HPLC detection of synthetic dyes in residual waters from wastewater treatment using adsorption on magnetic materials

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Abstract
Surface water contamination by synthetic dyes generates human and wildlife adverse health effects and causes photosynthesis activity decrease due to intense sun-light absorption of these pollutants. 50% of total discharged industrial effluents contain azo dyes. Congo Red is a benzidine based anionic azo dye that is usually employed in the rubber, paper, and plastic industries. Congo Red can cause eye and skin irritation and is potentially carcinogenic. This study explores the development of a new HPLC method to detect synthetic dyes in residual waters coming from wastewater treatment technologies based on magnetic material adsorption. Cobalt ferrite ($CoFe_2O_4$) and chitosan-coated cobalt ferrite ($CoFe_2O_4$-Chit) prepared by a simple co-precipitation method were tested as adsorbents for Congo Red (CR). The effect of contact time, solution pH, and initial dye concentration were studied with respect to Congo Red adsorption efficiency. The adsorption experiments were performed at pH = 4.5 and 10.8. The highest value for the removal efficiency using the magnetic material of cobalt ferrite coated with chitosan ($CoFe_2O_4$-Chit) was obtained at pH 10.8.

Keywords: adsorption, HPLC, magnetic materials, synthetic dyes, wastewater treatment

INTRODUCTION

Water is a very stable compound vital for all known forms of life. Industrialization and modernization have negatively influenced clean water resources. Many substances, such as heavy metals [1], dyes, pharmaceuticals [2], surfactants, pesticides, personal care products, and others, have contaminated water resources [3]. In recent years, the use of synthetic chemical dyes in various industrial processes has increased considerably. Some areas where these chemicals are frequently used are paper and pulp manufacturing, dyeing of cloth, plastics, leather treatment, printing, etc. Industrial wastewaters containing such dyes are generally discarded as effluents. Since some of these dyes are toxic, their removal from industrial effluents is a major environmental problem [4]. It has been found that these pollutants are carcinogenic, toxic, and harmful, in addition to causing many health problems such as allergy, skin irritation, nausea, hard breathing, sweating, vomiting, high bold pressure, confusion, headache, and mutations [5].

Dyes have complex aromatic molecular structures and are generally resistant to light, temperature, and oxidizers. This characteristic feature makes the dye non-degradable and therefore causes bioaccumulation in living organisms, leading to severe diseases and disorders [6]. Discarding synthetic dyes containing effluents into surface water can cause severe problems such as increasing the chemical oxygen demand (COD), delaying the growth of microorganisms, and reducing light penetration and visibility. Besides, dyes are resistant to light and moderate oxidative agents; therefore, they cannot be completely removed by conventional biological treatment processes, such as activated sludge and anaerobic digestion [7]. Normally, dyes are water-soluble or water-dispersible organic compounds that can be absorbed into the substrate by destroying the crystal structure of the substance. Dye molecules are chemically bound to the surface and after application to material, they become part of it [8]. Dyes may be classified as anionic (acid and color reagents), cationic (basic dyes), and non-ionic (dispersion dyes) dyes. However, reactive azo dyes constitute more than 50% of

all textile dyes used in industry and are characterized by the existence of double azo-nitrogen bonds [9]. There are more than 100,000 commercial dyes with over 7×10^5 tons of dyes produced annually and approximately 10% were discharged from textile and related industries [10].

