AppliedChem

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Hydroxyalkyl Amination of Agarose Gels Improves Adsorption of Bisphenol A and Diclofenac from Water: Conceivable Prospects

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Abstract: The hydroxyalkyl amination of agarose gels was studied as an approach to improve adsorption of polyphenols and pharmaceuticals from water. Three commercially available agarose gels, Zetarose FlashFlow4, ZetaCell-CL6B and Sepharose 4B were chemically modified using tris-(hydroxymethyl)aminomethane, TRIS, and ethanolamine, EA. The adsorbed amounts of bisphenol A and diclofenac were significantly higher on TRIS- and EA-derivatives compared with the parent gels. Regarding bisphenol A adsorption on TRIS-ZetaCell-CL6B, a maximal adsorption capacity, Q max of 16 μmol/mL gel and an equilibrium dissociation constant K_L of 2.7×10^{-4} mol/L were observed. Filtration of diclofenac-contaminated water through TRIS-Zetarose FlashFlow 4 resulted in a 10-fold reduction of the pollutant concentration within 64 column volumes of the effluent. The moderate binding affinity of polyphenols to TRIS- and EA-adsorbents facilitates efficient polyphenol desorption and column regeneration. The effects of TRIS- and EA-substituents in agarose gels, can be harnessed for the development of environmental adsorbents, as well as for the preparative separation of polyphenols and pharmaceuticals. We consider the physical shapes and textures of the prospective adsorbents with a particular focus on spongy macroporous cryogels. These innovative materials hold promise for future applications in liquid and air filtration.

Keywords: aromatic; cryogel; OH-π interaction; pollutants; water purification

Citation: Ljunggren, L.; Ivanova, S.; Ivanov, A.E. Hydroxyalkyl Amination of Agarose Gels Improves Adsorption of Bisphenol A and Diclofenac from Water: Conceivable Prospects. *AppliedChem* **2024**, *4*, 42–55. https://doi.org/10.3390/appliedchem4010004

Academic Editor: Dag Ekeberg

Received: 24 January 2024
Revised: 15 February 2024
Accepted: 21 February 2024
Published: 24 February 2024

1. Introduction

Adsorption of water-soluble aromatic compounds including phenolic pollutants, pesticides, and pharmaceuticals has gained much interest in the fields of water purification and preparative separation techniques. The most common adsorbents used to deplete the above compounds from water are activated carbons, low-cost lignocelluloses and natural aluminosilicates like zeolites or clays [1–3]. More recently, adsorption of phenols on graphene nanosheets and graphene oxide was studied [4,5] and reviewed [6]. The underlying adsorption mechanism was ascribed to π-π electronic interaction of the phenols with graphite basal planes as well as to hydrogen bonding in the case of graphene oxide [4]. In the aluminosilicates group, molecular adsorption was believed to be due to hydrogen bonding between the hydroxylic groups of the surface and the hydroxylic groups of the aromatic solutes [3].

Whereas the role of hydrogen bonding and π-π stacking in adsorption of aromatics was well recognized, the specific contribution of OH-π interactions [7] or NH_2^+-π interactions [8] to chromatographic retention of phenols was not discussed much before a systematic set of studies with highly cross-linked 12% agarose gel was performed [9,10]. In these studies, the separation of hydroxybenzoic acids was analyzed in the context of hydrogen bond formation between the aromatic hydroxyls of the acids and ether or hemiacetal

oxygens of the gel. At the same time, the aromatic π-electrons of the phenols were supposed to interact with hydrogen atoms of numerous hydroxyls of the chemically cross-linked agarose. It is relevant to note that OH-π interactions were recently shown to effectuate adhesion of bisphenol A diglycidyl ether to hydroxylated silica or γ-alumina surfaces [11].

To increase the number of surface hydroxyls able to act as hydrogen bond donors and to ensure their steric accessibility for the solutes, the synthesis of cross-linked agarose gels chemically modified with tris-(hydroxymethyl) aminomethane (TRIS) was reported in a recent patent application [12]. The gels exhibited significant adsorption capacities for aromatic compounds such as 2-napthol and bisphenol A. TRIS-functionalized agarose gels were studied earlier as adsorbents for bovine serum albumin and some other proteins [13], and the mechanism of protein adsorption included both electrostatic interaction and hydrogen bonding. The latter mechanism was supposed to come into play at zero net charge of the albumin globule (pH 5), where significant protein adsorption still took place.

The role of hydrophilic partition of aromatic compounds between highly hydrated TRIS-functionalized polymer-grafted silica and 80% aqueous acetonitrile mobile phase was studied via high performance liquid chromatography [14]. Chromatographic retention of certain substituted benzoic acids and nucleotide bases exhibited an increase with the polarity of the solutes. However, their adsorption at a low content of the organic solvent in the mobile phase or from pure water remained unreported. Similarly, the above-mentioned studies [9,10] described isocratic chromatographic separation of dihydroxybenzoic acids on the cross-linked 12% agarose gel column at relatively low (3 or 5%) acetic acid, ethanol, or acetonitrile content in the aqueous mobile phase but not in pure water. To the best of our knowledge, the adsorption behavior of low molecular weight aromatic compounds on TRIS-agaroses or TRIS-silicas from water has not yet been studied in comparison to that observed on the parent agarose or silica gels.

The aim of his work was to study hydroxyalkyl amination of agarose gels as an approach to improve adsorption of polyphenols and pharmaceuticals from water. In particular, the present work was aimed to investigate if the adsorption capacity of phenols reported in [12] was in fact due to the hydroxyalkyl amines covalently attached to agarose gel, and to evaluate the equilibrium binding constant for bisphenol A as a measure of interaction strength between the aromatic solute and the hydroxylated gels. Another aim of the study was to evaluate the possibility of removal of the emerging water pollutants such as bisphenol A and diclofenac using filtration through the TRIS-functionalized agarose gels. The possibility of using less expensive polysaccharides convenient for the production of technically feasible macroporous monolithic adsorbents is discussed.

