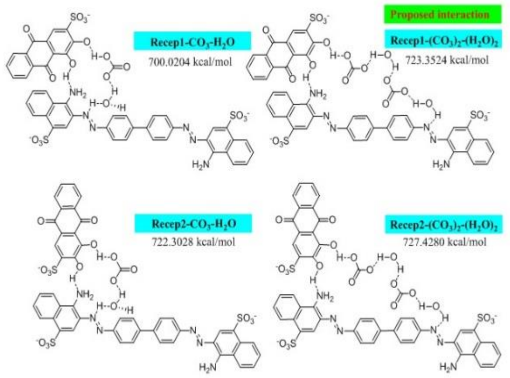

Fig. 10. Possible structures for dye-couple receptor and their calculated MM2 total energies.

3.8.Characterization of the chemosensor

ALRs/CR can be used as a novel receptor system for the detection of carbonate anion by absorbance intensity in aqueous solutions. A calibration curve was obtained from the plot of absorbance intensity with the single-stage addition of known various standard concentrations of carbonate anions. As can be noticed from the plotted calibration curve (Fig. 11), in the range of 1.21×10^{-6} to 9.51×10^{-5} mol L^{-1} of carbonate, a good linear relationship is attained between the absorbance intensity and the concentration of carbonate, with the Coefficient of Determination, r^2, of 0.9974. The Limit of Detection and the Limit of Quantification at λmax = 515 nm were determined mathematically to be 1.31×10^{-7} and 4.37×10^{-7} mol L^{-1}, respectively.

The repeatability of the method was evaluated by calculating the RSD from 10 replicates at the carbonate concentration levels of 2.38 × 10^{-5} mol L^{-1} and 6.20 × 10^{-5} mol L^{-1}, and the obtained values were 1.7% and 2.2%, respectively.

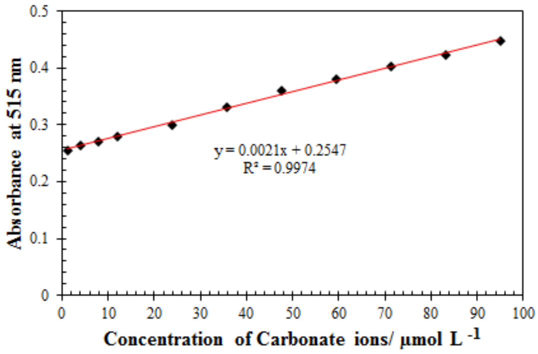

Fig. 11. The linearly proportional relationship between the absorbance of ALRs/CR (60:40 v/v, 50µmol L^{-1}) DMSO/H_2O (25:75 v/v, HEPES buffer solution 10 mmol L^{-1}, pH=8.5) receptor, at 515 media (standard deviation of 3 individual measurements = ± 0.0011)

3.9.Analytical application: Application of ALRs/CR as the solid-state indicator

The adaptability of the proposed receptor to the solid-state sensing was also examined using filter paper strips. The test strips were soaked in ALRs/CR solution (60:40 v/v, 50µmol L^{-1}), for a few minutes. The strips were then dried before being immersed in different concentrations of carbonate ion. As demonstrated in Fig. 12, the carbonate concentrations higher as 5.0×10^{-4} mol L^{-1} could easily be detected by naked eyes.

3.10.Analysis of carbonate ions in real samples of urban water

To investigate the practical applications, three samples of tap water were analyzed to determine the content of their CO_3^{2-} ions by the proposed method after 100 dilutions. To do this, the samples were analyzed using the acid-base titration method according to the manufacturer's instructions. Afterwards, the developed method was applied for the determination of carbonate ions in samples. The CO_3^{2-} content of the factual samples, which was developed by colorimetric and acid-base titration, is shown in Table 1. A good agreement was reached between the recovery percentages determined by the two methods. In addition to the percentage of retrieval, results were always more than 95 confidence, which confirmed the freedom of the method from matrix interference. On the other hand, the student t-statistics(Table1) [51] was used for analytical data of developed titration and acid-base methods. Application of the t-test to both sets of results showed that there is no significant difference at the 95% confidence level. As well the values of the one-way F statistic calculated in this analysis were less than the tabulated F value (6.39) for five replicate measurements. Statistical treatment of the data using the F test [51] showed that there are no significant differences between the two variances of the two methods. Considering that the proposed colorimetric method obtained a wider measurement range and a smaller detection range compared to the acid-base titration, the proposed method is a better way to measure the low concentration of CO_3^{2-} ions in water.