Multiple purification methods are used for dyes removal, namely filtration, chemical coagulation and precipitation, chemical oxidation, electrochemical removal and electrocoagulation, photocatalysis, ultrasonic or biological decomposition, and adsorption. Adsorbents are the main key for adsorption where a wide range of materials was used for this purpose. Conventional adsorbents such as clay, zeolite, bentonite, and montmorillonite were highly reported due to their abundance and low cost. However, finding new materials or modifying the classical ones were used to obtain high adsorption capacity and better adsorption conditions. For example, activated carbon is highly reported in terms of dyes and organics adsorption because of its remarkable effectiveness in this field, although its cost is relatively high and limits its usage [11]. There are numerous common approaches to eliminate the dyes from water/wastewater. These technologies can be divided into three categories: physical methods such as membrane filtration processes (nano-filtration, reverse osmosis, electrodialysis), sorption techniques, ion exchange, irradiation, electrokinetic coagulation; chemical methods such as oxidative process, H_2O_2 + Fe(II) salts (Fenton's reagent), ozonation, photochemical, electrochemical destruction; and biological methods such as decolorization by white-rot fungi, adsorption by living/dead microbial biomass, anaerobic textile-dye bioremediation systems and other microbial [12]. An adsorbent is a porous insoluble sponge-like substance with the ability to capture and trap adsorbate particles onto itself. This is the most crucial element of the adsorption process. The one issue frequently related to the adsorption technique is the cost of the adsorbent utilized. Adsorption is an exceptional dye removal technique as it does not require any additional special equipment and is easy to conduct. Adsorption is different, depending on solutions and membrane member properties, but is facilitated mainly in the hydrophobic region of membrane materials, due to hydrophobic interactions [13]. A good adsorbent should be able to function in varying dye concentrations, a wide range of pH as well as temperature [14]. Adsorption is one of the effective and economical methods for treating contaminants such as synthetic dyes, for example, Congo Red (CR). This involves the use of various materials (adsorbents) to remove contaminants from aqueous solutions. Congo Red is an azo dye most often used in the textile industry. It is believed to be the first dye with the ability to dye cotton, is very soluble in water, and is frequently used in the textile, plastic, and industrial industries. Congo Red becomes a contaminant when it is discharged into water bodies in the form of industrial effluent without being properly treated and therefore turns from a good product into a pest. It is difficult to degrade biologically due to its stable structure, which endangers the environment [15]. Water matrices have complex content from a physical and chemical point of view, and this makes it difficult to create an adequate analytical method [16]. The main innovation of the HPLC method is the possibility to analyze a wide variety of dyes (direct dispersed, reactive, acid, and basic dyes) with a single chromatographic method. Also, the inclusion of both Diode Array Detector (DAD) and MS analyzes allows a precise and sensitive analysis of the dyes. Other techniques used routinely in most laboratories are UV/VIS micro spectrometry (MSP) and Fourier-transform infrared spectrometry (FT-IR). Many laboratories use additional techniques, such as Raman spectrometry, thin layer chromatography, chemical, and melting tests [17].

The present paper was focused on the development of a new HPLC-DAD method for the detection of Congo Red dye from water samples. The HPLC-DAD method was applied to test the removal efficiency of Congo Red from synthetic aqueous solutions using adsorption on magnetic materials such as Cobalt ferrite ($CoFe_2O_4$) and chitosan-coated cobalt ferrite ($CoFe_2O_4$-Chit). Similar magnetic material adsorption experiments regarding Congo Red and other dyes will be extended in another study on real wastewater sample matrices.

EXPERIMENTAL PART

Chemicals and working standard solution

Acetonitrile, methanol, and ammonium acetate for liquid chromatographic analysis (HPLC) were purchased from Merck. $C_{32}H_{22}N_6Na_2O_6S_2$ was used as the source for the Congo Red stock solution. 25 mg of 99% $C_{32}H_{22}N_6Na_2O_6S_2$ (Sigma Aldrich) was dissolved in 25 mL of methanol to prepare 1000 mg/L of Congo Red stock solution. Samples of different concentrations of Congo Red were prepared from this stock solution by appropriate dilutions (Fig. 1).

Fig. 1. Congo Red samples of different concentrations

The raw materials such as $Fe(NO_3)_3 \cdot 9H_2O$ 96%, $Co(NO_3)_2 \cdot 6H_2O$, chitosan, glacial acetic acid (analytical grade, Sigma Aldrich) were used in the synthesis of magnetic materials. The solutions pH was changed with HCl 35% suprapure and NH_4OH 25% (Merck origin).