2. Materials and Methods

2.1. Materials

Zetarose FlashFlow4 (Zetarose FF4) and ZetaCell-CL6B were products of empBIOTECH (Berlin, Germany). Sepharose 4B was a product of Amersham Pharmacia Biotech AB (Uppsala, Sweden). Sodium bicarbonate (reagent grade, min. 99.5%), sodium hydroxide (puriss. p.a., min. 98%), potassium bromide (FT-IR Grade, min. 99%) and hydrochloric acid were products of Honeywell/Fluka (Seelze, Germany). Tris-(hydroxymethyl) aminomethane (TRIS) 96% was a product of Sigma-Aldrich (St. Louis, MO, USA). Ethanol 99,5% was a product of Solveco (Rosersberg, Sweden). "Klorin" liquid containing 27 g/L (0.36 M) sodium hypochlorite was a product of Colgate-Palmolive (Danderyd, Sweden); it was used to prepare aqueous 0.18 M bromine solution by oxidation of potassium bromide. Allyl bromide 99% was from Alfa Aesar (Heysham, UK). Bisphenol A > 99% and diclofenac sodium salt > 98% were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Experimental Methods

2.2.1. Chemical Modification of Agarose Gels

TRIS-derivatives of agaroses were synthesized using Zetarose FF4, ZetaCell-CL6B and Sepharose 4B according to the method described in the patent application [12]. Briefly, 19 g

of wet filtered-off gels were treated with allyl bromide (5.8 mL) in 4M NaOH (20 mL) under vigorous shaking at 21 °C overnight and rinsed in filter with aqueous 50% ethanol and deionized water to neutral pH to produce allyl-agarose (Step 1, Figure 1). Aqueous bromine solution (0.18 M) was added dropwise to a suspension of allyl-agarose in water (20 mL); the gel was filtered off and rinsed by water (Step 2). The thus synthesized bromohydrin-agarose was treated with 1M TRIS or 1M ethanolamine dissolved in 1M $NaHCO_3$ (pH 9.3 or 10.1, respectively) with shaking for 3 days at room temperature (Step 3).

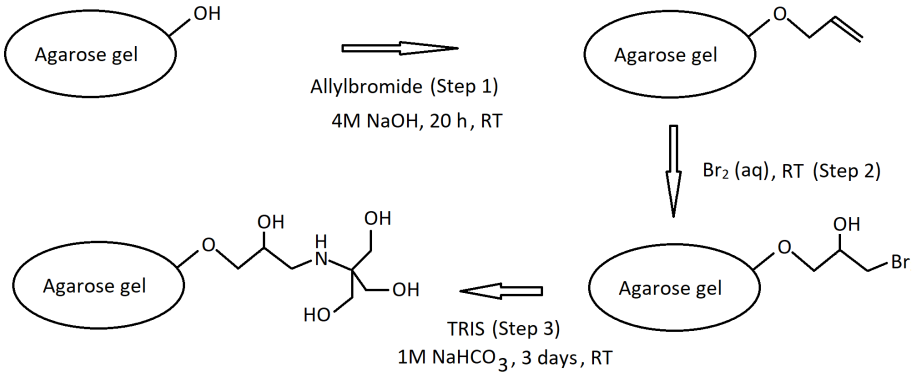

Figure 1. Scheme of agarose gel chemical modification with TRIS (tris-(hydroxymethyl) aminomethane).

The TRIS- and EA-derivatives of the agarose gels thus synthesized were washed to neutral pH, and kept in 0.02% sodium azide at 4 °C. To prepare the gel samples for elemental analysis, gels were further washed with deionized water and freeze dried. The content of allyl groups was estimated by titration with bromine water and calculated as number of Br_2 micromoles consumed by 1 g of wet allyl-agarose gel. The end point of bromine consumption was registered visually: the discoloration of bromine water upon its addition to the gel ended when the reaction was completed.

2.2.2. pH Measurements and Potentiometric Titration of TRIS-Agarose Gels

The pH was determined using a HACH LANGE sensION^{TM} + pH3 pH meter (HACH LANGE GMBH, Düsseldorf, Germany) and the same instrument was used for potentiometric titration.

Wet filtered-off agarose gels (0.2 g) were added to 10 mL 0.15 M NaCl solution, and the pH of suspension was adjusted to 10.5 ± 0.1 by gradually adding 1 M NaOH at magnetic stirring. Subsequently, the suspension was titrated by 5 or 10 µL aliquots of 0.1 M HCl. At each step, the pH was allowed to stabilize at the new value before introducing the next aliquot of HCl. A parallel titration was conducted with 10 mL of 0.15 M NaCl without beads adjusted to pH 10.5, as a reference.

From the amount of acid spent to reach a certain pH of the bead suspension, the amount of acid spent to reach the same pH in the reference sample was subtracted. The difference represented the amount of HCl needed to ionize TRIS units of the copolymer at the specific pH. The titration process continued until the pH value reached 3. Finally, the amount of HCl spent for the ionization of TRIS-agaroses was divided by the gels weight to get its ion-exchange capacity.