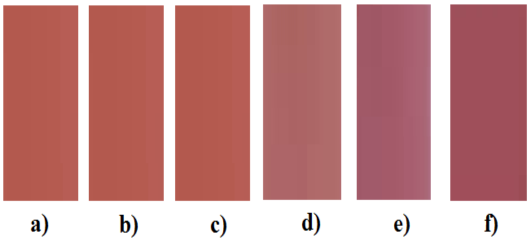

Fig. 12. Filter paper strips color changes in different concentration of CO_3^{2-} (Concentrations of CO_3^{2-} a): no carbonate, b) 5.0×10^{-5} mol L^{-1}, c) 1.0×10^{-5} mol L^{-1}, d) 5.0×10^{-4} mol L^{-1} e) 1.0×10^{-4} f) 5.0×10^{-3} mol L^{-1}

Table1. Determination of CO_3^{2-} µmol L^{-1} in some water samples by the developed UV–Vis spectroscopy (A) and the Acid-base titration (B) method (mean±standard deviation, n=5)

Sample[a,b]	**Added (CO_3^{2-} µmole L^{-1})**	**Found (CO_3^{2-} µmol L^{-1})**		**Recovery%**		**t-Test[a]**
		A	**B**	**A**	**B**	
Water	-	ND	ND	-	-	-
	5	5.1± 0.04	4.9 ± 0.05	102.0	98.0	0.45
	7	6.9± 0.06	7.2± 0.03	98.6	102.8	0.74
	10	10.2± 0.05	10.3± 0.05	102.0	103.0	1.79
	12	12.3± 0.06	12.6± 0.05	102.5	105.0	0.98

[a] Tabulated value at 95% confidence limit was 2.31.

[b] TapWater from Lamerd,Shiraz, Iran

[c] ND: Not detected.

3.11. Analysis of carbonate ions in Mineral water

The proposed receptor was used for the determination of carbonate in two commercially available brands of mineral water containing 5.0-6.0 mg L^{-1} carbonate. The sample solution was prepared by dilution of 50 mL of mineral water to 50 mL with deionized water. The analysis was carried out by UV-Vis spectroscopy using a calibration curve. It is seen from Table 2 that there was a satisfactory agreement between the determined values and the labeled carbonate content.

Table2. Determination of total carbonate ion in mineral water samples

Sample [a,b]	**Total carbonate ion determined (mg L^{-1})**	
	Chemo sensor	**Labelled**
Sample1(brand1)	5.32	5.0-6.0
Sample2(brand2)	5.98	6.0
Sample3(brand2)	6.04	6.0

[a] Values determined after10^2 times dilution.
[b] Average of three measurements (±S.D.).

4. CONCLUSION

To sum up, a new type of available, fast, highly sensitive and selective colorimetric assay for the detection of carbonate ions using a dye-based complex is demonstrated. The basis of this detection is the highly specific interaction between the anions, coexisting dyes, and weak acid and the colorimetric ALRs/CR probe in a competition assay template. In this study, ALRs/CR was proposed as an anion sensor for the first time. The chemosensor is simple in layout and rapid in function and is more comfortable and promising than the else methods. This new method no longer requires separation processes, chemical modifications and complex tools. The detection limits of this method for carbonate ions are lower or at least comparable to the previous methods [37, 38, 52-55], (Table 3). The presented method is an effective way to rapidly determine CO_3^{2-} ions in the water samples. The high sensitivity of ALRs/CR for carbonate ion was also demonstrated by the detection limit of 0.131 μmol L^{-1} which is lower than the corresponding national standard. The detection limit for CO_3^{2-} in the National Standard DZ/T 0064 49–1993 is 5mg L^{-1} [55]. This detection limit, combined with good precision and accuracy, makes the proposed chemosensor superior to the previous ones. In short, the method is sensitive, precise, simple to do, and therefore is suitable for the determination of water samples, and has a good perspective in agriculture, geonsorlogy, greenhouse, environment fields, and more.

DIVULGENCE STATEMENT

No possible contrast of interest was reported by the authors.

