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Poly(acrylamide-allyl glycidyl ether) Cryogel as a Novel Stationary Phase for Chlorophenol Adsorption

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Abstract

Poly(acrylamide-allyl glycidyl ether) [poly(AAm-AGE)] cryogel was prepared by bulk polymerization which proceeds in an aqueous solution of monomers frozen inside a glass column (cryo-polymerization). After thawing, the monolithic cryogel contains a continuous polymeric matrix having interconnected pores of 10-100 μm size. Cibacron Blue F3GA was immobilized by covalent binding onto poly(AAm-AGE) cryogel via epoxy groups. Poly(AAm-AGE) cryogel was characterized by swelling studies, FTIR, scanning electron microscopy (SEM) and elemental analysis. The equilibrium swelling degree of the poly(AAm-AGE) monolithic cryogel was 6.84 g H_2O/g cryogel. Poly(AAm-AGE) cryogel containing 68.9 μmol Cibacron Blue F3GA/g were used in the adsorption/desorption of chlorophenols (i.e., phenol, m-chlorophenol, p-chlorophenol and 2,4,6-trichlorophenol) from aqueous solutions. The maximum adsorptions of chlorophenols onto the Cibacron Blue carrying cryogels were 128.5 μmol/g for phenol, 144.3 μmol/g for 2,4,6-trichlorophenol, 155.8 μmol/g for p-chlorophenol and 164.7 μmol/g for m-chlorophenol. The affinity order was as follows: m-chlorophenol > p-chlorophenol > 2,4,6-trichlorophenol > phenol. The adsorption of chlorophenols decreased with increasing pH. Desorption of chlorophenols was achieved using methanol solution (30%, v/v). The cibacron Blue F3GA-carrying cryogels are suitable for repeated use for more than ten cycles without noticeable loss of adsorption capacity.

Key Words: Dye-affinity adsorption, cryogels, chlorophenols, environmental pollution.

INTRODUCTION

Wastewaters containing phenolic compounds present a serious problem [1]. Phenol containing waste-water may not flow into open water without treatment because of the toxicity of phenol. The toxic and hazardous nature of phenols and their associated derivatives, and their increasing amounts in industrial wastewaters have been well documented. They are known or suspected human carcinogens [2]. Loss of appetite, marasmus, headache, rapid fatigue and severe chronic insomnia are given as symptoms of chronic phenol intoxication in humans after long-term intake of excessive phenol concentrations. Phenolic compounds are present in the wastewater generated from paint, solvent, petroleum (petrochemical), coal-conversion, pharmaceutical, wood preserving chemicals, plastic, rubber-proofing, pesticide, iron-steel, phenol production, paper and pulp industries [3]. Large scale coal gasification and carbonization plants generate waste-water containing large quantities of high strength phenolic

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compounds [4]. The United States Environmental Protection Agency (EPA) regulates lowering phenol content in the waste-water to less than 1 mg/L from the several thousand mg/L.

Tradionally, activated carbon adsorption is the most widely used for the removal of phenols and their derivatives. The high cost of activated carbon has stimulated interest to use cheaper raw materials. Microorganisms have been considered as one of the promising adsorbents [5-9]. In the concept of biosorption, several chemical processes may be involved, such as adsorption, ion exchange, and co-valent bonding. The biosorptive sites on the microorganisms are carboxyl, hydroxyl, sulphydryl, amino and phosphate groups (10). Fungal cell walls and their components have a major role in biosorption [11-13]. Fungal biomass can also take up considerable quantities of organic pollutants from aqueous solution by adsorption or a related process, even in the absence of physiological activity [14]. Recently, polymer based reusable adsorbents are widely employed for the removal of phenols [15-18]. Recently, dye affinity adsorption has been used extensively in laboratory scale wastewater purification [19-24]. Dye-ligands are commercially available and inexpensive. Most of the reactive dyes consist of a chromophore (either azo dyes, anthraquinone, or phathalocyanine), linked to a reactive group (often a mono- or dichloro-triazine ring). The interaction between the dye-ligand and environmental pollutants (i.e., heavy metal ions or organic pollutans such as phenolic compounds) can be by complex combination of electrostatic, hydrophobic, hydrogen bonding. Cibacron Blue F3GA is an anthraquinone textile dye that interacts specifically and reversibly with different environmental pollutants [25].

Conventional packed-bed columns possess some inherent limitations such as the slow diffusional mass transfer and the large void volume between the beads [26]. Although some new stationary phases such as the non-porous polymeric beads [27] and perfusion chromatography packings are designed to resolve these problems, these limitations cannot be overcome in essence [28]. Recently, cryogel materials are considered as a novel generation of stationary phases in the separation science [29-35]. Cryogels are a very good alternative to wastewater treatment with many advantages. Several advantages of cryogels are large pores, short diffusion path, low pressure drop and very short residence time for both adsorption and elution. Cryogels are also cheap materials and they can be used as disposable avoiding cross-contamination between batches.

