Fe^{3+} Immobilized Cryogel as an Immobilized Metal Affinity Chromatography (IMAC) Support for Dye removal

Boya Uzaklaştırma İçin İmmobilize Metal Şelat Afinite Kromatografi Matriksi Olarak Fe^{3+} İmmobilize Kriyojeller

Research Article / Araştırma Makalesi

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ABSTRACT

In this study, the potential use of Fe^{3+} chelated Cibacron Blue F3GA immobilized poly(acrylamide-allyl glycidyl ether) [p(AAm-AGE)-CB-Fe^{3+}] cryogel to remove dyes from aqueous solutions was evaluated. Cryogel was prepared by radical polymerization. Cibacron Blue F3GA was covalently immobilized on p(AAm-AGE) cryogel and then chelated with Fe^{3+} ions. Maximum adsorption capacities were determined as 14.50 mg/g cryogel for Congo Red and 18.78 mg/g cryogel for Reactive Green. It was also determined that Freundlich isotherm was convenient for both dyes. More than 90% of the adsorbed dyes were desorbed in all cases. This study indicates that p(AAm-AGE)-CB-Fe^{3+} cryogelic material can be efficiently used as adsorbent for different dyes at high concentrations.

Key Words
Dye removal, congo red, reactive green, cryogel, IMAC.

ÖZET

Bu çalışmada, Fe^{3+} şelatlanmış Cibacron Blue F3GA immobilize poli(akrilamid-allil glisidil eter [p(AAm-AGE)-CB-Fe^{3+}] kriyojelin boya (Congo Red, Reactive Green) uzaklaştırma için potansiyel kullanımı araştırılmıştır. Kriyojel, radikalik polimerizasyon ile sentezlenmiştir. Cibacron Blue, kriyojel üzerine kovalent olarak bağlanmış ve Fe^{3+} iyonları ile şelatlanmıştır. Maksimum adsorpsiyon kapasiteleri, Congo Red için 14.50 mg/g kriyojel; Reaktive Green için 18.78 mg/g kriyojel olarak belirlenmiştir. Ayrıca, hem Congo Red hem de Reactive Green için Freundlich izoterminin uygun olduğu belirlenmiştir. Bu çalışma, p(AAm-AGE)-CB-Fe^{3+} kriyojelik materyalin yüksek derişimdeki farklı boyalar için adsorban olarak etkin bir şekilde kullanılabileceğini göstermiştir.

Anahtar Kelimeler
Boya uzaklaştırma, Congo Red, Reactive Green, kriyojel, İMAK.

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Abbreviations

IMAC	Immobilized metal chelate affinity chromatography
AAm	Acrylamide
AGE	Allyl glycidyl ether
MBAAm	N,N'-methylene-bis(acrylamide)
APS	Ammonium persulfate
TEMED	N,N,N',N'-tetramethylene diamine
p(AAm-AGE)	Poly(acrylamide-allyl glycidyl ether)
p(AAm-AGE)-CB	Cibacron Blue F3GA immobilized poly(acrylamide-allyl glycidyl ether)
p(AAm-AGE)-CB-Fe^{3+}	Fe^{3+}-chelated Cibacron Blue F3GA immobilized poly(acrylamide-allyl glycidyl ether)
SEM	Scanning electron microscope
FTIR	Fourier Transform Infrared
CB	Cibacron Blue F3GA
CR	Congo Red
RG	Reactive Green
P	Porosity Percent
PM	Porosity Percent for Macropores

INTRODUCTION

Dyes are important water pollutants which are generally present in the effluents of textile, leather, paper and dye manufacturing industries [1]. They usually have a synthetic origin and complex aromatic molecular structures which make them more stable and more difficult to biodegrade [2]. Due to the low biodegradability of dyes, a conventional biological wastewater treatment process is not very efficient in treating a dye wastewater. Color removal from textile effluents has been given much attention in the last few years, not only because of its potential toxicity, but also mainly due to its visibility problems [3,4].

Among the various classes of dyes, reactive dyes are the only colorants designed to bond covalently with the fabrics. Reactive dyes contain chromophoric groups such as azo, anthraquinone, triarylmethane, etc. and reactive groups, e.g., vinyl sulfone, chlorotriazine, trichloropyrimidine, difluorochloropyrimidine, etc. that forms covalent bonds with the fibers. Reactive Green is a dichlorotriazine dye which contains six sulfonic acid groups and five basic primary and secondary amino groups [5]. Congo Red is a diazo dye. Due to a color change from blue to red at pH 3.0-5.2, Congo Red can be used as a pH indicator. Furthermore, in biochemistry and histology, Congo Red is used to stain microscopic preparates, especially as a cytoplasm and erythrocyte stain. Congo Red has a strong, though apparently non-covalent affinity to cellulose fibers. However, the use of Congo Red in the cellulose industries (cotton textile, wood pulp & paper) has long been abandoned, primarily because of its tendency to change color when touched by sweaty fingers, to run and because of its toxicity. The release of azo dyes into the environment in effluent from dye-utilizing industries has become a major concern in wastewater treatment, since some azo dyes or their metabolites may be mutagens or carcinogens [6,7].