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Table 3 Comparison of the analytical characteristics of the methods for acetate anion determination

Method	**Reagent**	**Limit detection (μmol L^{-1})**	**Authentic sample/matrix**	**Refs.**
Fluorimetry	[Congo Red–(Cu)2]–SO_3	0.06	Water samples	[41]
Colorimetry	7,8-dihydroxy-3-(4-methyl phenyl) coumarin (DHMC)	1.47	Living cell	[42]
Titrimetry and spectrophotometry	Titrimetry and spectrophotometry	-	Uranium leach liquors -	[57]
Fluorimetry	Heterobimetallic metal-organic frameworks (Eu/Pt-MOFs)	0.021	Water samples	[58]
Automated spectrophotometry	Pb(II)	-	Seawater	[59]
Fluorimetry	7,8-dihydroxy-3-(4-methyl phenyl) coumarin (DHMC)	1.03	Living cell	[42]
Coupled Ion Selective Electrode	Chemical reactions of carbonate in water	-	Groundwater and lake water	[60]
Colorimetry	Alizarin Red S -Congo Red (ALRs/CR)	0.131	Water samples	Present work

REFERENCES

[1] R.M. Duke, E.B. Veale, F.M. Pfeffer, P.E. Kruger and T. Gunnlaugsson, Colorimetric and fluorescent anion sensors: an overview of recent developments in the use of 1,8-naphthalimide-based chemosensors, *Chem. Soc. Rev.* 39(10) (2010) 3936-53.

[2] P.A. Gale, E.N. Howe and X. Wu, Anion receptor chemistry, *Chem.* 1(3) (2016) 351-422.

[3] J.L. Sessler, P.A. Gale and W.-S. Cho, Anion receptor chemistry, *RSC* 2006.

[4] M. Maj-Żurawska, D. Ziemianek, A. Mikołajczuk, J. Mieczkowski, A. Lewenstam, A. Hulanicki and T. Sokalski, Improved selectivity and detection limit of the carbonate-selective electrode, *Anal. Bioanal. Chem.* 376(4) (2003) 524-526.

[5] M. Zougagh, A. Rios and M. Valcarcel, Direct determination of total carbonate salts in soil samples by continuous-flow piezoelectric detection, *Talanta* 65(1) (2005) 29-35.

[6] Y.S. Choi, L. Lvova, J.H. Shin, S.H. Oh, C.S. Lee, B.H. Kim, G.S. Cha and H. Nam, Determination of oceanic carbon dioxide using a carbonate-selective electrode, *Anal. Chem.* 74(10) (2002) 2435-40.

[7] H. Furukawa, N. Ko, Y.B. Go, N. Aratani, S.B. Choi, E. Choi, A.O. Yazaydin, R.Q. Snurr, M. O'Keeffe, J. Kim and O.M. Yaghi, Ultrahigh porosity in metal-organic frameworks, *Sci.* 329(5990) (2010) 424-8.

[8] N. Kallscheuer, Engineered Microorganisms for the Production of Food Additives Approved by the European Union-A Systematic Analysis, *Front. Microbiol.* 9 (2018) 1746.

[9] F. Devlieghere and J. Debevere, Influence of dissolved carbon dioxide on the growth of spoilage bacteria, *LWT- Food Sci. Technol.* 33(8) (2000) 531-537.

[10] T. Azad and S. Ahmed, Common milk adulteration and their detection techniques, *Int. J. Food Contam.* 3(1) (2016) 1-9.

[11] R.G. Amundson, J. Trask and E. Pendall, A rapid method of soil carbonate analysis using gas chromatography, *Soil Sci. Soc. Am. J.* 52(3) (1988) 880-883.

[12] A. Sola, A. Tárraga and P. Molina, A ferrocenyl-guanidine derivative as a highly selective electrochemical and colorimetric chemosensor molecule for acetate anions, *Dalton Trans.*41(27) (2012) 8401-8409.

[13] H.K. Lee, H. Oh, K.C. Nam and S. Jeon, Urea-functionalized calix [4] arenes as carriers for carbonate-selective electrodes, *Sens. Actuators B Chem.* 106(1) (2005) 207-211.

[14] A. Pietrzyk, W. Kutner, R. Chitta, M.E. Zandler, F. D'Souza, F. Sannicolo and P.R. Mussini, Melamine acoustic chemosensor based on molecularly imprinted polymer film, *Anal. Chem.* 81(24) (2009) 10061-70.

[15] E.E. Burt and A.H. Rau, The determination of the level of bicarbonate, carbonate, or carbon dioxide in aqueous solutions, *Drug Dev. Ind. Pharm.* 20(19) (1994) 2955-2964.