Equipment and chromatographic method

Congo Red analysis was performed on an Agilent 1200 series HPLC system equipped with: semipermeable membrane degasser, quaternary pump, autosampler with variable injection volume (0.1-100 µl), thermostatted column compartment, and a Diode Array Detector (DAD) with the ability to record simultaneously UV-VIS spectra (190-900 nm) and up to 8 discrete wavelengths in this range. Agilent ChemStation software was used for data acquisition, processing, and reporting. The optimum mobile phase composition was 100 mM Ammonium Acetate in water brought to pH 5.0 and Acetonitrile in the ratio 30/70 (v/v). All chromatographic runs were carried out on an Acclaim Surfactant Plus column (150 x 3.0 mm, 3.0 µm) from Thermo Scientific. The column was kept at 30°C. Experiments were performed in isocratic elution conditions at a flow rate of 0.5 mL/min. Injection volume was 10 µL and the chromatogram run-time was only 9 minutes (Congo Red retention time approx. 7.6 min). Detection of the target compound was performed at the absorption maximum of 506 nm which was observed in the UV-Vis spectrum obtained by HPLC-DAD. Figure 2 shows the structural formula of Congo Red, while in Figure 3 it is presented its UV-Vis spectrum obtained by HPLC-DAD.
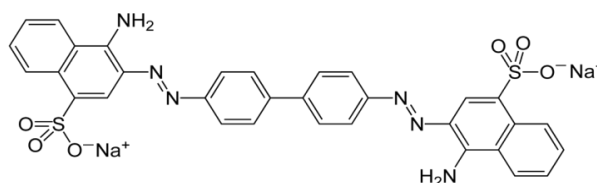

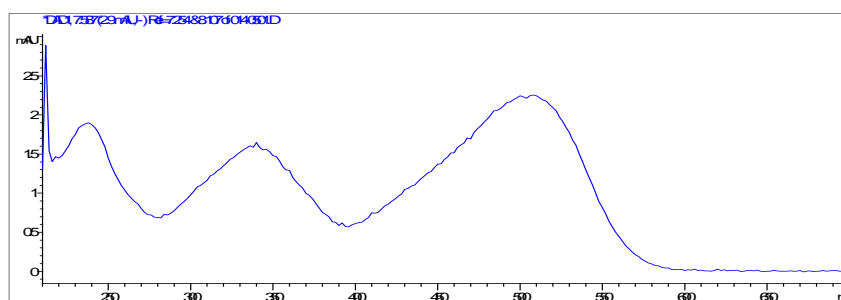**Fig. 2.** Structural formula of Congo Red ($C_{32}H_{22}N_6Na_2O_6S_2$)

Fig. 3. UV-VIS absorption spectra of Congo Red obtained by HPLC-DAD

The effects of operating conditions on the CR removal process were determined in batch experiments using a GFL 3015 orbital shaker at 150 rpm (rotation per minute). All the experiments were carried out at room temperature. The dye solution's pH was established with an Agilent 3200 laboratory pH-meter. The influence of CR solution's pH was established in experiments using a solution of 102.81 mg CR/L (experimentally determined). The volume of CR solution used in the experiments is 25 mL, and the amount of adsorbent was 0.01 g. The experiments were performed at room temperature over 6 hours to reach equilibrium. Tests were performed in the range of pH 2.22-10.8, the pH of the solution was changed using HCl and NH_4OH solutions of various concentrations. The equilibrium time was determined from tests performed in the time range of 5–360 min. The influence of initial CR concentration on the adsorption efficiency using $CoFe_2O_4$ and $CoFe_2O_4$-Chit was achieved from experiments conducted by varying the concentration in the range of 4.98–102.81 mg CR/L.

The CR removal efficiency (R, %) was calculated using the following equation:

$$R = \frac{C_0 - C_t}{C_0} \times 100\% \tag{1}$$

where R (%) is the CR removal efficiency, C_0 (mg/L) is the initial concentration of CR, and C_t (mg/L) is the concentration of CR at various contact times/different pH values.

RESULTS AND DISCUSSION

The cobalt ferrite ($CoFe_2O_4$) and chitosan-coated cobalt ferrite ($CoFe_2O_4$-Chit) were prepared by the co-precipitation method. The synthesis protocol and characterization of both materials were described in the previous publication [18].