There are many processes available for wastewater treatment such as chemical oxidation [7], foam flotation [8], electrolysis [9], biodegradation [10], adsorption [3,11], chemical coagulation [12,13] and photocatalysis [14]. Amongst several wastewater treatment technologies, adsorption is the most versatile process and widely used [15], for the pollutants removal from wastewaters. Activated carbon is the most commonly used method of dye removal by adsorption. Activated carbon is expensive, with high regeneration cost and 10–15% loss in the reactivation procedure [16]. Thus, many researchers researched for cheaper substitutes, which are relatively inexpensive, and are at the same time endowed with reasonable adsorptive capacity [17-23]. These studies include the use of coal [24], fly ash [25], activated clay [26], palm-fruit bunch [27], bagasse pith [28], cellulose based waste [29], peat, bentonite, slag and fly ash [30], activated sludge [31], rice husk and gram husk [32-34].

Among the polymeric materials utilized in bioseparation, gel matrices are the most widely used, for instance, as carriers in chromatography, media for electrophoresis, isotachophoresis and isoelectric focusing, as media for immunodiffusion assays, etc [35,36]. Cryogels are gel matrices that are formed in moderately frozen solutions of monomeric or polymeric precursors [37]. The unique macroporous morphology of cryogels, in combination with osmotic, chemical and mechanical stability, makes them attractive matrices for chromatography of large entities such as protein aggregates, membrane fragments, viruses, cell organells and even whole cells [38]. Cryogels are also cheap materials and have several advantages

such as large pores, short diffusion path, low pressure drop, and very short residence time for both adsorption and elution [39].

Immobilized Metal Ion Affinity Chromatography, IMAC, is a separation technique that uses covalently bound chelating compounds on solid chromatographic supports to entrap metal ions, which serve as affinity ligands for various proteins, making use of coordinative binding of some amino acid residues exposed on the surface [40]. The benefits of IMAC-ligand stability, high protein loading, rapid purification, mild elution conditions, simple regeneration and low cost [41]-are decisive when developing large-scale purification procedures for industrial applications.

This work reports on the removal of two different azo dyes from aqueous solutions by immobilized metal-chelate affinity chromatography with a cryogelic material. The novelty of this study is the first study for removal of dye molecules using cryogelic materials. Cryogel was prepared by radical polymerization of acylamide and allyl glycidyl ether. Cibacron Blue F3GA was covalently immobilized on p(AAm-AGE) cryogel, via the reaction between the chloride groups of the reactive dyes and the epoxide groups of the AGE and then Cibacron Blue F3GA immobilized p(AAm-AGE) cryogel was chelated with Fe^{3+} ions. Cibacron Blue F3GA immobilized cryogel carrying 25.8 μmol Fe^{3+} ions was used in dye adsorption studies under different conditions (pH, initial dye concentration, temperature, flow rate).

MATERIALS AND METHOD

Dyes and chemicals

Acrylamide (AAm, more than 99.9% pure, electrophoresis reagent), allyl glycidyl ether (AGE, 99%), N,N'-methylene-bis(acrylamide) (MBAAm), ammonium persulfate (APS) and N,N,N',N'-tetramethylene diamine (TEMED, more than 99%) were supplied from Sigma (St Louis, USA). Congo Red and Reactive Green were supplied from Sigma (St Louis, USA). All other chemicals were of the highest purity commercially available and were used without further purification. All water used in the experiments was purified using a All water used in the adsorption experiments was purified using a Millipore S.A.S 67120 Molsheim-France facility whose quality management system was approved by an accredited registering body to the ISO 9001. Before use the laboratory glassware was rinsed with deionised water and dried in a dust-free environment.

Production of p(AAm-AGE) Cryogel

Production of p(AAm-AGE) monolithic cryogel was performed using the Arvidsson et al's procedure by modification [42]. Allyl glycidyl ether (Allyl-2,3-epoxypropylether- AGE, % 99) was selected in order to insert reactive epoxy groups in the cryogel. Shortly, monomers (1.3068g AAm, 218 μL AGE (0.97 g/mL)) were dissolved in 30 mL deionized water. Total concentration of monomers was 6% (w/v). The cryogel was produced by radical polymerization initiated by TEMED (24 μL) and APS (14.4 mg). After adding APS the solution was cooled in an ice bath for 15 min. TEMED was added and reaction mixture was stirred for 30 min. Then, reaction mixture was poured into plastic syringe (4 mL) with closed outled at the bottom. The polymerization solution in the plastic syringe was frozen at -12°C for 24 h and then thawed at room temperature. After washing with 200 ml of water, the cryogel was stored in buffer containing 0.02% sodium azide at 4°C until use.