The purpose of this investigation was to examine the extent of removal of model phenol or chlorophenol pollutants (i.e., phenol, m-chlorophenol, p-chlorophenol and 2,4,6-trichlorophenol) with a cryogel column. Poly(acrylamide-allyl glycidyl ether [poly(AAm-AGE)] cryogel was prepared by bulk polymerization which proceeds in aqueous solution of monomers frozen inside a glass column (cryo-polymerization). Cibacron Blue F3GA carrying poly(AAm-AGE) cryogel was characterized using FTIR, scanning electron microscope (SEM), elemental analysis and swelling test.

EXPERIMENTAL

Materials

Acrylamide (AAM, more than 99.9% pure, electrophoresis reagent), allyl glycidyl ether (AGE, 99%), N,N'-methylene-bis(acrylamide) (MBAAm) and amonium persulfate (APS) were supplied from Sigma (St Louis, USA). N,N,N',N'-tetramethylene diamine (TEMED) was obtained from Fluka A.G. (Buchs, Switzerland). Cibacron Blue F3GA was obtained from Polyscience (Warrington, USA) and used without further purification. Phenol and

chlorinated phenols for adsorption studies were also obtained from Sigma and used without further purification.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 Barnstead (Dubuque, IA) ROpure LP® reverse osmosis unit with a high flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANOpure® organic/colloid removal and ion exchange packed bed system. Laboratory glassware was kept overnight in a 5% nitric acid solution. Before use the glassware was rinsed with deionised water and dried in a dust-free environment.

Production of poly(AAm-AGe) cryogel

Production of poly(AAm-AGe) cryogel was performed using the Arvidsson et al's procedure [36]. AGE was selected in order to insert reactive epoxy groups in the cryogel. Briefly, monomers (10 ml of AAm, 1 ml of AGE) were dissolved in deionized water and the mixture was degassed under vacuum for about 5 min to eliminate soluble oxygen. Total concentration of monomers was 6% (w/v). The cryogel was produced by free radical polymerization initiated by TEMED (120 µl) and APS (100 mg). After adding APS (1% (w/v) of the total monomers) the solution was cooled in an ice bath for 2-3 min. TEMED (1% (w/v) of the total monomers) was added and the reaction mixture was stirred for 1 min. Then, the reaction mixture was poured into a plastic syringe (5 ml, id. 0.8 cm) with closed outled at the bottom. The polymerization solution in the syringe was frozen at - 12°C for 24 h and then thawed at room temperature. Extensive cleaning procedure for removal of unconverted monomers and initiator was performed. Briefly, washing solutions (i.e., a dilute HCl solution, and a water-ethanol mixture) were recirculated through the monolithic cryogel column, until to be assured that the cryogel column is clean. Purity of the monolithic cryogel was followed by observing the change of optical densities of the samples taken from the liquid phase in the recirculation system, and also from the DSC thermograms of the cryogel obtained by using a differential scanning microcalorimeter (Mettler, Switzerland). Optical density of the original monolithic cryogel was 1.67. But after the applying of cleaning procedure this value was reduced to 0.02. In addition, when the thermogram of the uncleaned monolithic cryogel was recorded, it has a peak around 50°C. This peak might be originated from TEMED. But after applying of this cleaning procedure, between 30-100°C any peak was not observed on this thermogram. After washing, the cryogel was stored in buffer containing 0.02% sodium azide at 4°C until use.

Cibacron Blue F3GA Immobilization

Cibacron Blue F3GA immobilization studies were carried out in a recirculating system equipped with a water jacket for temperature control. The cryogel was washed with 30 ml of water. Then, 100 ml of Cibacron Blue F3GA solution (5 mg/ml) containing NaOH (5 g) was pumped through the glass column under recirculation at 80°C for 2 h. Under these experimental conditions, a chemical reaction took place between the chloride group of the Cibacron Blue F3GA and the epoxide group of the poly(AAm-AGE) cryogel. The adsorption was followed by monitoring the decrease in UV absorbance at 630 nm. After incubation, the Cibacron Blue F3GA-attached poly(AAm-AGE) cryogel was washed with distilled water and methanol until all the physically adsorbed Cibacron Blue F3GA were removed. The modified cryogel was then stored at 4°C with 0.02% sodium azide to prevent microbial contamination.

Characterization of cryogel

The swelling degree of the cryogel (S) was determined as follows: cryogel sample was washed on porous filter until washing was clear. Then it was sucked dry and then transferred to pre-weighed vial and weighed ($m_{wet\ gel}$). After drying to constant mass in the oven at 60°C, the mass of dried sample was determined ($m_{dry\ gel}$). The swelling degree was calculated as:

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

The morphology of a cross section of the dried cryogel was investigated by scanning electron microscope (SEM). The sample was fixed in 2.5% gluaraldehyde in 0.15 M sodium cacodylate buffer overnight, post-fixed in 1% osmium tetroxide for 1 h. Then the sample was dehydrated stepwise in ethanol and transferred to a critical point drier temperated to 10°C where the ethanol was changed for liquid carbon dioxide as transitional fluid. The temperature was then raised to 40°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°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 Cibacron Blue F3GA, the poly(AAm-AGE) cryogel and Cibacron Blue F3GA-attached poly(AAm-AGE) cryogel were 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.