2.2.3. Bisphenol A Batch Adsorption Kinetics

An aqueous solution of bisphenol A (10 mg/L or 100 mg/L, from 2.75 mL to 19.875 mL) was combined with 0.125 mL or 0.25 mL TRIS- or EA-modified or non-modified agarose beads and agitated on an orbital mixer for various times. After the agitation the gels were allowed to sediment for 10 min, the aliquots (1 mL) of the supernatants were taken and centrifuged. The concentration of bisphenol A was estimated spectrophotometrically at 276 nm or 215 nm, see Supplementary Materials. The studies were performed at room temperature, 21 °C. After the UV measurement, the aliquots were returned to the adsorption

mixture and the measurements were repeated at various times. The sensitivity limit for determination of bisphenol A was 0.2 mg/L, and the standard error of bisphenol A estimation was 0.073 mg/L, see Supplementary Materials.

2.2.4. Adsorption Isotherms of Bisphenol A

Adsorbed amounts of bisphenol A (Q_{BP}) were calculated from the equilibrium concentrations of the substance in contact with the chemically modified and non-modified agaroses for 24 h using the following equation:

$$Q_{BP} = (C_0 \times V_0 - C_{EQ} \times V_{EQ})/V_G \quad (1)$$

where C_0 and C_{EQ} are the starting and equilibrium concentrations of bisphenol A in the adsorption mixture, respectively, V_0—volume of the bisphenol A solution, V_{EQ}—volume of the equilibrium adsorption mixture and V_G—volume of the swollen gel.

2.2.5. Column Adsorption of Bisphenol A and Diclofenac

Aqueous solutions of bisphenol A (10 mg/L) or diclofenac (10 mg/L) in deionized water were applied at a flow rate of 0.4 mL/min to 1 mL column (∅ 0.8 cm × 2 cm) packed with TRIS-Zetarose 4FF, and 2 mL fractions of the effluent were collected. To detect bisphenol A, the absorbance at 215 nm was measured in the fractions to detect bisphenol A, and after the starting bisphenol A solution absorbance of the starting bisphenol A solution was reproduced in the effluent, the column was rinsed with deionized water, 50% ethanol and deionized water. The amount of adsorbed substance was found assuming that the column was saturated at 50% breakthrough. The absorbance at 276 nm was measured in fractions to detect diclofenac. The sensitivity limit for determination of diclofenac was 0.7 mg/L, and the standard error of diclofenac estimation was 0.043 mg/L, see Supplementary Materials.

2.2.6. Fourier-Transform Infrared Spectroscopy (FTIR) Spectrometry

Infrared spectra were recorded using a Thermo Nicolet Nexus 6700 instrument (Thermo Scientific, Waltham, MA, USA) equipped with MCT/B detector, and KBr beam splitter. The powder was placed on the ATR crystal and pressed down. The presented scans are averages of 100 collected scans with data spacing of 3.857 cm^{-1} in wavelength range of 4000–525 cm^{-1}. All spectra were processed and analyzed using the OMNIC™ 8 Spectra Software 833-036200.

2.2.7. Scanning Electron Microscopy

SEM micrograph was obtained as described earlier [15], i.e., directly from uncoated dry samples (no sputtering) using a Zeiss EVO LS10 scanning electron microscope equipped with a LaB6 filament (Carl Zeiss Microscopy GmbH, Jena, Germany). Imaging was carried out in variable pressure mode at 10 Pa using a backscatter detector, at 20 kV accelerating voltage, with a 250 pA probe current and 6–7 mm working distance.

3. Results and Discussion

3.1. Characteristics of TRIS- and EA-Agarose Gels

The chemical modification of agarose gels is illustrated in Figure 1. Alkylation of the gels with allyl bromide (Step 1) was followed by the formation of a bromohydrin (Step 2) and its amination with TRIS (Step 3) or ethanolamine. FTIR spectra confirmed the formation of allyl ester (see Figure 2). The absorption band at 930 cm^{-1} is a characteristic of the attached allyl group. A similar band at 922 cm^{-1} ascribed to the in-plane scissoring (δ) of the allyl group was recently observed in the spectrum of the allylated cellulose [16]. The alkylation of the soluble polysaccharide by 1-bromopropane in a strong alkali, i.e., under conditions like ours, has been quantitively described elsewhere [17].

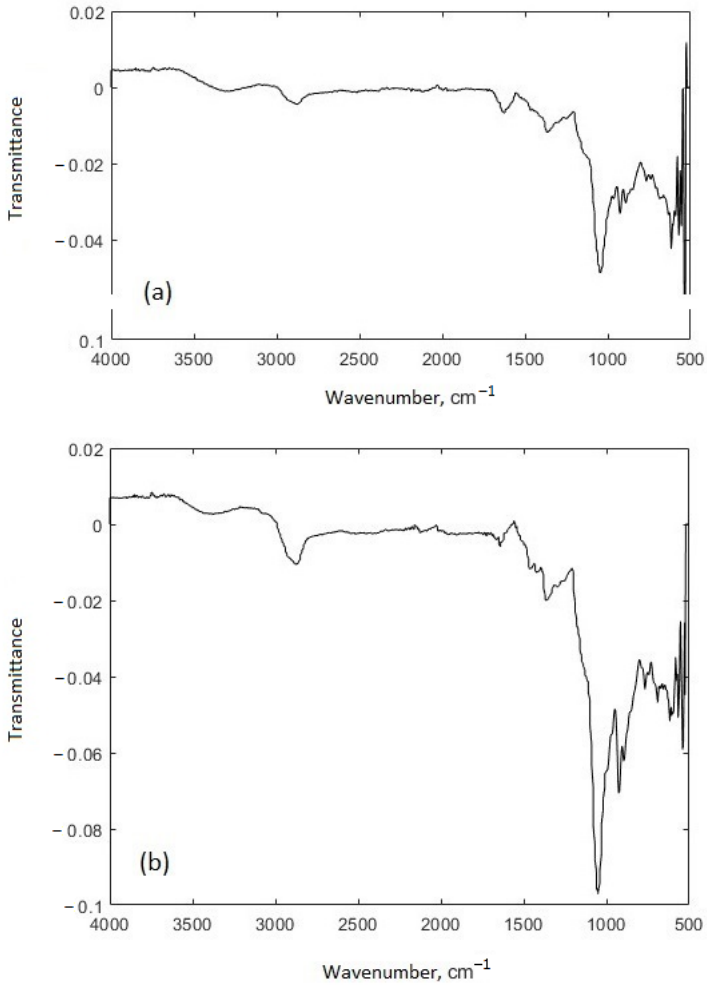

Figure 2. FTIR spectra of freeze-dried Zetarose FlashFlow 4 (**a**) and allyl-Zetarose Flash. Flow 4 (**b**) gels.