Cibacron Blue F3GA Immobilization

P(AAm-AGE) cryogels were washed with 2 L water to remove unreacted monomers. Cibacron Blue F3GA immobilization was performed according to related literature [43]. P(AAm-AGE) cryogels were bottled up into Cibacron Blue F3GA solution (100 mg Cibacron Blue F3GA dissolved in 30 mL deionized water) and shaken at 150 rpm for 30 min at 60°C. 1.5 g NaCl was added to reaction mixture and shaken for 1 h at 60°C. Then, temperature of reaction was increased to 70°C and 0.15 g Na_2CO_3 added to reaction mixture. Reaction was continued for 2 h at 70°C. Reaction mixture was cooled to room temperature and cryogels were washed with water until washings are colorless. Finally, cryogels were washed with 1.0 M NaCl and water. Cibacron Blue F3GA immobilized cryogels were stored in 0.02% sodium azide at 4°C.

Incorporation of Fe^{3+} Ions

Chelates of Fe^{3+} ions with Cibacron Blue F3GA immobilized p(AAm-AGE) cryogels were carried

out in a re-circulating system with 50 mL of aqueous solutions containing 100 ppm Fe^{3+} solution. Fe^{3+} solution was prepared in universal buffer at pH 5 which was the optimum pH for Fe^{3+} chelate formation at room temperature ($Fe(NO)_3.9H_2O$ was used as the source of Fe^{3+} ions). Cibacron Blue F3GA immobilized p(AAm-AGE) cryogels were conditioned with pH 5.0 universal buffer before Fe^{3+} immobilization. Then, Fe^{3+} solution was passed through the cyrogels for 7 hours. Fe^{3+} ion concentrations in initial solution and at equilibrium were determined with Perkin-Elmer AA 700 atomic absorption spectrophotometer. Fe^{3+} leakage from p(AAM-AGE)-CB-Fe^{3+} cryogels was investigated as a function of medium pH (5.0-8.0) and also in the dye desorption medium (50% ethanol).

Characterization of Cryogel

The swelling degree of the cryogels (S) was determined. Cryogels were dried to constant mass at vacuum oven at 55^{0}C and 100 mbar and masses of dried cryogels were determined ($m_{dry\ gel}$). The dried cryogels were bottled up to 50 mL ionized water and mass of swollen cryogel was determined regularly for 24 h period ($m_{wet\ gel}$).

The swelling degree was calculated as:

$$S = (m_{wet\ gel} - m_{dry\ gel}) / m_{dry\ gel} \quad (1)$$

Percentage of porosity and porosity for macropores were also calculated.

$$P = [(m_{swollen\ gel} - m_{water\ bound})/m_{swollen\ gel}] \times 100 \quad (2)$$

$$PM = [(m_{swollen\ gel} - m_{squeezed\ gel})/m_{swollen\ gel}] \times 100 \quad (3)$$

The morphology of a cross section of the dried cryogel was investigated by scanning electron microscope (SEM). The sample was fixed in 2.5% glutaraldehyde in 0.15 M sodium cacodylate buffer overnight, post-fixed in 1% osmium tetraoxide for 1 h. Then the sample was dehydrated stepwise in ethanol and transferred to a critical point drier temperated to +10^{0}C where the ethanol was changed for liquid carbon dioxide as transitional fluid. The temperature was then raised to +40^{0}C and the pressure to ca. 100 bar. Liquid CO_2 was transformed directly to gas uniformly throughout the whole sample without heat of vaporization or surface tension forces causing damage. Release of the pressure at a constant temperature of +40^{0}C resulted in dried cryogel sample. Finally, it was coated with gold-palladium (40: 60) and examined using a JEOL JSM 5600 scanning electron microscope (Tokyo, Japan). FTIR spectra of the p(AAm-AGE)-CB-Fe^{3+} cryo-gel was obtained by using a FTIR spectrophotometer (FTIR 8000 Series, Shimadzu, Japan). The dry cryogel (about 0.1 g) was thoroughly mixed with KBr (0.1 g, IR Grade, Merck, Germany), and pressed into a tablet, and the spectrum was then recorded.

Adsorption studies

Effects of pH, initial dye concentration, temperature and flow rate on the adsorption capacity were studied. To observe the effects of pH medium, pH of the solution was changed between pH 4.0-9.0 adjusted with NaOH and HCl (4.0-9.0 for Reactive Green, 5.0-9.0 for Congo Red). To determine the effects of initial dye concentration on adsorption, it was changed between 25-5000 ppm. To observe the effects of temperature on the adsorption, temperature of the medium was changed between 15^{0}C and 35^{0}C. Finally, to determine the effects of flow rate, the flow rate of the solution was changed in the range of 0.3–3.0 mL/min. Initial and final concentrations of Reactive Green and Congo Red were spectroscopically determined at 673 and 498 nm, respectively.