To evaluate Cibacron Blue F3GA immobilization amount, the poly(AAm-AGE) cryogel was subjected to elemental analysis using a Leco Elemental Analyzer (Model CHNS-932, USA).

Chromatographic Procedures

Chlorophenols Adsorption from Aqueous Solutions

The chlorophenols (i.e., phenol, m-chlorophenol, p-chlorophenol and 2,4,6-trichlorophenol) adsorption studies from the single species aqueous solutions were carried out in a column system equipped with a water jacket for temperature control. The cryogel was washed with 30 ml of water and then equilibrated with 25 mM phosphate buffer containing 0.1 M NaCl (pH 7.4). Then, the prepared chlorophenol solution (50 ml) was pumped through the column for 2 h. Effects of the initial concentration of chlorophenols and pH of the medium on the capacity were studied. The flow rate of the solution was changed in the range of 0.2-2.0 ml/min. 50 ml of aqueous chlorophenol solutions with different concentrations (in the range of 25-1000 mg/L) were treated with the unmodified and/or Cibacron Blue F3GA-carrying cryogel at different pH (in the range of 2.0-12.0, adjusted with HCl-NaOH) at room temperature, in the flasks agitated magnetically at 600 rpm. Stock solutions of chlorophenols were 1000 mg/L and all were prepared daily. The adsorption time was selected as 60 min in the preliminary experiments, which was assumed as the equilibrium adsorption time, because there was no significant change in the amount of adsorption after 60 min. The concentration of the chlorophenols in the aqueous phase was measured by high performance liquid chromatography. The LC equipment consisted of a Cecile CE1100 liquid chromatograph pump and Hewlett Packard HP 3395 integrator. Cecile CE1220 LC UV variable wavelength monitor was used as dedector. In the

chromatographic determination, Spherisorb ODS1 column (Length: 25 cm; inside diameter: 4.6 mm) containing 5 µm particles was used. Samples were injected through a Rheodyne injector with a 20 µL loop. For the detection of the chlorophenols the wavelength was set at high absorption wavelength. The mobile phase was prepared by addition of phosphoric acid to deionized water until a pH of 2.35 was obtained. The aqueous phosphoric acid was modified with 38% methanol. Tetrabutylamonium bromide was used as ion-pair reagent with the concentration of $1.4x10^{-4}$ M. The mobile phase was degassed in an ultrasonic water bath immediately before use. Prior to use, the mobile phase was also filtered throgh a 0.45 µm filter. A flow rate of 1.2 ml/min was used in all experiments. The concentration of adsorbed chlorophenols was obtained by using the mass balance.

Desorption and Repeated Use

In order to determine the reusability of the Cibacron Blue F3GA-carrying cryogel, consecutive adsorption-desorption cycles were repeated five times by using the same adsorbent. Desorption of chlorophenols was achieved by using methanol solution (30% v,v). The Cibacron Blue F3GA-carrying cryogels loaded 88.8 µmol phenol/g, 94.6 µmol 2,4,6-trichlorophenol/g, 97.6 µmol p-chlorophenol/g and 109.1 µmol m-chlorophenol/g were placed in this desorption medium and stirred at 600 rpm for 30 min at room temperature. The final chlorophenol concentration in the aqueous phase was determined by using a HPLC. The desorption ratio was calculated from the amount of chlorophenols initially loaded on the cryogels and the final chlorophenols concentration in the desorption medium.

RESULTS AND DISCUSSION

A supermacroporous monolithic cryogel was produced by polymerization 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 of the pores in monolithic cryogels allowed their modification with the ligand, Cibacron Blue F3GA. The scanning electron micrograph of the internal structure of the monolithic cryogel is shown in Figure 1. Poly(AAm-AGE) cryogel produced in such a way have non-porous and thin polymer walls, large continuous inter-connected pores (10-100 µm in diameter) that provide channels for the mobile phase to flow through. Pore size of the matrix is much larger than the size of the chlorophenol molecules, allowing them to pass easily.