Considering the significant water solubility and polarity of Congo Red, which are mainly given by its sulfonic groups, it was decided to separate this compound using a chromatographic column dedicated to surfactant separation (Acclaim Surfactant Plus). The reason for this column choice was the structural similarity between surfactants and some synthetic dyes when considering the highly ionized polar groups in their molecules (sulfonic group, quaternary ammonium, etc.). To obtain a rapid and sensitive HPLC method, multiple optimization tests were performed using different injection volumes (2 µL, 10 µL), different values of the mobile phase flow-rate (0.5 mL/min, 0.6 mL/min). Also, for the elution under an isocratic regime, different compositions of the mobile phase were tested with variable proportions between the aqueous and organic solvent (50/50, 30/70, 25/75, 20/80). The optimum composition for the mobile phase was 30% aqueous phase (100 mM ammonium acetate, pH 5) and 70% organic solvent (Acetonitrile), which resulted in narrow and high symmetry Congo Red peak. The detection wavelength was also optimized considering maximum sensitivity. Accordingly, 506 nm was chosen as Congo Red detection wavelength in the Visible domain. Selected wavelength allowed quantitation of Congo Red down to 0.02 mg/L (20 µg/L) with a S/N ratio of approx. 10.

Final optimized LC parameters (surfactant column, 10 µL injection volume, 0.5 mL/min flow-rate, 30/70 mobile phase composition, 506 nm detection wavelengths) allowed for rapid

and sensitive detection of Congo Red which presented increased peak efficiency and the reduced retention time (7.5 min). Method calibration curve was obtained using Congo Red aqueous solutions of: 0.1 mg/L, 0.2 mg/L, 0.5 mg/L, 1 mg/L, 2.5 mg/L, 5 mg/L and 10

mg/L (Fig. 4). Expected levels of Congo Red in spiked water samples (laboratory-controlled) before and after adsorption (removal) on magnetic materials did not require a lower calibration level.
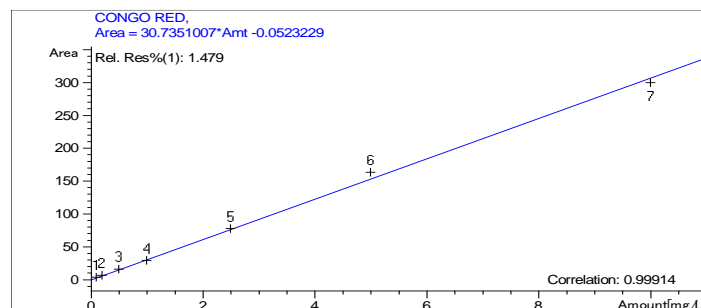

Fig. 4. HPLC-DAD calibration curve of Congo Red

The influence of the process control parameters such as contact time, dye solution pH, and initial CR concentration on removal efficiency

was examined. The results regarding the influence of CR solutions pH on removal efficiency is depicted in Figure 5.
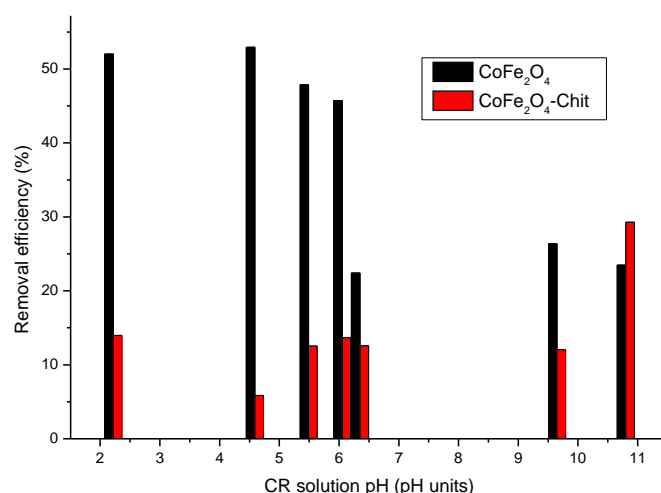