[16] K. Tsukada, Y. Miyahara, Y. Shibata and H. Miyagi, An integrated chemical sensor with multiple ion and gas sensors, *Sens. Actuators B Chem.* 2(4) (1990) 291-295.

[17] L. He, C. Liu and J.H. Xin, A novel turn-on colorimetric and fluorescent sensor for Fe^{3+} and Al^{3+} with solvent-dependent binding properties and its sequential response to carbonate, S*ens Actuators B Chem.* 213 (2015) 181-187.

[18] X. Huang, Y. He, Z. Chen and C. Hu, Colorimetric sensors for anion recognition based on the proton transfer signaling mechanism, *Chin. J. Chem.* 27(8) (2009) 1526-1530.

[19] Y. Li, H. Lin, Z. Cai and H. Lin, Colorimetric Sensing of Biologically Important Acetate Ion Based on Indole Derivation, *Mini. Rev. Org. Chem.* 8(1) (2011) 25-30.

[20] S. Kumar, S. Bothra and S.K. Sahoo, Fluoride selective colorimetric sensor based on cefetamet pivoxil drug, *J. Fluorine Chem.* 164 (2014) 51-57.

[21] D. Sharma, S.A. Kumar and S.K. Sahoo, Vitamin B6 cofactor derived chemosensor for the selective colorimetric detection of acetate anions, *Tetrahedron Lett.* 55(4) (2014) 927-930

[22] M. Yıldız, Ö. Karpuz, C.T. Zeyrek, B. Boyacıoğlu, H. Dal, N. Demir, N. Yıldırım and H. Ünver, Synthesis, biological activity, DNA binding and anion sensors, molecular structure and quantum chemical studies of a novel bidentate Schiff base derived from 3, 5-bis (triflouromethyl) aniline and salicylaldehyde, *J. Mol. Struct.* 1094 (2015) 148-160.

[23] Ö. Arslan, B. Aydıner, E. Yalçın, B. Babür, N. Seferoğlu and Z. Seferoğlu, 8-Hydroxyquinoline based push-pull azo dye: Novel colorimetric chemosensor for anion detection, *J. Mol. Struct.* 1149 (2017) 499-509.

[24] Q.X. Liu, Z.L. Hu and Z.X. Zhao, A new fluorescent-colorimetric chemosensor for fluoride anion based on benzimidazolium salt, *Tetrahedron* 74(46) (2018) 6710-6716.

[25] L. Liu, X. Shang, R. He, J. Li, Y. Chen, H. Chen and T. Wang, Anion binding ability and cytotoxicity of a selective colorimetric chemosensor for H_2S based on Zn (Ⅱ) complex, *Inorg. Chim. Acta* 495 (2019) 118994.

[26] H. So, J.B. Chae and C. Kim, A thiol-containing colorimetric chemosensor for relay recognition of Cu^{2+} and S^{2-} in aqueous media with a low detection limit, *Inorg. Chim. Acta* 492 (2019) 83-90.

[27] S. Dalapati, S. Jana and N. Guchhait, Anion recognition by simple chromogenic and chromo-fluorogenic salicylidene Schiff base or reduced-

Schiff base receptors, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 129 (2014) 499-508.

[28] J. Zhao, D. Yang, X.-J. Yang and B. Wu, Anion coordination chemistry: From recognition to supramolecular assembly, *Coord. Chem. Rev.* 378 (2019) 415-444.

[29] P. Piatek, J. Jurczak, A selective colorimetric anion sensor based on an amide group containing macrocycle, *Chem Commun* 20 (2002) 2450-1.

[30] H. Khanmohammadi, K. Rezaeian and A. Abdollahi, Colorimetric detection of anions in aqueous media using N-monosubstituted diaminomaleonitrile-based azo-azomethine receptors: real-life applications, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 139 (2015) 405-12.

[31] H. Khanmohammadi, M. Pass, K. Rezaeian and G. Talei, Solvatochromism, spectral properties and antimicrobial activities of new azo–azomethine dyes with $N_2S_2O_2$ donor set of atoms, *J. Mol. Struct.* 1072 (2014) 232-237.

[32] V. Reena, S. Suganya and S. Velmathi, Synthesis and anion binding studies of azo-Schiff bases: Selective colorimetric fluoride and acetate ion sensors, *J. Fluorine Chem.* 153 (2013) 89-95.