The characteristics of the modified agarose gels are listed in Table 1. The bromohydrin content of the 190 μmol/mL gel estimated during the titration of the attached allyl groups by bromine water, see Section 2.2.1, corresponded to ca. 1.5 bromohydrin groups per agarobiose disaccharide repeating unit of agarose. Further covalent attachment of the TRIS- and EA-groups due to the bromohydrin groups' amination resulted in incorporation of the amine nitrogen estimated via elemental analysis in the dried gels, see Table 1, and via potentiometric titration.

Table 1. Characteristics of bromohydrin- and hydroxyalkyl amine-agaroses.

Type of Agarose Gel	Bromohydrin, μmol/g Wet Gel; μmol/mL	TRIS or EA Content (*), μmol/g Wet Gel; μmol/mL	%N	TRIS or EA Content (**), μmol/g Wet Gel; μmol/mL
TRIS-Sepharose 4B	220; 150	80; 55	0.28	18; 12
TRIS-ZetaCell-CL6B	290; 190	140; 92	0.48	31; 20
TRIS-Zetarose FF4	290; 190	75; 49	1.03	46; 30
EA-Zetarose FF4	290; 190	144; 94	1.55	67; 44

(*) according to potentiometric titration; (**) according to elemental analysis.

The titration of the hydroxyalkyl aminated agaroses with 0.1 M HCl, as illustrated in Figure 3, revealed their ion-exchange capacities within the range of 75 to 144 μmol/g wet gel. Notably, EA-Zetarose FF4 exhibited the highest number of ion-exchanging groups among

the studied agarose derivatives. The titration of TRIS- and EA-agaroses led to estimates of higher immobilized amine content compared to the values calculated from elemental analysis. This discrepancy may arise from the experimental errors associated with nitrogen estimation, especially at low amine content in the chemically modified agaroses. Further insights can be drawn from Table 1: amination of the bromohydrin groups was not fully complete, possibly due to steric hindrance caused by the bulky TRIS reagent or competitive hydrolysis of the bromohydrin under alkaline conditions. Conversely, amination with ethanolamine resulted in a higher yield of the hydroxyalkyl derivative, with a substitution density ca. 0.75 group per agarobiose unit.

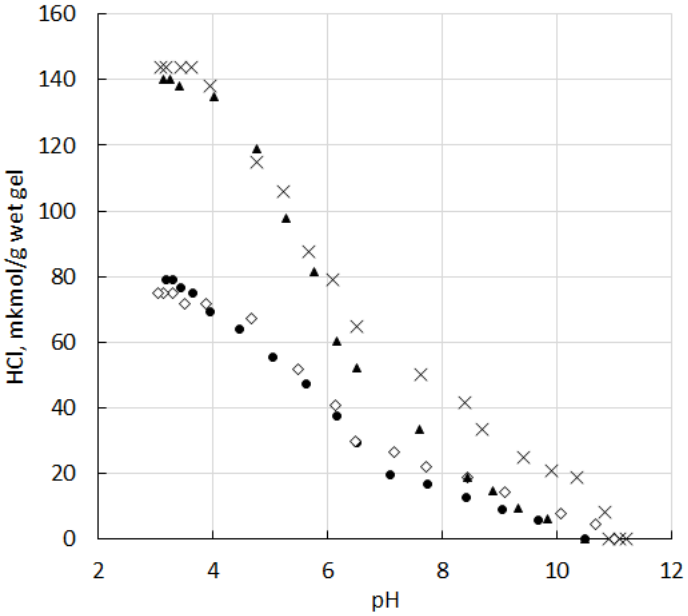

Figure 3. Potentiometric titration curves: (●) TRIS-Sepharose 4B, (◊) TRIS-Zetarose FlashFlow4, (▲) TRIS-ZetaCell CL6B, and (×) EA-Zetarose FlashFlow4.

The titration of hydroxyalkyl aminated agaroses occurred across a wide range of pH values, despite the relatively high pK_a values of TRIS (8.1) and ethanolamine (9.5). This phenomenon is typical of weak anion-exchanger titrations and results from the repellency of the positively charged polymer toward approaching hydrated protons—a phenomenon known as the polyelectrolyte effect. At the realistic pH of deionized or distilled water (6.0–6.5), approximately half of the amino groups exist in the positively charged protonated form. This protonation may enhance adsorption of solutes with opposite electric charges.

3.2. Kinetics of Bisphenol A Adsorption and the Equilibrium Adsorbed Amounts

The time-dependent concentration of bisphenol A solution in contact with the gels is illustrated in Figure 4. The TRIS-agaroses were clearly more adsorptive than the non-modified gels; the adsorption capacities of the latter were like those of neutral poly (vinyl alcohol) hydrogels [15]. The adsorption equilibrium was established in ca. 5 h. The adsorption tests were made for 5 h with TRIS-ZetaCell 6B and 10 mg/L bisphenol A solutions in water or in 0.15 M sodium chloride or in 0.15 M ammonium sulphate which resulted in similar equilibrium bisphenol A concentrations, which suggests that ion exchange does not significantly contribute to the binding mechanism. Indeed, the pKa of bisphenol A is ca. 10.3 [18], which means that the compound exists in an electrostatically neutral form in pure water. Further, since the agarose derivatives do not bear large hydrophobic groups to interact with phenyl rings, see Figure 1, and since the ammonium salt did not affect the binding, the interaction may be ascribed to hydrogen bonding, perhaps of the OH-π type.