Desorption and Reusability Studies

Desorption of Reactive Green from p(AAm-AGE)-CB-Fe^{3+} cryogel was performed with pH 7.0 phosphate buffer and desorption of Congo Red was performed with 50% ethanol-50% ionized water mixture. To determine the reusability of Fe^{3+} immobilized cryogel, adsorption-desorption cycle was repeated for ten times. In order to regenerate and sterilize, after elution cryogel was washed with pH 7.0 phosphate buffer and ionized water.

RESULTS AND DISCUSSION

Characterization of p(AAm-AGE)-CB-Fe^{3+} Cryogel

A supermacroporous cryogel was produced by copolymerization in the frozen state of monomers, acrylamide (AAm) and allyl glycidyl ether (AGE)

with N,N'-methylene-bis(acrylamide) (MBAAm) as a cross-linker in the presence of amonium persulfate (APS)/N,N,N',N'-tetramethylene diamine (TEMED) as initiator/activator pair. The functional epoxy groups on the surface in the pores of the cryogels allowed their modification with the ligand, Cibacron Blue F3GA. The scanning electron micrograph of the internal structure of the p(AAm-AGE)-CB-Fe^{3+} cryogel is shown in Figure 1. p(AAm-AGE)-CB-Fe^{3+} cryogel produced in such a way have non-porous and thin polymer walls, large continuous interconnected pores (10-100 μm in diameter, supermacroporous) that provide channels for the mobile phase to flow through. The equilibrium swelling degree of the p(AAm-AGE)-CB-Fe^{3+} cryogel was 21.8 g H_2O/g cryogel. p(AAm-AGE)-CB-Fe^{3+} cryogel is opaque, sponge like and elastic. This cryogel can be easily compressed by hand to remove water accumulated inside the pores. When the compressed piece of cryogel was submerged in water, it soaked in water and within 1-2 s restored its original size and shape. Percentage of porosity and porosity for macropores were also determined as 4.4% and 77.2%, respectively.

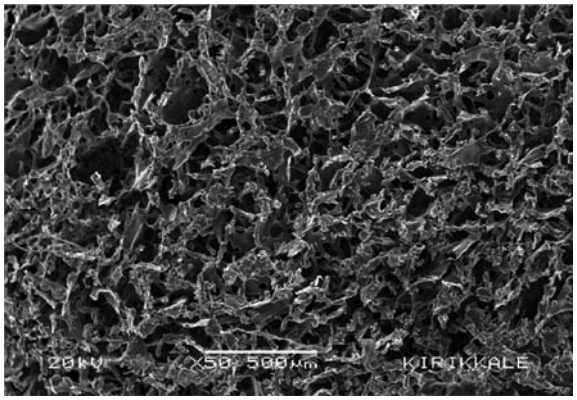

Figure 1. Scanning electron micrograph of the inner part of the p(AAm-AGE)-CB-Fe^{3+} cryogel.

The schematic diagram for the production of p(AAm-AGE) cryogel and immobilization of Cibacron Blue F3GA molecules through the p(AAm-AGE) cryogel was given in Figure 2. Cibacron Blue F3GA was used as the dye-ligand. Then, Fe^{3+} ions covalently incorporated onto matrix. The incorporation of Cibacron Blue F3GA was found to be 168.2 μmol/g cryogel. The studies of Cibacron Blue F3GA leakage from the p(AAM-AGE) cryogel showed that there was no dye leakage in any medium used throughout this study. Maximum Fe^{3+} loading was found to be 25.8 μmol/g cryogel. The number of locations of surface-exposed electron-donating amine groups and their ability to coordinate with chelated metal ions dictate the adsorption of proteins on metal-immobilized adsorbents.