[33] W. Radchatawedchakoon, W. Sangsuwan, S. Kruanetr and U. Sakee, Synthesis and evaluation of simple naked-eye colorimetric chemosensors for anions based on azo dye-thiosemicarbazones, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 121 (2014)

[34] S. Dini and H. Khanmohammadi, A new azo-azomethine sensor for detection of CN- and AcO- anions: Highly selective chemosensor for naked eye detection of sodium diclofenac, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 222 (2019) 117157.

[35] C.H. Dos Santos, N.M. Uchiyama and I.A. Bagatin, Selective azo dye-based colorimetric chemosensor for F^-, $CH_3COO^-$and PO_4^{3-}, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 210 (2019) 355-361.

[36] G. Hennrich and H. Sonnenschein, U. Resch-Genger, Fluorescent anion receptors with iminoylthiourea binding sites—selective hydrogen bond mediated recognition of $CO_3^{2-,}$ HCO_3^- and HPO_4^{2-}, *Tetrahedron Lett.* 42(15) (2001) 2805-2808.

[37] H. Tavallali, G. Deilamy-Rad, A. Parhami and S. Lohrasbi, A novel and simple fluorescent and colorimetric primary chemosensor based on Congo-Red for sulfite and resultant complex as secondary fluorescent chemosensor towards carbonate ions: Fluorescent probe mimicking INHIBIT logic gate, *Talanta* 149 (2016) 168-177.

[38] Ş.N.K. Elmas, F. Ozen, K. Koran, A.O. Gorgulu, G. Sadi, I. Yilmaz and S. Erdemir, Selective and sensitive fluorescent and colorimetric chemosensor for detection of CO_3^{2-}anions in aqueous solution and living cells, *Talanta* 188 (2018) 614-622.

[39] Y. Marcus, Thermodynamics of solvation of ions. Part 5.—Gibbs free energy of hydration at 298.15 K, *J. Chem. Soc. Faraday Trans.* 87(18) (1991) 2995-2999.

[40] Y.P. Chin, S.F.A. Raof, S. Sinniah, V.S. Lee, S. Mohamad and N.S. Abdul Manan, Inclusion complex of alizarin red s with β-cyclodextrin: synthesis, spectral, electrochemical and computational studies, *J. Mol. Struct.* 1083 **(**2015) 236-244.

[41] P. Zucca, C. Vinci, F. Sollai, A, Rescigno and E. Sanjust, Degradation of Alizarin Red S under mild experimental conditions by immobilized 5, 10, 15, 20-tetrakis (4-sulfonatophenyl) porphine–Mn (III) as a biomimetic peroxidase-like catalyst, *J. Mol. Catal. A: Chem.* 288 (2008) 97-102.

[42] P.K. Gautam,P.M. Shivapriya, S. Banerjee, A.K. Sahoo and S.K. Samanta, Biogenic fabrication of iron nano adsorbents from mixed waste biomass for aqueous phase removal of alizarin red S and tartrazine: Kinetics, isotherm, and thermodynamic investigation, *Environ. Prog. Sustain. Energy* 39 (2020)13326.

[43] H.S. Freeman and A.T. Peters, Colorants for non-textile applications, Elsevier 2000.

[44] P. Cooper, Removing color from dyehouse wastewaters—a critical review of technology available, *J. Soc.Dye.Colour.* 109 (1993) 97-100.

[45] G. Vijayakumar, M. Dharmendirakumar, S. Renganathan,.S. Sivanesan, G. Baskar and K.P. Elango, Removal of Congo red from aqueous solutions by perlite, *Clean–soil, air, water* 37 (2009) 355-364.

[46] F. Amourizi, K. Dashtian and M. Ghaedi, Polyvinylalcohol-citrate-stabilized gold nanoparticles supported congo red indicator as an optical sensor for selective colorimetric determination of Cr (III) ion, *Polyhedron* 176 (2020) 114278.

[47] H. Tavallali, G. Dilamy-Rad, A. Parhami and S. Lohrasbi, Copper Coordinated Congo-Red as a Solvent Assisted Selective Fluorometric and Colorimetric Chemosensor for Determination and Naked-Eye Detection of Multiple Analytes in Nanomolar Scale: A Reversible Fluorescent CN^-/CO_3^{2-} Switch that Works as Keypad Lock, *Iran. J. Anal. Chem.* 3 (2016) 76-87.

[48] H. Bensei, The Benesi–Hildebrand method for determination of Kf for DA association and e values for DA CT absorption, *J. Am. Chem. Soc.* 71 (1949) 2703-2708.