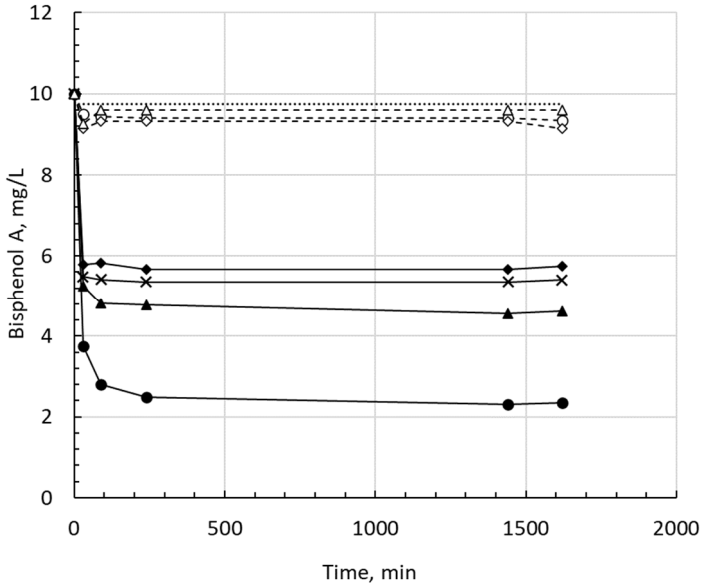

Figure 4. Kinetics of bisphenol A adsorption on TRIS-containing agarose gels (filled symbols) and the corresponding parent gels (open symbols). (▲)—TRIS-ZetaCell CL6B; (●)—TRIS-Sepharose 4B; (♦)—TRIS-Zetarose FF4; and (×) EA-Zetarose FF4. The gels (0.25 mL sediment) were added to 9.75 mL bisphenol A (10 mg/L). The upper dotted line designates 9.75 mg/L bisphenol A concentration resulting from diluting with 0.25 mL of water.

As follows from Figure 4, the highest adsorbed amount was found with TRIS-Sepharose 4B despite its low content of coupled TRIS. This could be due to the different modes and degrees of cross-linking involved in the different agarose beads. Ethanolamine- and TRIS-modified Zetarose FF4 adsorbed similar amounts of bisphenol A.

The TRIS-ZetaCell 6B with intermediate adsorption capacity was chosen for the adsorption study at various starting and equilibrium concentrations of bisphenol A. The obtained adsorption isotherm is illustrated in Figure 5. From the linear part of the isotherm, a distribution coefficient of ca. 50 could be calculated, which indicated significant affinity of bisphenol A to TRIS-ZetaCell 6B. The adsorption isotherm complied with a Langmuir model can be described by the following equation:

$$Q_{BP} = Q_{max}\, C_{EQ}/(K_L + C_{EQ}) \quad (2)$$

where Q_{BP} is the adsorption capacity calculated according to Equation (1) at the equilibrium concentration C_{EQ}, Q_{max}—maximum adsorption capacity at saturation and K_L—equilibrium dissociation constant. The same graph was plotted using the following inverted coordinates:

$$1/Q_{BP} = K_L/Q_{max}\, C_{EQ} + 1/Q_{max} \quad (3)$$

allows calculation of the maximal adsorption capacity, Q_{max} = 16 µmol/mL gel, and the equilibrium dissociation constant, $K_L = 2.7 \times 10^{-4}$ mol/L. The value of Q_{max} is below the content of TRIS in the TRIS-ZetaCell 6B (92 µmol/mL), see Table 1, which indicates limited accessibility of TRIS-groups to the solute. The value of K_L is within the range reported for the interaction of the 12% cross-linked agarose gel with several polyphenols in water [19], where resveratrol showed the strongest interaction, $K_L = 7 \times 10^{-6}$ M, and gallic acid showed the weakest, $K_L = 1.2 \times 10^{-2}$ M.

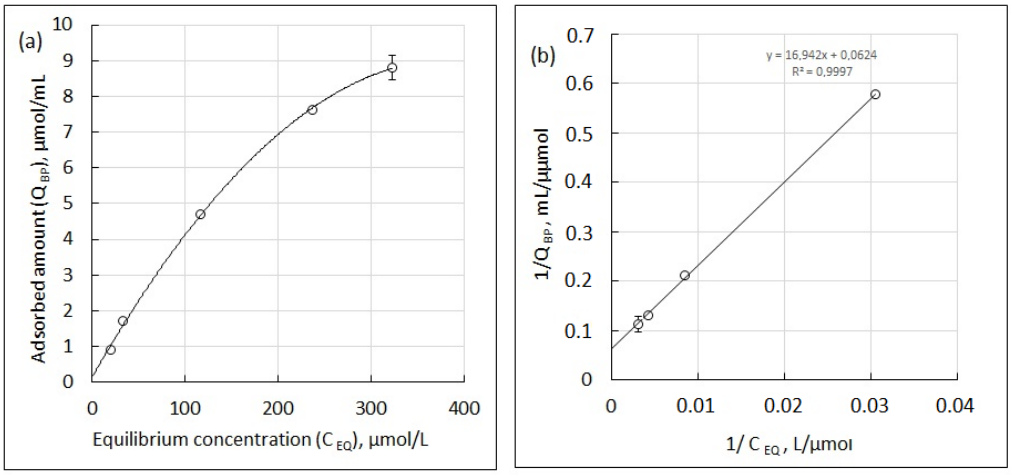

Figure 5. Adsorption isotherm of bisphenol A on TRIS-ZetaCell CL6B: (**a**) Q BP as a function of c EQ BP; (**b**) 1/Q BP as a function of 1/c EQ; and the intercept equals to 1/Q max. The error bars are standard deviations for two independent measurements.