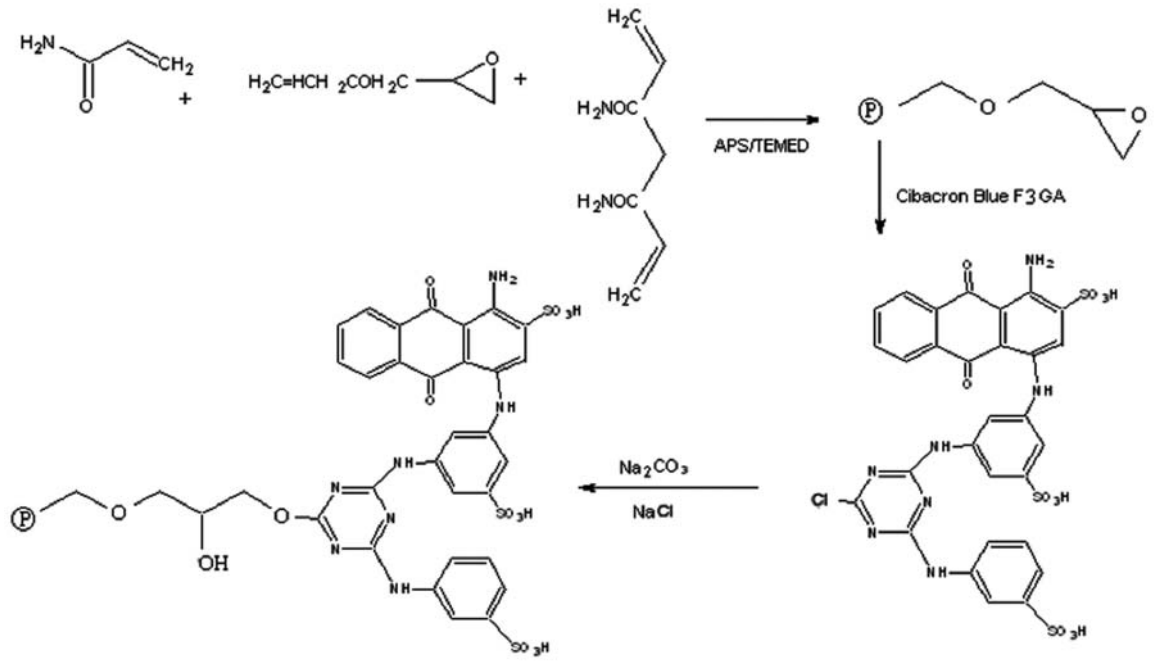

Figure 2. Schematic diagram for the production of p(AAm-AGE) cryogel and immobilization of dye molecules through the p(AAm-AGE) cryogel.

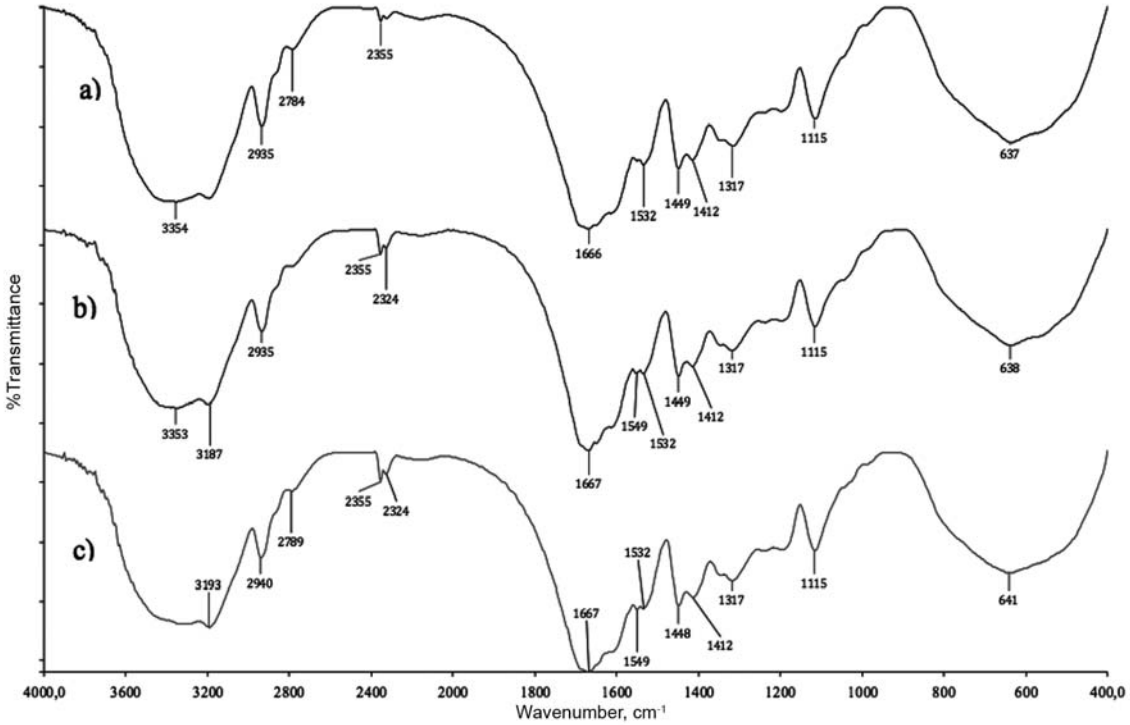

Figure 3. FTIR spectrum of p(AAm-AGE) (a), p(AAm-AGE)-CB (b) and p(AAm-AGE)-CB-Fe^{3+} (c) cryogels.