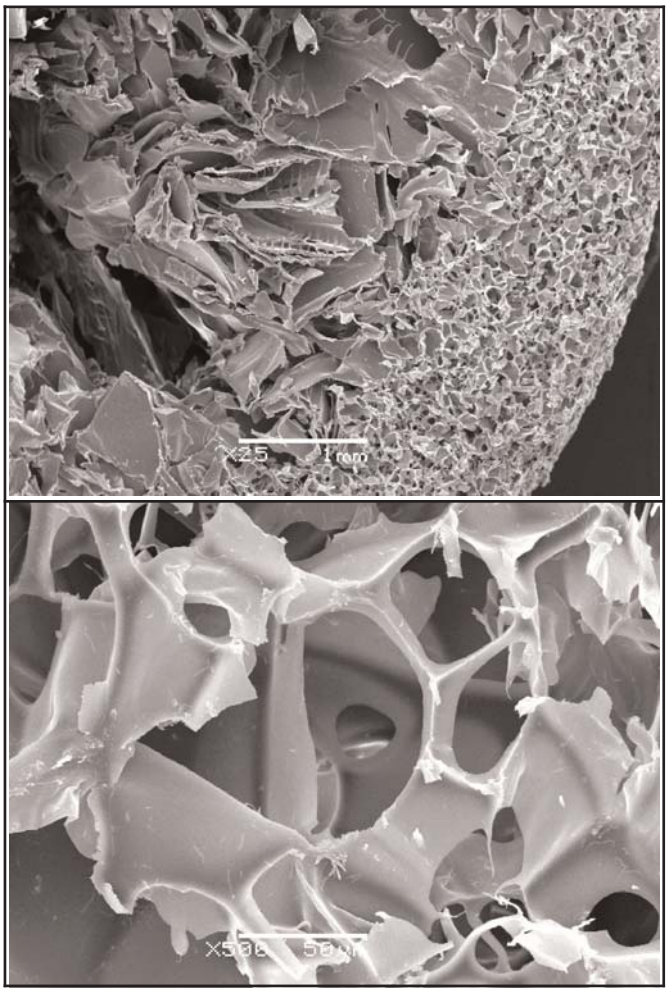

Figure 1. Scanning electron micrograph of the inner part of the supermacroporous poly(AAm-AGE) monolithic cryogel matrix.

As a result of the convective flow of the solution through the pores, the mass transfer resistance is practically negligible. The equilibrium swelling degree of the poly(AAm-AGE) monolithic cryogel was 6.84 g H_2O/g dry cryogel. Poly(AAm-AGE) monolithic 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.

Cibacron Blue F3GA is covalently attached on poly(AAm-AGE) cryogel. Figure 2 shows the FTIR spectra of Cibacron Blue F3GA attached poly(AAm-AGE) cryogels. The FTIR bands observed at 1160 cm^{-1} was assigned to symmetric stretching of S=O, as also pointed out on the chemical structure of the Cibacron Blue F3GA (Figure 3). The split of the band at 3300-3500 cm^{-1} indicates also SO_3H and NH_2 groups.

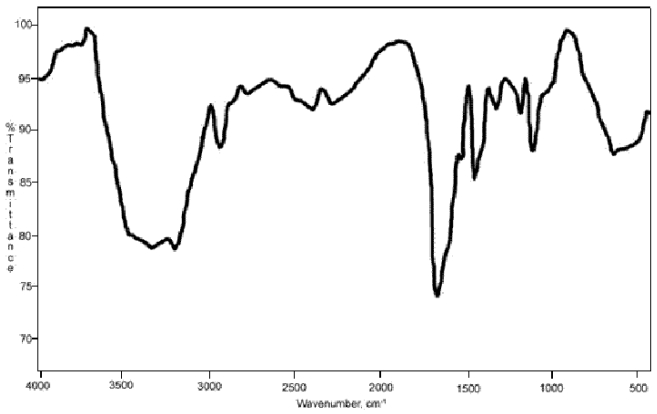

Figure 2. FTIR spectrum of Cibacron Blue F3GA attached poly(AAm-AGE).

(These bands show the attachment of Cibacron Blue F3GA within the poly(AAm-AGE) cryogel. The visual observations (the colour of the cryogel) ensured attachment of dye molecules. The dye content was 68.9 μmol/g dry cryogel. Note that AAm, AGE and other chemicals in the polymerization formula do not contain sulphur. This sulphur amount determined by elemental analysis originated from only immobilized dye into the polymeric structure.

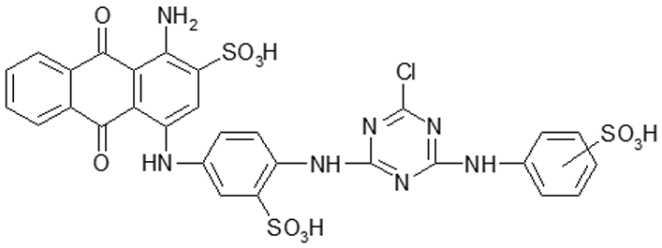

Figure 3. Chemical Structure of Cibacron Blue F3GA.

The Cibaron Blue F3GA-immobilized cryogel was extensively washed with methanol until to ensure that there is no dye leakage from any of the dye-immobilized cryogel and in any media used at adsorption-desorption steps. The release of dye-molecules was also measured in three different kinds of media. There was no measurable release of dye into the acidic medium (pH 2.0). Dye was released in the neutral medium while some was released in the alkaline medium too. The release in the strongly alkaline medium indicates the existance of strong ionic interactons. The release in neutral medium might just be the physically occluded dye along with any weakly/physically bonded dye. The studies of Cibacron Blue F3GA leakage from the poly(AAM-AGE) cryogel showed that there was no dye leakage in any medium used throughout this study, even after long period of time (more than 24 weeks).