Expressed in mg/g dry polymer, and compared with other functional polysaccharide adsorbents, the maximal specific adsorption capacity of TRIS-ZetaCell CL6B is 62 mg/g, e.g., it is lower than that of phosphonated levan (105 mg/g [20]) and higher than that of chitosan (34 mg/g [21]).

Interestingly, a water-soluble TRIS-functionalized poly (amido amine) dendrimer has previously been reported to bind the polyaromatic hydrocarbon phenanthrene from an aqueous solution at neutral pH [22]. The dendrimer, with a molecular weight of approximately 18,000 g/mol demonstrated the ability to bind up to 10 phenanthrene molecules at ca. 0.1 mM hydrocarbon concentration. The apparent dissociation constant of the complexes can be roughly evaluated as 5×10^{-5} M based on the data presented in Figure 4c of Ref. [22]. These findings confirm the efficacy of TRIS-functionalized polymers in binding aromatic compounds from water. Furthermore, the significant interaction between bisphenol A and TRIS-agaroses suggests the potential for its removal from polluted water through filtration using these gels.

3.3. Removal of Bisphenol A and Diclofenac from Water by Filtration through TRIS-Zetarose 4FF Columns

Deionized water spiked with 10 mg/L bisphenol A was frontally applied to a 1 mL column with TRIS-Zetarose FF4 swollen in water (see Section 2.2.4). Figure 6a illustrates the obtained elution profile. As follows from the figure, the filtration resulted in at least a 10-fold reduction of the pollutant concentrations within 18 column volumes of the effluent. The binding capacity of TRIS-Zetarose FF4 at bisphenol equilibrium concentration of 5 mg/L (50% from the breakthrough, 22 µmol/L) was ca. 1 µmol/mL gel, which agreed with the prediction from the adsorption isotherm (see Figure 5a). Practically, this moderate adsorption capacity can still be employed for water purification in case of successful column regeneration. Indeed, the capacity was completely restored after rinsing the column with 50% aqueous ethanol and water: see the breakthrough curve from repeated bisphenol A application in Figure 6a. Lower chromatographic retention of polyphenols on Superose ^{TM} 12 cross-linked agarose gel column in the presence of higher concentration of ethanol in the mobile phase has been registered elsewhere [19], while the adsorbed amounts of polyphenols calculated from batch experiments accordingly decreased with increasing ethanol concentration.

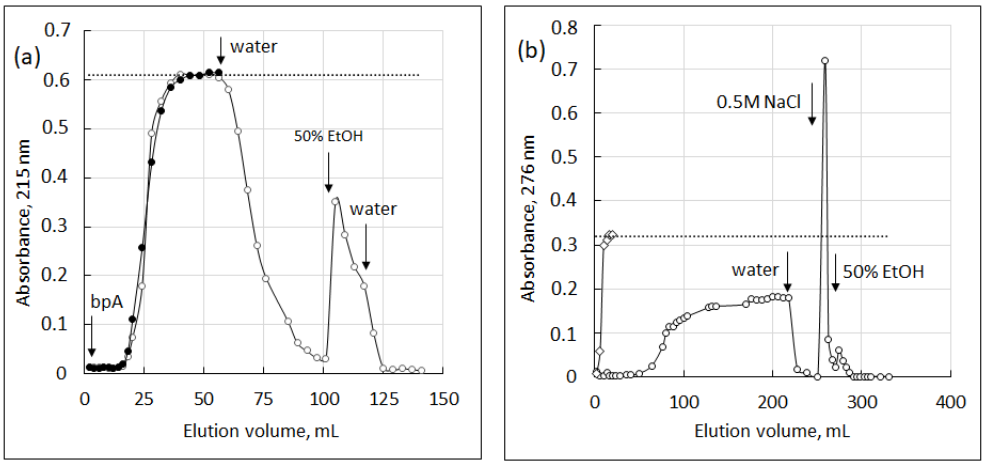

Figure 6. Breakthrough curves of (**a**) bisphenol A (10 mg/L) and (**b**) diclofenac (10 mg/L) obtained on 1 mL columns with TRIS-Zetarose 4FF (○). Closed circles relate to repeated application of bisphenol A to the column. (**b**): Breakthrough curve of diclofenac on 1 mL column with Zetarose-4FF (◊). Flow rate 0.4 mL/min. Dotted lines indicate absorbances of the starting bisphenol A and diclofenac solutions.

A similar filtration experiment has been completed with water spiked with diclofenac, and its chemical structure is shown in Figure 7. Figure 6b illustrates the elution profile of diclofenac frontally applied to a 1 mL column. The filtration resulted in at least a 10-fold reduction of the pollutant concentrations within 64 column volumes of the effluent, unlike filtration through the Zetarose 4FF column where the breakthrough was much faster. Desorption of the substance was achieved by rinsing with 0.5 M NaCl, which indicated an ion-exchange mechanism of adsorption. This was probably due to the interaction of opposite charges on the TRIS-groups (positively charged secondary amine) and on the diclofenac molecule (negatively charged carboxylic acid) in an aqueous medium. Weak anion exchangers, including ovalbumin-polyethyleneimine hydrogels [23] have previously been studied as adsorbents for diclofenac removal from water. The synergistic combination of ion-exchange interactions, hydrogen bonding and π-π stacking led to efficient removal of the pharmaceutical from water.