[49] D.G. Themelis, S.C. Karastogianni and P.D. Tzanavaras, Selective determination of cyanides by gas diffusion-stopped flow-sequential injection analysis and an on-line standard addition approach, *Anal. Chimica Acta* 632(1) (2009) 93-100.

[50] C.M. Zvinowanda, J.O. Okonkwo and R.C. Gurira, Improved derivatisation methods for the determination of free cyanide and cyanat e in mine effluent, *J. Hazard. Mate.* 158(1) (2008) 196-201.

[51] J. Miller and J.C. Miller, Statistics and chemometrics for analytical chemistry, *Pearson education*2018.

[52] D.S.R. Murty, A. Thangaraj and R. Radhamani, Determination of carbonate, bicarbonate and phosphate in uranium leach liquors containing $KMnO_4$ as oxidant, *Talanta* 42 (1995) 945-948.

[53] N.N. Sun and B. Yan, Rapid and facile ratiometric detection of CO_3^{2-} based on heterobimetallic metal-organic frameworks (Eu/Pt-MOFs), *Dyes Pigm.* 142 (2017) 1-7.

[54] Q. Shangguan, H. Shu, P. Li, K, Lin, R.H. Byrne, Q. Li, D.Yuan and J. Ma, Automated spectrophotometric determination of carbonate ion concentration in seawater using a portable syringe pump-based analyzer, *Marine Chem.* 209 (2019) 120-127.

[55] Z. Nan, Y. Huang, R. Zhu and Z. Xue-Liang, Fast detection of carbonate and bicarbonate in groundwater and lake water by coupled ion selective electrode, *Chinese J. Anal. Chem.* 44(3) (2016) 355-360.

استفاده جدید از دو رنگ کنگو رد و آلیزارین رد S به عنوان حسگر شیمیایی رنگ سنجی سریع و گزینش پذیر برای تشخیص با چشم غیر مسلح و تعیین کربنات در نمونه های آبی

حسین توللی*، ابولفتح پرهامی، عباس کریمپور، محمدعلی کریمی

دانشکده شیمی، دانشگاه پیام نور ۴۶۹۷۰-۱۹۳۹۵ تهران، ایران

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چکیده

در این مطالعه، یک حسگر رنگ سنجی جدید بر اساس دو رنگ کنگورد (CR) و آلیزارین رد S(ALRs) برای تشخیص کربنات در سطوح ردیابی در محیط آبی آماده شد. محدوده خطی کربنات به دست آمده از نتایج این تعیین ساده، انتخابی و سریع، ۱,۲۱ × ۱۰–۶–۹,۵۱ × ۱۰–۵ mol L^{-1} با حد تشخیص ۱,۳۱ × ۱۰–۷ mol L^{-1} بود. در موارد مورد بررسی، کاتیون‌ها، آنیون‌ها و اسیدهای ضعیف ($I^-, Br^-, F^-, Cl^-, SCN^-, SO_3^{2-}, S_2O_3^{2-}, S_2O_8^{2-}, OAc^-, H_2PO_4^-, HPO_4^{2-}, NO_3^-, C_2O_4^{2-}, HCO^{3-}, BrO^{3-}, IO^{3-}, Cit^{3-}$), ($K^+, Na^+, <1.5 \times 10^{-4}\ \text{mol L}^{-1}$ نشان داد که وجود این یون های شیمیایی، در اندازه گیری آنیون کربنات مزاحمتی نداشتند. بازیابی های نشان داده شده در محدوده ۹۸/۶–۱۰۲/۵٪ نشان دهنده اعتبار بالای روش در نمونه های آب است. به طور معمول، این ترکیب ارائه شده مقرون به صرفه و سریع، با عملکرد بدون مشکل بود که می تواند به عنوان یک گیرنده کربناته خوب در گلخانه های آب جاری و زمین های زراعی محسوب شود. علاوه بر این، با توجه به تغییرات پیچیده انجام شده در نوارهای کاغذ فیلتر، این حسگر شیمیایی ظرفیت بالقوه ای را دارد که به عنوان یک نشانگر رنگ سنجی حالت جامد آنیون کربنات استفاده شود.

واژه‌های کلیدی

حسگر شیمیایی رنگ سنجی; تشخیص با چشم غیر مسلح؛ آلیزارین رد s; کنگورد؛ تشخیص کربنات؛ آب آشامیدنی