Chlorophenols Adsorption from Aqueous Solutions

Effects of pH

The medium pH affects the solubility of phenol or chlorophenols and the ionisation state of the functional groups (sulphonate and amino groups) of the dye structure. The amino groups carry positive

charges that allow the dye molecules to be potential binding sites for chlorophenols. The effect of pH on adsorption of chlorophenols is shown in Figure 4. It was observed that the adsorption capacities

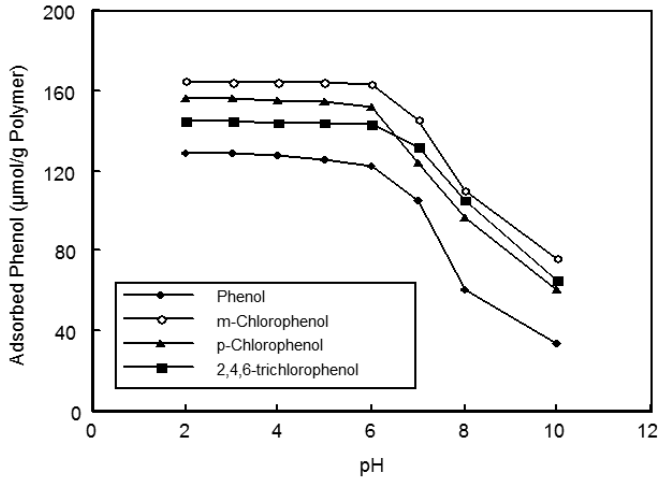

Figure 4. Effect of pH on adsorption of chlorophenols on the Cibacron Blue F3GA-carrying cryogels; Cibacron Blue F3GA content: 68.9 µmol/g; Chlorophenol concentration: 500 mg/ml; Flow rate: 0.2 ml/min; T: 20°C. *Each point is as average of five parallel studies.*

decreased with increasing pH. The highest adsorption of chlorophenols occured at pH 2.0 for all species. However within the pH range of 2.0-6.0 there is no significant decrease in the equilibrium adsorption capacity. But, adsorption capacity was considerably decreased when the pH of the initial solution was above 7.0. The interaction forces between chlorophenols and Cibacron Blue F3GA are rather weak in the neutral solutions. The decrease in adsorption capacity may also be due to the competing hydroxide ions.

Effects of Concentration

The chlorophenols adsorption capacities of the Cibacron Blue F3GA-carrying cryogels are given as a function of the initial concentration of chlorophenols within the aqueous phase in Figure 5. It was observed that the amount of adsorption was significantly increased with the initial chlorophenols concentration. The maximum adsorption capacities of the Cibacron Blue F3GA-carrying cryogels in the studied range are 128.5 µmol/g for phenol, 144.3 µmol/g for 2,4,6-trichlorophenol, 155.8 µmol/g for p-chlorophenol and 164.7 µmol/g for m-chlorophenol at pH 2.0, which are corresponding an initial concentration of 500 mg/L. This binding may have resulted from cooperative effect of different interaction mechanisms such as hydrophobic, electrostatic and hydrogen bonding caused by the acidic groups and aromatic structures on the Cibacron Blue F3GA and chlorophenol molecules. The structure of Cibacron Blue F3GA consists of aromatic rings (Figure 3). It is assumed that when a phenolic molecule is adsorbed on Cibacron Blue F3GA-attached cryogel, the aromatic p electrons of the phenolic molecule interact directly with the p electrons of the Cibacron Blue F3GA aromatic ring. In other words, adsorption is a result of the interaction (or overlapping) of the two p-electron orbitals. Note that the sulphonate and amino groups of Cibacron Blue F3GA are also available for interaction with phenolic compounds.

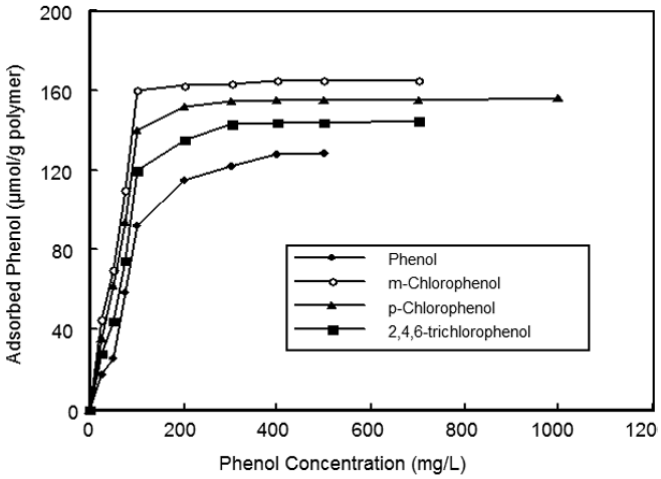

Figure 5. Chlorophenols adsorption capacity of the Cibacron Blue F3GA-carrying cryogels; Cibacron Blue F3GA content: 68.9 µmol/g; pH: 2.0; Flow rate: 0.2 ml/min; T: 20°C. *Each point is an average of five parallel studies*.