Cibacron Blue F3GA is covalently immobilized on p(AAm-AGE) cryogel, via the reaction between the chloride groups of the reactive dyes and the epoxide groups of the AGE**.** The FTIR bands observed around 1549 cm^{-1} was assigned to N-H bending (Figure 3). The FTIR bands observed around 1400 cm^{-1} and 2900 cm^{-1} were assigned to bending of C-H and stretching of C-H, respectively. It should be noted that the bending band of C-H in epoxide groups of AGE observed 2784 cm^{-1} was absent in Figure 3.b. The band observed at 3500 cm^{-1} was assigned to the -OH functional group. It is clearly seen that the intensity of the -OH band increases after Cibacron Blue F3GA attachment. The band observed at 3187 cm^{-1} indicates also streching of N-H groups of the dye molecule. These bands show the attachment of Cibacron Blue F3GA within the p(AAm-AGE) cryogel. The visual observations (the color of the cryogel) ensured attachment of dye molecules**.** The shift observed at the band around 3200 cm^{-1} might be due to the chelation between $-NH_2$ groups of the dye and Fe^{3+} ions (Figure 3c).

Adsorption Studies

Effect of Contact Time

Effect of contact time on dye adsorption onto p(AAm-AGE)-CB-Fe^{3+} cryogel is shown in Figure 4. Adsorption increased with time and dye adsorption reached a plateau at about 10 minutes for both of dyes. Because of filling up all ligands on the surface of IMAC matrix designed in this study, adsorption capacity of p(AAm-AGE)-CB-Fe^{3+} cryogel was not changed after 10 minute.

Effect of pH

The effect of pH on dye adsorption onto p(AAm-AGE)-CB-Fe^{3+} cryogel was studied in the pH range 5.0- 8.0 for Congo Red and 4.0-8.0 for Reactive Green in universal buffer adjusted with NaOH and HCl. The effects of pH on adsorption are presented in Figure 5. As seen Figure 5, the amounts of Congo Red and Reactive Green adsorbed onto the p(AAm-AGE)-CB-Fe^{3+} cryogel showed a maximum at pH 6.0. Specific interactions (hydrophobic, electrostatic and hydrogen bonding) between the newly prepared IMAC matrix and dye molecules at pH 6.0 may be resulted both from the ionization states of several groups on both the Congo Red and Reactive Green (i.e., sulfonic acid and amino groups), and from the conformational changes at this pH. Increase in the electrostatic repulsion effects between the opposite charged groups may also cause a decrease in adsorption (Figure 5).

Effect of Initial Dye Concentration

Figure 6 shows the effect of initial dye concentrations on adsorption. It was observed that the amount of adsorbed dye was increased with the initial dye concentration. Maximum adsorption capacities were found to be 14.50 mg/g cryogel for Congo Red and 18.78 mg/g cryogel for Reactive Green, respectively and the adsorbed amounts per unit mass of cryogel reached to a plateau value at about 1000 ppm dye concentration. As seen Figure 6, it was observed that newly developed IMAC matrix exhibited higher affinity to Reactive Green. It should be also noted that non-specific adsorption is independent of the initial concentration and it is observed to be similar at all the concentration ranges studied.

Adsorption Isotherms

An adsorption isotherm is used to characterize the interactions of each protein molecule with the adsorbent. This provides a relationship between the concentration of the protein in the solution and the amount of protein adsorbed on the solid phase when the two phases are at equilibrium.

The Langmuir adsorption model assumes that the molecules are adsorbed at a fixed number of well-defined sites, each of which is capable of holding only one molecule. These sites are also assumed to be energetically equivalent and distant from each other so that there are no interactions between molecules adsorbed on adjacent sites. Adsorption isotherms were used to evaluate adsorption properties. The corresponding transformations of the equilibrium data for dyes gave rise to a linear plot, indicating that the Langmuir model could be applied in these systems and described by the equation:

$$Q = Q_{max}. b . C_{eq} / (1 + bC_{eq}) \quad (4)$$

where; Q is the adsorbed amount of dye molecules (mg/g), C_{eq} is the equilibrium dye concentration (mg/mL), b is the Langmuir constant (mL/mg) and, Q_{max} is the maximum adsorption capacity (mg/g). This equation can be linearized as follows:

$$C_{eq}/Q = 1/(Q_{max}. b) + C_{eq}/Q_{max} \quad (5)$$

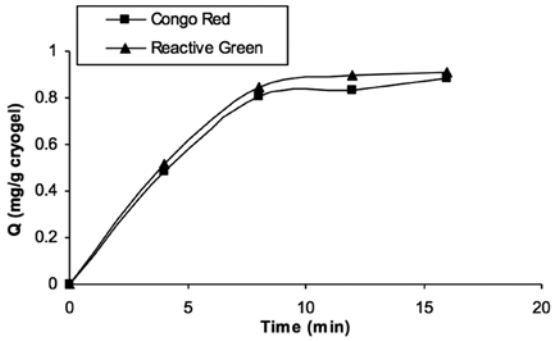

Figure 4. Effect of contact time on dye adsorption equilibrum onto p(AAm-AGE)-CB-Fe^{3+} cryogel. c: 50 ppm, T: 25°C, flow rate: 0.3 mL/min.