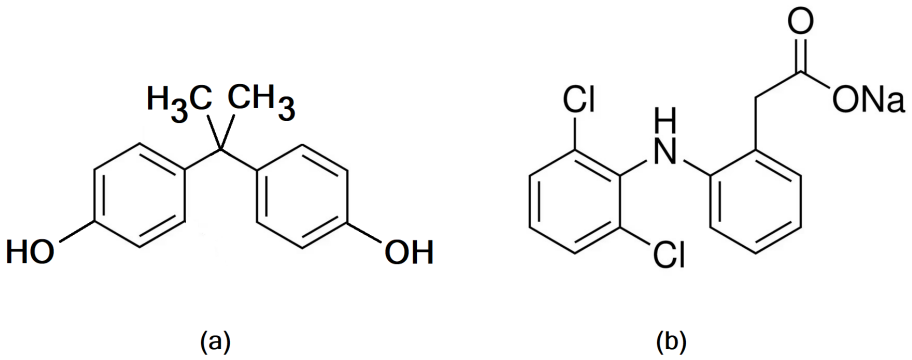

Figure 7. Chemical structures of bisphenol A (**a**) and diclofenac (**b**).

Besides bisphenol A and diclofenac, some other aromatic compounds such as acetanilide and resorcinol were found to adsorb on TRIS-Zetarose 4FF (see Supplementary Materials). Their adsorbed amounts were, however, lower than those discussed above, possibly due to the presence of a single aromatic ring in their molecules.

3.4. Future Trends

Permeable porous materials with an open, three-dimensional structure and high specific surface area play a crucial role in environmental purification technologies. Two notable

examples are aerogels [5,24] and hydrogels [25], which serve as valuable adsorbents. Polymer networks offer various possibilities for introducing specific adsorption sites, such as reactive functional groups, into these materials [25]. Our research focuses on cross-linked agaroses, which serve as an operational model for developing adsorbents using less expensive natural carbohydrate polymers like cellulose or chitosan. Commercial agaroses with their well-defined porosity and particle size, allow us to evaluate the adsorption capacity of both the parent and chemically modified gels. We have demonstrated that the increase in adsorption capacity is indeed due to the immobilized TRIS- or EA-groups. Looking ahead, our research aims to create porous materials with pre-defined shapes and permeabilities that are better suited for technical implementation in purification technologies. Potential avenues include macroporous membranes [26] and spongy flow-through monoliths [15,27], both of which may exhibit hydroxyalkyl amino groups.

Some membranes and monoliths were produced in various shapes using cryogelation techniques [28,29]. These methods involve either the radical polymerization of vinyl monomers or the cross-linking of water-soluble polymers in a semi-frozen state. During this process, ice crystals form and displace the chemical reagents into thin, non-frozen areas. As a result, highly variable macropore structures emerge through polymerization, with interconnected pores of sub-millimeter size forming in the areas where the crystals melt after defrosting [28,29]. These porous materials known as cryotropic hydrogels or cryogels hold great promise for diverse purification techniques. These include frontal adsorption of emerging environmental pollutants [15,27,30], particularly during water filtration through spongy porous structures.

Importantly, the mass-transfer process in these structures primarily relies on convective flow, which accelerates both adsorption and desorption [28]. Figure 7a illustrates the macroscopic view, while Figure 7b provides insight into the macropores of poly (vinyl alcohol) cryogel produced as described in our earlier study [15]. The cryogel exhibits slit-shaped macropores, which serve as primary conduits for convective flow. Additionally, smaller pores populate the walls of these macropores, contributing to the overall specific surface area of the material and potentially accommodating the adsorption sites.

Experimental evidence supports the preparation of functional cryogels based on carbohydrate polymers, which are convenient for chemical functionalization with TRIS- or EA-groups as described in our present work. One notable example is a composite cryogel composed of agarose and poly (hydroxyethyl methacrylate) [31]. This material exhibits inherent interconnectivity of its pores and high diffusive mobility of solutes within the pore structure. An alternative, potentially more cost-effective option, involves the cryogels produced from chitosan [32]. These materials have demonstrated effectiveness in removing metal ions, dyes, and other contaminants from water. The primary amino groups present in each repeating unit of chitosan may facilitate the alkylation step (see Figure 1), which can likely be performed under milder conditions compared to the alkylation of agarose's hydroxylic groups.

Apart from water purification techniques, there are well-recognized challenges in isolating valuable phenolic compounds from food industry by-products [33,34]. While styrene-divinylbenzene and acrylic resins are commonly used for this purpose, these adsorbents may exhibit drawbacks due to slow phenols desorption caused by strong binding to the surface. This is reflected in their low apparent equilibrium dissociation constants ($K_L \approx 10^{-6}$ M for chlorogenic acid and XAD7 resin [33]). Consequently, elution times become lengthy, and substantial volumes of organic solvents are consumed during the desorption of target phenols and column regeneration. Furthermore, the achieved desorption is often not fully quantitative. In contrast, desorption of bisphenol A from TRIS-Zetarose 4FF occurs relatively quickly, leading to complete restoration of the column's binding capacity (as shown in Figure 6a). The less tight binding of phenols to TRIS-agaroses ($K_L \approx 2.7 \times 10^{-4}$ M) and possibly to the cross-linked 12% agarose gels offer advantages over using more hydrophobic resins for phenol isolation.

In our assessment, a challenging option is represented by the combination of the studied TRIS- and EA-agarose derivatives with permeable macroporous textures and spongy monolithic shapes illustrated by Figure 8. Besides the superior mass-transfer phenomena discussed above, these shapes lend themselves to adaptation for various technical equipment employed in adsorption processes. For example, one practical application involves filling of cartridges used in solid-phase extraction for the cleanup and concentration of the phenolic analytes before quantification. The insertion of monolithic adsorbents into the small columns eliminates the need for supporting filters, streamlining construction, and making the packing procedure easier.