Fig. 5. The effect of CR solution's pH on removal efficiency

From the examination of the results presented in Figure 5, it can be observed that magnetic materials tested are characterized by different adsorption behavior. A decrease in removal efficiency with the increase of CR solution pH was noticed in the case of $CoFe_2O_4$. This may be due to the fact that at lower pH, the CR has a cationic form, and thus there can be a strong electrostatic attraction between the positively charged CR molecules and negatively charged $CoFe_2O_4$ surface. This has as result the higher removal efficiency at lower pH. At higher pH, the removal efficiency is reduced due to the strong electrostatic repulsion between the

negative surface of $CoFe_2O_4$ and the anionic form of CR molecules that can be found in the basic medium. The higher removal efficiency was observed at a pH of around 4.5 for $CoFe_2O_4$. A different behavior was found in the case of $CoFe_2O_4$-Chit. At lower pH, there is a high concentration of protons that protonate the amine ($-NH_2$) and hydroxyl groups ($-OH$) from the chitosan structure. These protonated sites will interact by electrostatic forces with negatively charged sulfonic functional groups of Congo Red. However, there can occur repulsive forces with protonated functional groups of Congo Red (Fig. 6).
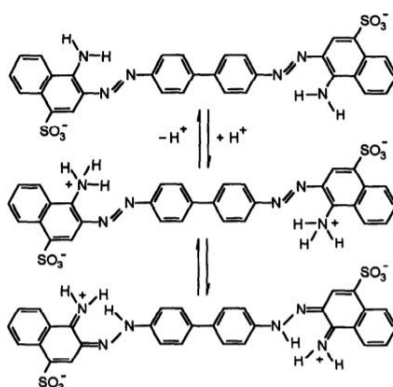
Fig. 6. Protonation and tautomeric equilibrium of the Congo red in acidic solution [19]

At higher pH, the protonation of the CR is reduced and the sulfonate groups can interact electrostatically with the positive functional groups from the surface of chitosan. Also, hydrogen bonding and van der Waals forces can occur with effect on the removal efficiency in a neutral or slightly acid medium. The higher value of removal efficiency has been registered at pH equal with 10.8. Similar trends regarding the effect of the CR solutions pH are presented in the literature data [20]. Another conclusion that can be drawn from these results is that the $CoFe_2O_4$ has a higher CR removal efficiency than $CoFe_2O_4$-Chit. This can be explained by the fact that $CoFe_2O_4$-Chit has been prepared as macroparticles while $CoFe_2O_4$ was obtained as nanoparticles (Fig. 7). Consequently, $CoFe_2O_4$-Chit has a lower specific surface area, adsorption capacity, and efficiency than $CoFe_2O_4$.

Fig. 7. The image of $CoFe_2O_4$ particles (left) and $CoFe_2O_4$-Chit particles (right)

The variation of removal efficiency with the contact time is displayed in Figure 8. The experiments have been performed at pH = 4.5 and 10.8.
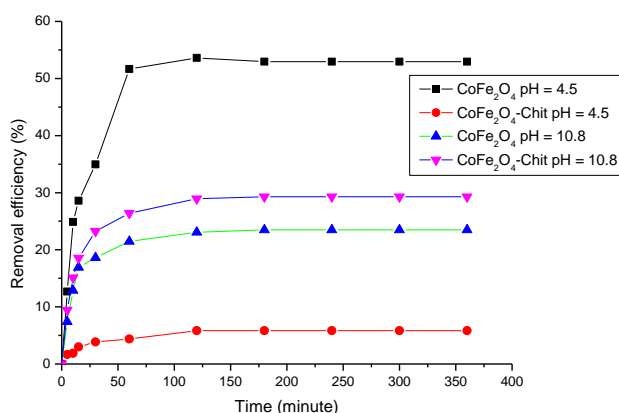

Fig. 8. The effect of contact time on the removal efficiency of $CoFe_2O_4$ and $CoFe_2O_4$-Chit

According to the results from Figure 8, it can be concluded that the time to reach the equilibrium is 180 minutes for both materials tested. The rate of the CR removal process is higher in the

first 30 minutes due to the numerous free active sites on the surface of the adsorbent. After this period the removal rate increases slowly as a consequence of the saturation of active sites.