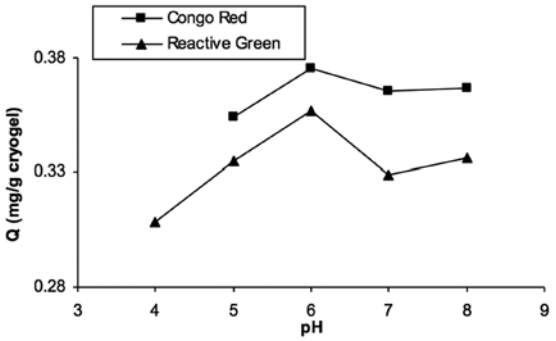

Figure 5. Effect of pH on dye adsorption onto p(AAm-AGE)-CB-Fe^{3+} cryogel. c: 20 ppm t: 8 min, T: 25°C, flow rate: 0.3 mL/min.

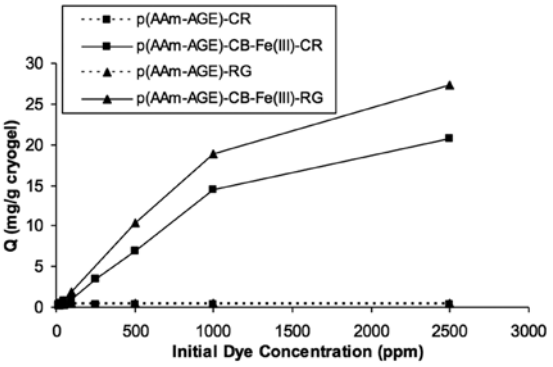

Figure 6. Effect of initial concentration on dye adsorption onto p(AAm-AGE)-CB-Fe^{3+} cryogel. pH: 6.0, T: 25°C, flow rate: 0.3 mL/min, t: 8 min.

The plot of C_{eq} versus C_{eq}/Q was employed to generate the intercept of $1/Q_{max}$.b and the slope of $1/Q_{max}$. The maximum adsorption capacities (Q_{max}) data for the adsorption of Congo Red and Reactive Green were obtained from the experimental data. The correlation coefficients were found as 0.7988 for Congo Red and 0.8210 for Reactive Green, respectively (Table 1).

Table 1. Langmuir constants for dye adsorption.

	Reactive Green	Congo Red
b, mL/g	322.58	53.48
Q_{max}, mg/g	0.074	0.576
R^2	0.8210	0.7988

Table 2. Frendlich constants for dye adsorption.

	Reactive Green	Congo Red
n	1.052	1.216
K_F	20.67	14.79
R^2	0.9995	0.9832

The other well-known isotherm, which is frequently used to describe adsorption behavior, is the Freundlich isotherm. This isotherm is another form of the Langmuir approach for adsorption on a heterogeneous surface. The amount of adsorbed molecule is the summation of adsorption on all binding sites. The Freundlich isotherm describes reversible adsorption and is not restricted to the formation of the monolayer. This empirical equation takes the form:

$$Q_{eq} = K_F (C_{eq})^{1/n} \quad (6)$$

where, K_F defines adsorption capacity and n is degree of adsorption favorability.

The adsorption isotherms of dyes were found to be linear over the whole concentration range studies and the correlation coefficients were high (Table 2). According to the correlation coefficients of isotherms, Freundlich adsorption model is favorable. Freundlich adsorption isotherm constants, n and K_F and the correlation coefficients were found as 1.216, 14.79 and 0.9832 for Congo Red and 1.052, 20.67 and 0.9995 for Reactive Green, respectively. The magnitude of K_F and n values of Freundlich model showed easy uptake of dyes from aqueous medium with a high adsorption capacity of p(AAm-AGE)-CB-Fe^{3+} cryogel.

Effect of Temperature

Effect of temperature on dye adsorption onto p(AAm-AGE)-CB-Fe^{3+} cryogel was studied in the range of 15-35°C. As seen in Figure 7, adsorption capacity of cryogel was not change significantly by changing temperature for both Congo Red and Reactive Green. This result implicates that dye removal with this novel IMAC matrix can be efficiently achieved at whole temperature range.

Effect of Flow Rate

The adsorption capacities at different flow-rates are given in Figure 8. Results showed that the dye adsorption capacity of the p(AAm-AGE)-CB-Fe^{3+} cryogel decreased significantly with the increasing flow-rate from 0.75 to 3.0 mL/min from 0.498 to 0.385 mg/g for Congo Red and 0.448 to 0.414 mg/g for Reactive Green, respectively. This is due to decrease in contact time between the dye molecules and the p(AAm-AGE)-CB-Fe^{3+} cryogel at higher flow-rates.