It should be mentioned that the adsorption capacity

for the chlorophenols is higher than that of phenol, possibly due to the higher solubility of phenol in water (Table 1). The affinity order is as follows: m-chlorophenol > p-chlorophenol > 2,4,6-trichlorophenol > phenol. The difference in adsorption behavior of the chlorophenol species compared to each others can be explained by the different affinity of the phenolic species for the reactive functional groups in the ligand Cibacron Blue F3GA. A difference in geometrical structure is most probably also case for the immobilized-Cibacron Blue F3GA ligand resulting in a relatively high adsorption of m-chlorophenol.

Table 1. Aqueous solubility of phenol and chlorophenols.

Substance	Solubility, C_s (mol/L)
Phenol	638.4
m-Chlorophenol	198.4
p-Chlorophenol	186.0
2,4,6-Trichlorophenol	4.3

Note that one of the main requirements in dye-affinity adsorption is the specificity of the affinity adsorbent for the target molecule. The non-specific interaction between the support, which is the poly(AAm-AGE) cryogel in the present case, and the molecules to be adsorbed, which are the chlorophenol molecules here should be minimum in order to consider the interaction as specific. The chlorophenols adsorption on the unmodified cryogels (carrying no Cibacron Blue F3GA) are relatively low, about 7.9 μmol/g for phenol, 11.8 μmol/g for 2,4,6-trichlorophenol, 16.4 μmol/g for m-chlorophenol and 18.5 μmol/g for p-chlorophenol. Note that these cryogels are highly swellable and also porous, which therefore may absorb (or entrap) chlorophenols within the matrix of the swollen cryogel.

Comparison with Other Supports

In literature, different sorbents with a wide range of adsorption capacities for phenolic compounds have been used. Aksu and Yener investigated the biosorption of phenol, o-chlorophenol and p-chlorophenol from aqueous solutions on dried activated sludge [5]. Maximum adsorption capacity was found to be 1.7 mmol/g. Azanova and Hradil investigated the adsorption of phenol by porous copolymers of different porous structure and polarity with hyper-crosslinked poly(ethylene glycol dimethacrylate) and commercial samples including Lewatit EP63 and Amberlite XAD4 [16]. They obtained maximum adsorption capacity in the range of 0.46-1.6 mmol/g. Singh and Misra studied iron(III) hydroxide-loaded marble as an adsorbent to remove phenolic compounds (i.e., phenol, 2-chlorophenol, p-nitrophenol, pyrogallol, resorcinol, quinol, payrocatechol) from aqueous solutions [37]. They reported adsorption capacity between 14.7-76.5

Table 2. Adsorption parameters obtained from Langmuir, Freundlich and Redlich-Peterson isotherms.

Phenol type	Langmuir model			Freundlich model			Redlich-Peterson model			
	q_m (μmol/g)	b (L/mg)	R^2	K_F	1/n	R^2	A (L/mg)	B (mg/L)	β	R^2
Phenol	322.6	0.0023	0.915	2.56	0.675	0.863	7.32	19.7	0.325	0.612
o-CP	227.3	0.0103	0.955	19.8	0.360	0.746	0.48	11.5	0.640	0.896
p-CP	222.2	0.0079	0.962	20.0	0.362	0.736	0.70	14.1	0.638	0.916
2,4,6-TCP	232.6	0.0055	0.964	8.08	0.485	0.815	1.23	11.2	0.515	0.908

μmol/g. Ravi et al reached adsorption capacity between 3.2-4.4 mmol/g with activated carbon for phenol and cresol isomers [38]. Furuya et al used chloro- and nitrophenols as the test adsorbates, and granular activated carbon was used as the adsorbent [39]. They achieved up to 4 mmol/g adsorption capacity. Dargaville et al found up to 0.1 mmol/g adsorption capacity for multinuclear phenolic compounds by activated carbon [40]. Shu et al used aluminosilicate-based microporous materials (pillared clays, silicalite and zeolite beta) and they reported selective phenol adsorption capacity up to 1 mmol/g [41]. Streat and Sweetland reported up to 1.5 mmol/g adsorption capacity for phenol and chlorophenols with a new series of hypercrosslinked porous polymeric ion-exchange sorbents (Hypersol-MacronetTM) [42]. Gupta et al showed 60 μmol/g adsorption capacity for phenol and p-nitrophenol with a low cost adsorbent i.e., bagasse fly ash (a waste generated in local sugar plants) sorbents [43]. Lin and Cheng reported that organobentonites are rather effective adsorbents in removing phenol and chlorophenol pollutants from industrial wastewaters [44]. However, Notice that the adsorption capacities that we achieved are comparable with the values reported in the previous publications.