The influence of the CR concentration in the initial solutions/wastewater was analyzed in experiments with CR solutions with a concentration in the range 4.98 – 102.91 mg/L at 240 minutes contact time. The results are summarized in Figure 9.
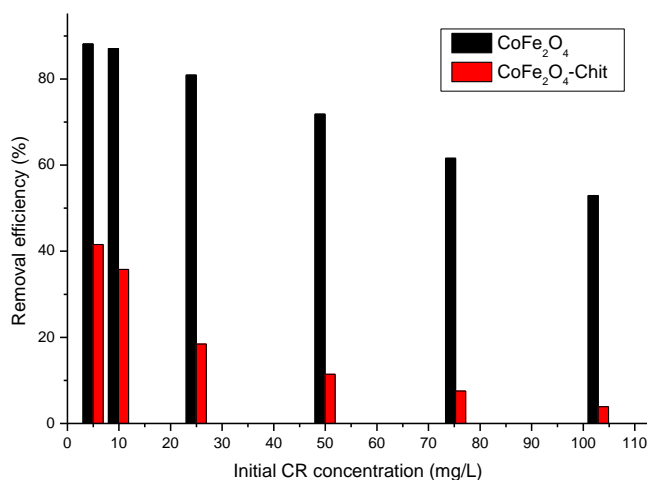

Fig. 9. The effect of initial CR concentration on removal efficiency of $CoFe_2O_4$ and $CoFe_2O_4$-Chit

Figure 9 shows that the removal efficiency decreases from 88.15 to 52.91% as the initial Congo Red concentration increases from 4.98 to 102.91 mg/L for adsorption onto $CoFe_2O_4$. A decrease from 41.57 to 3.91% was observed for adsorption onto $CoFe_2O_4$-Chit. This can be explained based on the ratio of the available active sites of magnetic materials/adsorbents to the initial number of moles of Congo Red is large at low initial concentration. According to this, the fractional adsorption does not depend on initial concentration [21].

CONCLUSIONS

The present study was developed a highly sensitive and rapid HPLC-DAD method for the detection of Congo Red dye from spiked water samples. Chromatographic separation was achieved in 9 minutes. All HPLC method parameters (nature and composition of the mobile phase, flow-rate, injection volume, detector wavelength, and column temperature) were optimized for the rapid separation and sensitive detection of Congo Red to determine this synthetic dye at concentration levels down to parts per billion (μg/L) from aqueous solutions. Due to several UV-Vis chromophores and conjugated aromatic rings present in Congo Red molecule, highly sensitive detection of this analyte was achieved at 506 nm with an instrumental quantitation limit (HPLC-DAD) of 0.02 mg/L (20 μg/L). Nonetheless, a 5 time's higher concentration level was employed as the lowest level for the Congo Red calibration curve of (0.1 – 10 mg/L).

Cobalt ferrite and chitosan-coated cobalt ferrite were employed for the adsorption removal of Congo Red dye from synthetic water samples. In the case of cobalt ferrite, the maximum retention efficiency (R) was recorded at a pH value of 4.5 As the pH value increases, there is a decrease in retention efficiency. In the case of cobalt ferrite coated with chitosan, it was found that the maximum retention value was recorded at a pH value of 10.8. As the pH increases, the amount of Congo Red retained per gram of cobalt ferrite coated with chitosan increases. With increasing pH, the electrostatic attraction interactions between the negatively charged surface of the chitosan-coated cobalt ferrite and the Congo Red anionic molecules also increase. As a result of low pH values, the adsorption of Congo Red, which is an anionic dye, is reduced and increases with increasing pH. As a result, experiments to establish the equilibrium time were performed at two pH values (4.5 and 10.8), resulting in a decrease in values from 41.57 to 3.91% for adsorption on $CoFe_2O_4$-Chit.

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