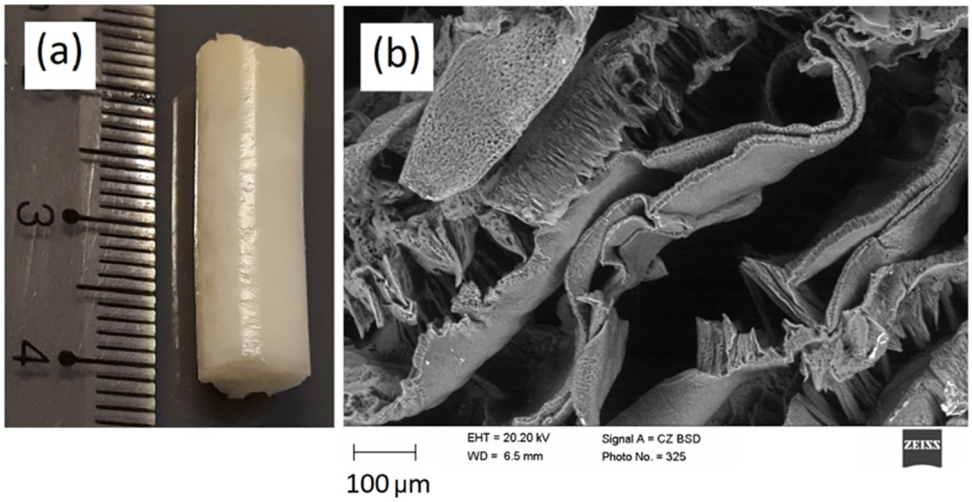

Figure 8. The macroscopic (**a**) and microscopic (**b**) views of poly (vinyl alcohol) cryogels produced according to the study [15]. The monolithic gel was cut perpendicular to the axis to get the slice shown in (**b**).

Another viable option involves the removal of toxic phenolic constituents from smoke, including cigarette mainstream smoke [35]. In a patent application from some years ago, filters composed of dry cylindrical cryogels made from agarose and sodium alginate demonstrated remarkable efficiency [36]. These filters removed ten thousand times more submicron smoke particles compared to conventional cigarette filters, all while maintaining a similar pressure drop across the filter. The resistance to airflow can be adjusted and optimized by varying the gel composition or the concentration of the cross-linking agent. Additionally, the carbohydrate composition of adsorbent material facilitates its natural degradation. The incorporation of TRIS- and EA-groups may further enhance its capacity for binding and removing phenols from smoke or polluted air.

The current and proposed techniques face potential limitations due to the high costs associated with commercial agaroses. To address this, incorporating more cost-effective carbohydrate polymers into the adsorbent structure appears economically viable. However, scaling up cryogelation techniques to industrial levels poses a challenge for developers. Achieving rapid and consistent freezing across all production samples becomes crucial in this context.

4. Conclusions

The synthesized TRIS- and EA-derivatives of the agarose gels had much higher adsorption capacities for bisphenol A and diclofenac compared with the parent gels. Binding of both the compounds was due to their interaction with the immobilized TRIS and EA groups. Hydrogen bonding may underlie bisphenol A adsorption, whereas diclofenac was adsorbed because of ion-exchange interactions with the TRIS groups positively charged at neutral pH. The effects of TRIS and EA coupling may be used for the development of environmental adsorbents as well as for the preparative separation of polyphenols and phar-

maceuticals. The moderately strong binding of polyphenols to TRIS- and EA-adsorbents facilitates easy polyphenol desorption and column regeneration. When considering the physical shapes and textures of the TRIS- and EA-functionalized adsorbents, one promising technical solution for the future is the use of monolithic macroporous cryogels. These adsorbents could play a crucial role in the purification of both liquids and air.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/appliedchem4010004/s1, Figure S1: Linear calibration for estimation of bisphenol A at concentrations below 10 mg/L; Figure S2: Linear calibration for estimation of bisphenol A at concentrations above 10 mg/L; Table S1: Summary output obtained in Excel for the linear regression of Figure S1; Figure S3: Linear calibration for estimation of diclofenac; Table S2: Summary output obtained in Excel for the linear regression of Figure S3; Table S3: Breakthrough curves of bisphenol A (10 mg/L) obtained on 1 mL column with TRIS-Zetarose 4FF (Figure 6a): Raw data. Table S4: Breakthrough curves of diclofenac (10 mg/L) obtained on 1 mL column with TRIS-Zetarose 4FF (Figure 6b): Raw data; Figure S4: Breakthrough curves of acetanilide and resorcinol.

Author Contributions: Conceptualization, L.L.; methodology, L.L., S.I. and A.E.I.; validation, S.I. and A.E.I.; formal analysis, A.E.I.; investigation, S.I. and A.E.I.; resources, L.L.; writing—original draft preparation, A.E.I.; writing—review and editing, L.L.; visualization, A.E.I.; supervision, L.L.; project administration, L.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data are contained within the article and Supplementary Material.

Acknowledgments: This work was carried out in memory of and as a tribute to Jerker Porath. During the last years of his life, we had interesting discussions on agarose modifications for new applications as this was in line with his earlier research. We deeply regret we are not able to continue his ambitions. The authors are also thankful to Viktoria Toldi, a trainee at Malmö University, for participating in adsorption studies, to Tobias Halthur (CR Competence AB, Lund, Sweden) for carrying out SEM experiments and to Ekaterina Bogdanova (Malmö University) for her assistance with FTIR spectrometry of the agarose gels.

Conflicts of Interest: The authors declare no conflicts of interest. Author Alexander E. Ivanov was employed by VitroSorb AB, a Swedish medical device company. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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