Adsorption Isotherms

Two important physico-chemical aspects for evaluation of the adsorption process as a unit operation are the kinetics and the equilibria of adsorption. Modelling of the equilibrium data has been done using the Langmuir, Freundlich and Redlich-Peterson isotherms [45]. The Langmuir and Freundlich equations are represented as follows Equation 2 and Equation 3, respectively.

$$1/q_e = (1/q_{max}) + [1/(q_{max}\, b)].(1/C_e) \quad (2)$$

$$\ln q_e = 1/n\ (\ln C_e) + \ln K_F \quad (3)$$

where, b is the Langmuir isotherm constant, K_F is the Freundlich constant, and n is the Freundlich exponent. 1/n is a measure of the surface heterogeneity ranging between 0 and 1, becoming more heterogeneous as its value gets closer to zero. The ratio of qe gives the theoretical monolayer saturation capacity of cryogel.

The Redlich-Peterson equation describes adsorption on heterogeneous surfaces, as it contains the heterogeneity factor β. This equation has three parameters, A, B and β. Parameter β ranges between 0 and 1. It reduced to the Langmuir equation as β approaches 1. A, B and β were determined by curve fitting.

$$C_e/q_e = (B/A) + (1/A)\, C_e^{\beta} \quad (4)$$

Some model parameters were determined by nonlinear regression with commercially available software and are shown in Table 2. Comparison of all theoretical approaches used in this study shows that the Langmuir and Redlich-Peterson equations fit the experimental data best.

It must be also noted that the standard deviation of the values determined by regression analysis is comparatively low. It must be also pointed out that the experimental adsorption capacities for poly(AAm-AGE) cryogels are lower than to the theoretical adsorption capacities (i.e., obtained from adsorption models). This difference is due to the steric/geometric hindrances (i.e., accessibility) between the chlorophenol compounds and the binbing sites (i.e., Cibacron Blue F3GA) on the surface of poly(AAm-AGE) cryogels.

Adsorption Dynamics

In order to quantify the extent of uptake in adsorption kinetics, the kinetic models (Pseudo-first- and second-order equations) can be used in

Table 3. The first- and second-order kinetic constants for poly(Am-AGE) cryogel.

	Exp	First-order kinetic			Second-order kinetic		
	Q_{exp} (μmol/g)	k_1 (1/min)	q_e (μmol/g)	R^2	k_2 (g/mg.min)	q_e (μmol/g)	R^2
Phenol	128.5	0.050	186.9	0.921	0.00014	178.6	0.965
o-CP	164.7	0.067	246.8	0.943	0.00030	192.3	0.990
p-CP	155.8	0.068	292.6	0.898	0.00021	191.3	0.982
2,4,6-TCP	144.3	0.071	324.2	0.898	0.00016	192.3	0.970

this case assuming that the measured concentrations are equal to adsorbent surface concentrations [46]. The first-order rate equation of Lagergren is one of the most widely used for the adsorption of solute from a liquid solution. It may be represented as follows:

$$\log(q_e - q_t) = \log(q_e) - (k_1 t)/2.303 \quad (5)$$

where q_e is the experimental amount of adsorbed chlorophenol at equilibrium (μmol/g); q_t is the amount of chlorophenol adsorbed at time t (μmol/g); k_1 is the equilibrium rate constant of first order adsorption (1/min); and q_e is the adsorption capacity. A plot of log (q_e- q_t) versus t should give a straight line to confirm the applicability of the kinetic model. In a true first-order process logq_e should be equal to the interception point of plot of log (q_e - q_t) via t. The rate constant for the second-order adsorption could be obtained from the following equation:

$$(t/q_t) = (1/k_2 q_e^2) + (1/ q_e)\, t \quad (6)$$

where k_2 is the equilibrium rate constant of pseudo-second-order adsorption (g/μmol.min).

A plot of t/q_t versus t should give a linear relationship for the applicability of the second-order kinetics. The rate constant (k_2) and adsorption at equilibrium (q_e) can be obtained from the intercept and slope, respectively.

Table 3 shows the results for both first order and the second order kinetic models. The results show that the second order mechanism is applicaple (R^2 values are the highest). These results suggest that the pseudo-second order mechanisms is predominant and that chemisorption might be the rate-limiting step that controls the adsorption process. The rate-controlling mechanism may vary during the course of the biosorption process with three possible mechanisms rate-limiting occuring [47]. There is an external surface mass transfer or film diffusion process that controls the early stages of the adsorption process. This may be followed by a reaction or constant rate stage and finally by a diffusion stage where the adsorption process slows down considerably [48].

Effect of Flow-Rate

The adsorption capacities at different flow-rates are given in Figure 6. Results show that the chlorophenol adsorption capacity onto the poly(AAm-AGE)/Cibacron Blue F3GA cryogel decreases when the flow-rate through the column increases. An increase in the flow rate reduces the solution volume treated efficiently until breakthrough