Desorption and Reusability studies

Desorption of dye molecules from the p(AAm-AGE)-CB-Fe^{3+} cryogel was carried out in continuous system with different desorption media and more than 90% of adsorbed dye molecules can be effectively desorbed. In order to show the

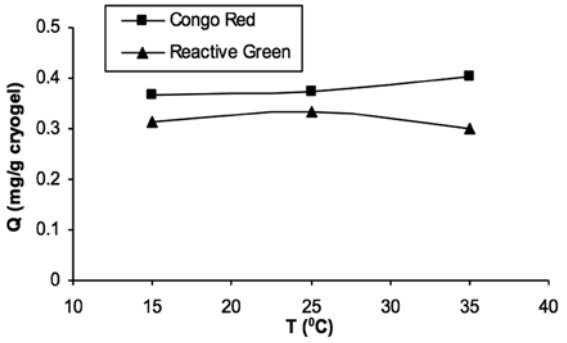

Figure 7. Effect of temperature on dye adsorption onto p(AAm-AGE)-CB-Fe^{3+} cryogel. c: 20ppm, pH: 6.0, flow rate: 0.3mL/min, t: 8 min.

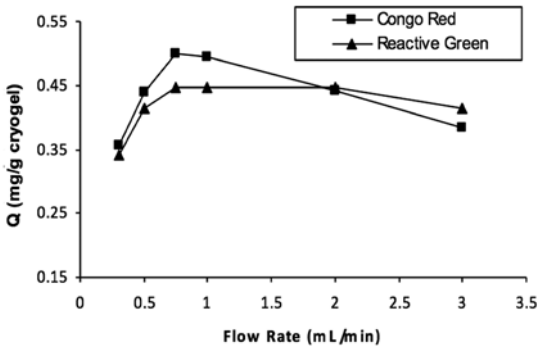

Figure 8. Effect of flow rate on dye adsorption onto p(AAm-AGE)-CB-Fe^{3+} cryogel. c: 20 ppm, pH: 6.0, T: 25°C, t: 8 min.

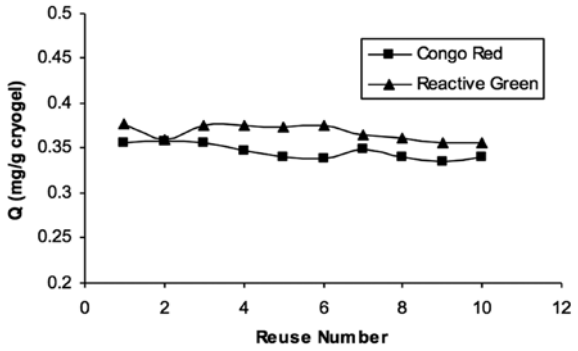

Figure 9. Reuseability of p(AAm-AGE)-CB-Fe^{3+} cryogel for dye adsorption. c: 20ppm, pH: 6.0 for RG & CR, T: 25°C, flow rate: 0.3 mL/min, t: 8 min.

reusability of the p(AAm-AGE)-CB-Fe^{3+} cryogel, the adsorption-desorption cycle was repeated ten times using the same cryogel. As seen in Figure 9, there was no remarkable reduce in the adsorption capacity of the cryogel. This result showed that Fe^{3+} immobilized p(AAm-AGE)-CB cryogel can be repeatedly used in the adsorption of different dye molecules without detectable losses in its initial adsorption capacity. The reusability of this novel IMAC matrix can provide economic advantages for large-scale biotechnological applications.

CONCLUSION

From the presented study, it may be concluded that the removal of dyes from aqueous solutions by adsorption on the p(AAm-AGE)-CB-Fe^{3+} cryogel has been found to be useful means for controlling the water pollution due to dyes. The important characteristics and results of the methodology are:

1. The results of the present column studies show that the adsorption of Congo Red and Reactive Green on the p(AAm-AGE)-CB-Fe^{3+} cryogel quite satisfactory.

2. It could be concluded that the linear fits for Freundlich equation were better than those for Langmuir equation. Therefore, the adsorption processes of dyes on the p(AAm-AGE)-CB-Fe^{3+} cryogel was complicating and the probability of multilayer adsorption was higher than that of monolayer adsorption.

3. Adsorption was found to be pH dependent. Maximum adsorption capacities, under the studied experimental conditions, were found to be 14.50 mg/g cryogel for Congo Red and 18.78 mg/g cryogel for Reactive Green, respectively. It was also observed that newly developed IMAC matrix exhibited higher affinity to Reactive Green.

4. Adsorption was found to be temperature independent. So, this novel IMAC matrix can be efficiently applied at whole temperature range for controlling the water pollution due to dyes.

5. The p(AAm-AGE)-CB-Fe^{3+} cryogel was applied in ten sequential cycles of adsorption–desorption with a limited loss of adsorption capacity, only 5.3% for Congo Red and 2.5% for Reactive Green, respectively.

The presented adsorption method used newly developed IMAC matrix offers many advantages for potential industrial use such as high efficiency, simplicity, universality, stability and cheapness of the chelating supports and also an economic feasibility since it does not require high costs for chemicals and equipment.

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