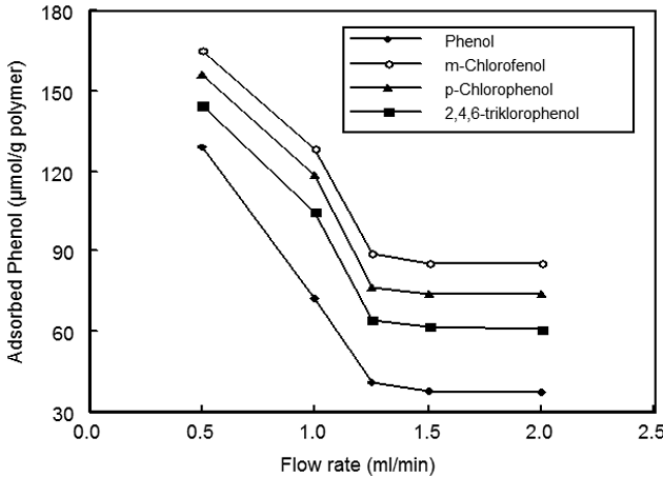

Figure 6. Effect of flow-rate on chlorophenol adsorption: Cibacron Blue F3GA content: 68.9 µmol/g; Chlorophenol concentration: 500 mg/ml; pH: 2.0; T: 20°C. *Each point is an average of five parallel studies.*

point and therefore decreases the service time of cryogel column. This is due to decrease in contact time between the chlorophenol molecules and the poly(AAm-AGE)/Cibacron Blue F3GA cryogel at higher flow rates. These results are also in agreement with those referred to the literature [49]. When the flow-rate decreases the contact time in the column is longer. Thus, chlorophenol molecules have more time to diffuse to the pore walls of cryogel and to bind to the ligand, hence a better

adsorption capacity is obtained. In addition, for column operation the cryogel is continuously in contact with a fresh solution. Consequently the concentration in the solution in contact with a given layer of cryogel in a column is relatively constant.

Desorption Studies

To be useful in separation and removal processes, adsorbed species should be easily desorbed under suitable conditions and adsorbents shuld be used many times in order to decrease material costs. Desorption of chlorophenols from the Cibacron Blue F3GA-attached poly(AAm-AGE) cryogel was also carried out in column system. Desorption experiments were performed with methanol solution (30%, v/v) as the desorption agent. This means that methanol breaks down the interaction forces between chlorophenols and binding sites onto the dye structure. Note that there was no Cibacron Blue F3GA release in this case which shows that dye-molecules are bonded strongly to poly(AAm-

Table 4. Adsorption-desorption cycles for chlorophenols. Initial concentrations of chlorophenols: 500 mg/L; pH: 2.0. T: 20°C.

Cycle No	Phenol		2,4,6-trichlorophenol		p-chlorophenol		m-chlorophenol	
	Adsorption (µmol/g)	Desorption (%)	Adsorption (µmol/g)	Desorption (%)	Adsorption (µmol/g)	Desorption (%)	Adsorption (µmol/g)	Desorption (%)
1	128.5	97.8	144.3	96.6	155.8	98.4	164.7	95.9
2	128.2	97.5	144.0	96.9	155.6	98.2	164.1	96.6
3	127.6	98.0	143.8	96.5	155.0	97.6	164.0	97.1
4	127.2	98.2	143.5	97.5	154.7	97.5	163.8	97.9
5	127.0	97.5	143.2	97.7	154.4	97.9	163.4	98.2
6	126.4	97.1	143.0	97.4	154.2	96.8	163.0	99.0
7	126.0	97.3	142.9	98.0	154.0	96.4	162.9	98.5
8	126.0	96.9	142.5	98.3	153.2	97.3	163.1	96.9
9	125.7	96.8	142.6	98.7	153.5	98.0	162.3	98.1
10	125.2	96.7	142.0	98.1	153.0	97.2	162.0	98.0

AGE) cryogel. Table 4 shows the adsorption-desorption values of chlorophenols by Cibacron Blue F3GA-carrying cryogels after several cycles of consecutive adsorption and desorption. There was no remarkable reduce in the adsorption capacity of the cryogel. This table clearly shows that the Cibacron Blue F3GA-carrying cryogels can be used repeatedly without loosing significantly their adsorption capacities for all chlorophenols studied here.

CONCLUSION

Wastewater's containing phenolic compounds present a serious problem. Phenol containing wastewater may not be conducted into open water without treatment because of the toxicity of phenol. At present, polymeric adsorbents are widely employed for the removal of phenols. Poly(AAm-AGE) cryogels carrying 68.9 μmol Cibacron Blue F3GA/g polymer were used for adsorption/desorption of chlorophenols (i.e., phenol, m-chlorophenol, p-chlorophenol and 2,4,6-trichlorophenol) from aqueous solution. The present studies lead to the following conclusions: The maximum adsorption capacities of these dye-affinity cryogels from their single solutions were 128.5 μmol/g for phenol, 144.3 μmol/g for 2,4,6-trichlorophenol, 155.8 μmol/g for p-chlorophenol and 164.7 μmol/g for m-chlorophenol. The affinity order was as follows: m-chlorophenol > p-chloro-phenol > 2,4,6-trichlorophenol > phenol. The adsorption of chlorophenols decreased with increasing pH. Consecutive adsorption and desorption cycles showed the feasibility of this Cibacron Blue F3GA-carrying cryogels for chlorophenol removal from aqueous solutions.

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